

Augmenting efficacy of the commercial probiotic consortium, Ecotrax® on soil, water quality, survival, growth and feed transformation on the semi-intensive pond culture system of the white leg shrimp, *Litopenaeus vannamei* (Boone, 1931)

Edward Gnana Jothi George^{a*}, Godfred Ponraj Jayaraj^b, Deivasigamani Balaraman^a and Arulselvam Soundarapandy^c

^aCAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Portonovo, Tamilnadu, India
^bTIL Biosciences (Animal Health Division of Tablets India Limited), 72, Marshalls Road, Chennai, Tamilnadu, India
^cMinerva Aqua Farms Limited, Alluru, Nellore, Andhra Pradesh, India

ABSTRACT

*The competence of the commercial probiotic consortium, Ecotrax® as water and soil probiotic in enhancing the water quality, growth, survival and feed conversion of *Litopenaeus vannamei* were investigated for better yield with lesser feed conversion ratio (FCR). Three test ponds treated with Ecotrax® and three control ponds were used in the study for the duration of 119 days. Weekly investigation at regular intervals were conducted on both experimental groups for physicochemical parameters of water involving temperature, pH, salinity, transparency, dissolved oxygen (DO), hardness, and average body weight (ABW) to favour proper feed management and pond management strategies. Total production, survival numbers, survival percentage, ABW, feed conceded and feed conversion ratio (FCR) were analysed post harvesting in both test and control ponds. Significant differences ($P < 0.001$) were observed in the parameters like transparency and hardness between the test and control ponds. Water quality was observed to be nutritive in the test ponds treated with Ecotrax®. This was also proven by the productivity with significant difference ($P < 0.001$) between the experimental groups. Test ponds treated with Ecotrax® had exhibited higher production, survival percentage, ABW, feed consumption with attractively lesser FCR. Ecotrax® (TIL Biosciences) was highly effective in maintaining the soil and water quality parameters, engendering better growth, survival, ABW, yield and impressive FCR in *L. vannamei* shrimp aquaculture. Thus generating higher revenue to the aquaculturists and contributing to the economic growth of the country.*

Keywords: Ecotrax®, Aquaculture, Soil probiotics, Water probiotics, and TIL Biosciences.

INTRODUCTION

In recent years, aquaculture has grown immensely and due to high economic and export value, penaeid shrimps are of high significance in the global aquaculture market. The annual production of shrimp is approximately 5 million metric tons. However, the current global demand for both the wild and farmed shrimp is more than 6.5 million metric tons per annum [1]. In order to meet the demand and increase production, water quality is a major concern to be taken care of. Water quality is the central factor for a successful shrimp aquaculture as water serves as the culture medium for the growth of the shrimps. The quality of water deteriorates with the progress of culture and increase in biomass which may lead to less nutritive water and other microbial infections as a result of the accumulation of metabolic waste of cultured organisms, decomposition of unutilized feed and decay of biotic materials [2]. Antibiotics were once considered to be a remedy and currently its usage in shrimp aquaculture was prohibited as this leads to the emergence of various drug resistant microorganisms through adaptation or by genetic exchange [3]. Hence, the use of probiotics as biological control agents is increasing with the demand for more environment

friendly aquaculture practices. In order to be considered as biological control agents in aquaculture, probiotics should be non-pathogenic and biochemically and physiologically well characterized. They should be normal inhabitants of the host and able to survive and grow at the site of application while exerting their beneficial effect. Finally, they should maintain their viability and activity throughout product manufacturing and storage [4]. Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes [5]. Irianto and Austin, [6], reported that the application of beneficial microorganisms (*Bacillus*, *Lactobacillus*, *Nitrosomonas*, *Cellulomonas*, *Nitrobacter*, *Rhodoseudomonas*, *Nitrosomonas* and *Acinetobacter*) would be very useful for controlling the pathogenic microorganisms and water quality.

The present work investigates the efficacy of the commercial probiotics consortium, Ecotrax®, a flagship product of TIL Biosciences (An Animal Health Division of Tablets India Limited), in enhancing the water quality, growth, survival and feed transformation of the white leg shrimp, *L. vannamei* in semi-intensive pond culture system.

MATERIALS AND METHODS

Study area

The study was carried out in Minerva Aqua Farm located at Kurru Island (14°42'50"N and 80°7'5"E), Alluru, Nellore, Southeast coast of India (Figure 1) during the period of March to June. Each pond selected for the study was of 1h area. Water used for the semi-intensive culture was pumped from the creek in to the pond post bleaching.

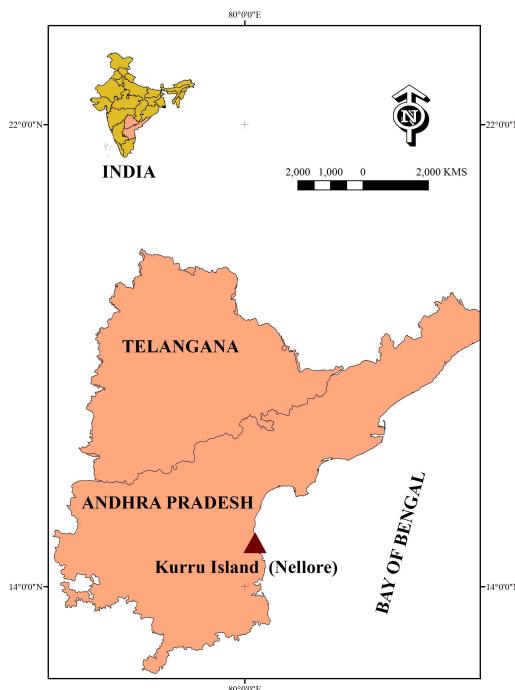


Figure 1: Map showing the study area, Kurru Island, Nellore, Andhra Pradesh

Probiotic strains in Ecotrax®

After repeated screening for the probiotic characteristic features as water and soil probiotics, seven potential *Bacillus* strains were selected out of several hundred strains based on its efficacy as biological control agents, biochemical and physiological properties, viability and activity throughout product manufacturing and storage. A consortium was formed and checked for the above said properties in order to develop them into a product in commercial scale. The probiotic consortium composition of the product, Ecotrax® (TIL Biosciences, Animal Health Division of Tablets India Limited, Chennai, India) is furnished in the Table 1.

Table 1: Composition of Ecotrax® (per 1000 gms)

S. No.	Strains	Cell count
1.	<i>Bacillus sp</i> ABPL 135	2×10^{10}
2.	<i>Bacillus sp</i> ABPL 136	2×10^{10}
3.	<i>Bacillus sp</i> ABPL 025	2×10^{10}
4.	<i>Bacillus sp</i> ABPL 026	2×10^{10}
5.	<i>Bacillus sp</i> ABPL 142	2×10^{10}
6.	<i>Bacillus sp</i> ABPL 144	2×10^{10}
7.	<i>Bacillus mesentericus</i> TOA	2×10^8

Experimental design

Trials were carried out with six ponds categorized into two groups' test and control group with three ponds for each group. Three test ponds were treated with Ecotrax®. Water source, surface topography, seed procurement, maintenance, pond preparation, seeding strategy and physicochemical parameters of the water in all six ponds were maintained alike in order to make rational investigation with least possible variation. Physicochemical parameters of water responsible for the growth of the animals, feed intake, Feed Conversion Ratio (FCR), and survival were analysed.

Pond preparation

The waste materials in the ponds were removed by scrapping from the excavated ponds and tilled for proper preparation. It is then allowed to sun dry in order to oxidize the hydrogen sulphide and eliminate other obnoxious gases. Post drying, required amount of burnt lime (CaO) was applied in order to elevate the temperature and thereby eliminating the microbes and other organisms. Soil pH was recorded in the pond using pH pen (Erma, Japan). Upon calculating the average pH of the ponds, required amount of agricultural lime was applied to maintain the optimum pH. Initially, water levels in the ponds were maintained at 1.5 m level. Ponds were then bleached with 30 ppm bleaching containing 30% chlorine. In order to provide proper aeration, four paddle wheel aerators (2 HP each) were fixed in the four corners of the pond. Subsequently, for enhancing the growth of planktons and to supplement other minerals and carbon source, fermentation juice (Table 2) was prepared and added to the ponds along with commercial probiotic, Ecotrax® and other pre seeding constituents in the test ponds and without Ecotrax® in the control ponds. Physicochemical parameters of the water were checked.

Table 2: Required constituents for pond preparation per hectare

S. No.	Ingredients	Quantity (kg)
Fermentation juice constituents		
1.	Jaggery	10
2.	Dried yeast	0.250
3.	Ground nut cake	15
4.	Rice bran	15
Pond preparation		
5.	Diamino phosphate (DAP)	15
6.	Agricultural lime	500
7.	Ecotrax®	1
8.	Urea	20
9.	Extramin (Minerals)	10

Physicochemical parameters of test and control ponds

The physicochemical parameters exhibiting the water quality of the shrimp culture pond was monitored once in a week with regular intervals at different corners of the pond. Water salinity was measured using a portable hand-held refractometer (Erma, Japan), pH was measured using electronic pH pen (Erma, Japan), and temperature was measured using standard Celsius Thermometer. The dissolved oxygen was estimated by modified Winklers' method as described by Strickland and Parsons, [7]. The total hardness of the water was estimated by complexometric titration using EDTA [8]. Transparency was measured based on the penetration of light using a Secchi disc.

Seed stocking

Healthy *L. vannamei* seeds were procured from a commercial hatchery and were transported in oxygenated double layered polythene bags. Pre stocking, the seeds were acclimatized to the pond ambience and water by allowing the seed bags to float on the water surface in each pond for 30 min. The bags were then opened and the pond water was slowly introduced to the bag by sprinkling on to the bags for 60 min to match with the pond water quality. Consequently, the bags were shuttled to various parts of the pond and the seeds were released slowly. The seeds were stocked at a density of 30/m².

Feeding profile

The shrimps were fed with Godrej White Diamond feed (Godrej Agrovet Limited, India). The feeding schedule was based on the feed chart provided by the company (Table 3). Blind feeding was done for initial 25 days. Later, the feeding was adjusted based on the check tray observation and sampling conducted periodically on every Saturday. Two check trays were mounted on two sides of the pond over catwalk alongside the feeding area for monitoring the health status of the animal and feed intake. In order to cover wider area in the pond surface, feed was broadcasted over the pond through rope method using floats four times a day.

Table 3: Feeding profile as prescribed by the manufacturer (Godrej Agrovet Limited, India)

Feed No.	Feed type	Shrimp weight (gms)	Feeding rate (as % of body wt.)
1	Crumble	PL 15-0.6	20-9%
2	Crumble	0.5-2.0	10-5%
3	Mini Pellet	2-5	6-4%
3P	Pellet	4-12	4.5-3.5%
4S	Pellet	10-18	3.5-2.2%
4	Pellet	>18	2.2-1.8%

Ecotrax® application profile

The recommended dosage of Ecotrax® for stocking density of 30/m² was 400 grams/acre at a frequency of 7 days. The recommended dosage level of Ecotrax® for various pond sizes, is mentioned in the Table 4. The recommended dosage of Ecotrax® was mixed with the pond water of the same pond and left out for 20 mins with mild aeration during morning hours. Later, the mixture was gently sprinkled over the pond via., the rope method using float evenly over the pond and the pond was properly aerated following the addition of Ecotrax®.

Table 4: Standardized dosage of Ecotrax® as per manufacturer (TIL Biosciences, Animal Health Division of Tablets India Limited, India) instruction

S. No.	Stocking Density (SD) / m ²	Quantity (gm) / acre	Frequency (days)
1.	10 – 20 / m ²	200	7
2.	20 – 30 / m ²	400	7
3.	40 – 50 / m ²	600	7

Growth, survival and feed transformation analysis

Sampling was done in test and control ponds once in a week with regular intervals during the early hours of the day with a cast net of known diameter (3.2m). The total number of shrimps caught per haul and individual weight of the shrimps was recorded. Growth of the animal was analysed using the data acquired from weekly sampling reports.

$$\text{Average Body weight (ABW)} = \text{Wet weight of the animals / No. of animals}$$

$$\text{Weight Gain (\%)} = [(\text{Final weight} - \text{Initial weight})/\text{Initial weight}] \times 100$$

Post harvesting, the total production, animals survived and the percentage of survival was calculated based on the harvesting data acquired. The data acquired from the test and control ponds were compared and analysed.

$$\text{Survival Nos./pond} = \text{Animal count} \times \text{Wet weight of produced shrimp (Biomass)}$$

$$\text{Survival (\%)} = (\text{No. of animals survived} / \text{No. of animals stocked}) \times 100$$

At the end of the culture period, shrimps in both the test and control ponds were harvested. A bag net was fitted on outlet canal with 20 numbers mesh of width 1m and a length of 4 m. The water was released and the shrimps were caught and collected. The cumulative feed consumed was calculated for every pond and the feed conversion ratio (FCR) was calculated based on the data acquired post harvesting.

$$\text{Feed Conversion Ratio (FCR)} = \text{Cumulative dry weight of feed} / \text{Wet weight of produced shrimp (Biomass)}$$

Statistical analysis

Data are presented as mean \pm SE. The results were analysed by the Student's *t*-test to determine the significant difference ($P < 0.001$) between the control and test ponds. All statistical calculations were performed using SPSS for Windows version 11.5 (SPSS Inc, Chicago, IL, USA). All column charts were plotted using Origin 6.1 (OriginLab Corporation, Massachusetts, USA).

RESULTS AND DISCUSSION

It is vital to provide shrimp with a healthy environment and probiotics has a great deal of potential [9]. In this study, we ascertained the augmenting efficiency of the commercial probiotic consortium, Ecotrax® on soil, water quality, growth, survival and feed conversion ratio of semi-intensive pond culture system of *L. vannamei* for a period of 119 days with a control group without administering Ecotrax® and test group with Ecotrax® administration. Addition of probiotic as water cleanser was found to be highly beneficial in the water quality management [2]. However, the efficacy of a probiotic depends on many factors such as species composition, application level, frequency of application and environmental conditions. Hence, significant physicochemical parameters of water influencing the growth, survival and feed transformation was scrutinized following standard application procedure of the probiotic prescribed by the manufacturer upon various testing.

Water quality plays a pivotal role in the successful *L. vannamei* culture. As the culture proceeds, the quality of water deteriorates due to the accumulation of metabolic organic and inorganic waste materials, biotic materials and unused feed decomposition [10]. The growth of the shrimps remains unaffected until equilibrium is maintained between the internal and external system of the animal. However, this equilibrium is distressed when there is impairment in the water quality parameters, which consequently makes the animal vulnerable to diseases due to stress and thus stunts growth and survival. Despite of the above discussed facts, probiotics are reported to effectively deal with these issues and improve water quality, growth rate, survival and feed transformation in farmed shrimps [11].

It has previously been reported that, improved water quality has especially been associated with *Bacillus* species [11]. The fundamental fact is that, gram positive bacteria are better converters of organic matter back to carbon dioxide and involved in the conversion of ammonia to nitrate through nitrite as well thus favouring the growth. Stability of commercial probiotics is a key factor and Ecotrax® is a consortium of seven potential *Bacillus* species selected after various testing procedures and is scientifically proven to be stable for two years.

The seeds were stocked with stocking density of 30/m². The physicochemical parameters were analysed once in a week with regular intervals in both experimental groups. The values are represented as means with standard errors of three replicates for each group (mean \pm SE; n = 3). The data obtained were then analysed statistically and determined for significant differences ($P < 0.001$) by Student's *t*-test between the control and test groups and is presented in the Table 5.

Table 5: The values of physicochemical parameters recorded on control and test ponds

S. No.	Parameters	Values ^a		
		Control ponds	Test ponds	P value
1.	Temperature (°C)	29.666 \pm 0.2	29.769 \pm 0.2	0.02954
2.	pH	8.027 \pm 0.1	8.005 \pm 0.1	0.66373
3.	Salinity (ppt)	31.277 \pm 0.3	31.055 \pm 0.3	0.85182
4.	Transparency (cm)	34.388 \pm 0.5	27.0 \pm 0.5	1.1185E-12*
5.	Dissolved Oxygen (ppm)	4.261 \pm 0.2	4.233 \pm 0.2	0.47993
6.	Total Hardness (mg/L)	1344.389 \pm 15	1110.944 \pm 13	3.92364E-13*

^a Values are means with standard errors of three replicates for each experimental group (mean \pm SE; n = 3)

* Significance at $P < 0.001$ as determined by Student's *t*-test

Temperature is one of the most important environmental parameters in shrimp aquaculture as it directly influences the metabolism, oxygen consumption, growth, moulting and survival. Sudden change in temperature may affect the shrimp immune system. But, Rengipat *et al.*, [12], mentioned that the use of *Bacillus* species provided disease protection by activating both the cellular and humoral immune defense mechanism in tiger shrimp (*Penaeus monodon*). Maximum temperature in the control group throughout the study period was reported to be 34°C and the minimum was observed to be 25°C. Maximum and minimum temperature was reported to be the same in both the experimental groups and hence, no significant difference ($P < 0.001$) was observed between the control and test group (Figure 2). However, the animals in the test ponds remained healthier than the control. This may be due to the seven potential *Bacillus* species enriched in the product Ecotrax®.

pH is yet another significant physicochemical parameter effecting the growth of the shrimps to a higher extent by impelling the metabolism and other physiological processes in the biological system. pH and photosynthesis are almost directly linked as higher pH infers higher photosynthesis and higher fertility. Perhaps, this is due to the fact that, water fertility leads to healthier plankton bloom, which subsequently leads to higher photosynthesis. Ramanathan *et al.*, [13], reported that, the optimum range of pH required for maximum growth and production of penaid species is 6.8 to 8.7. pH in control and test ponds ranged from 7.8 to 8.5 and hence no significant difference ($P < 0.001$) was observed between the control and test ponds (Figure 3). This could be considered as the optimum

pH required for healthy shrimp culture as certain salts like bicarbonate has to be present fundamentally in the water medium for growth, reproduction and other physiological activity.

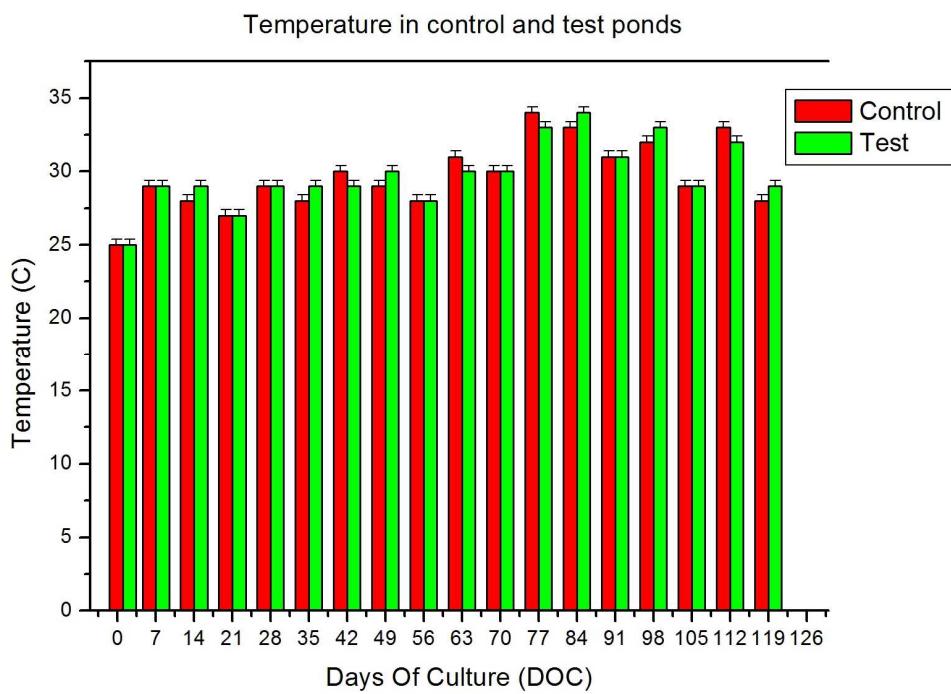


Figure 2: Values of temperature recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean \pm SE; n = 3)

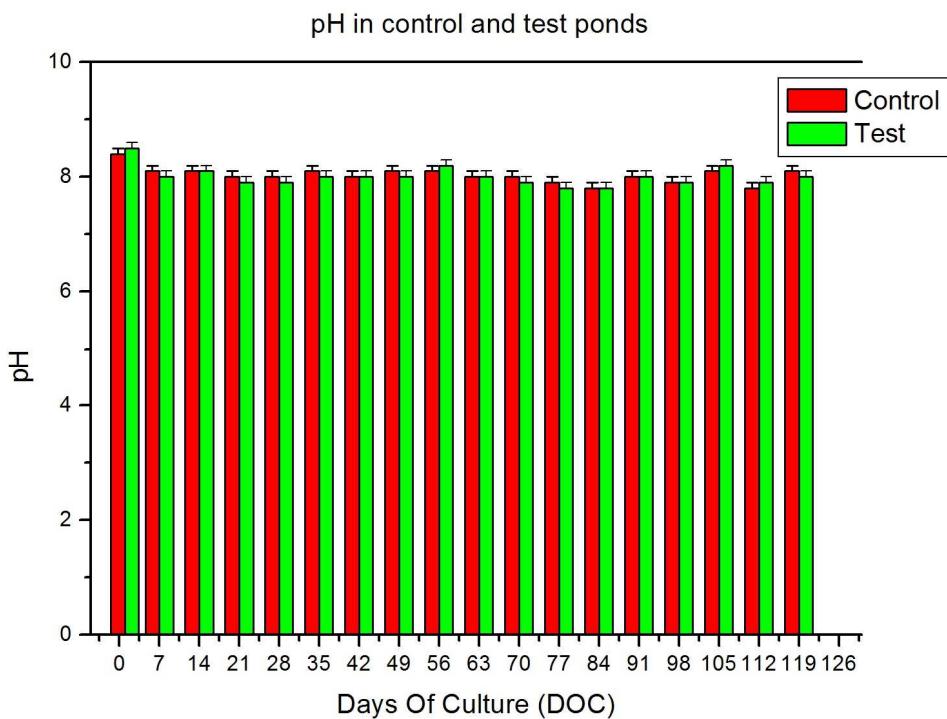


Figure 3: Values of pH recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean \pm SE; n = 3)

The above discussed two physicochemical parameters; temperature and pH were dependent on each other to an extent. According to Le Chatelier's Principle, the forward reaction involving the formation of hydrogen ions (hydroxonium ions) and hydroxide ions from water is an endothermic process absorbing heat as follows,



If the condition of a reaction in dynamic equilibrium is changed, the position of equilibrium moves to counter the change made. Hence, when the temperature of the water increases, the equilibrium will move to lower the temperature. This process takes place by absorbing the extra heat. This exemplifies that the forward reaction is favoured leading to increased formation of hydrogen and hydroxide ions.

Salinity is considered to be the most vital factor in propelling many functional responses of the shrimp biological system as metabolism, growth, migration, osmotic behaviour, and reproduction. High salinity confers increased immunity to the shrimps against diseases, but on the other side, stunts growth. In low salinity the shell becomes weak and the animal may become susceptible to diseases. Though, *L. vannamei* is familiar for its wide range of salinity tolerance, maintaining an optimal salinity is a must for successful shrimp culture. Nevertheless, salinity depends upon various factors like season, rainfall, temperature and water condensation. Salinity ranged from 26 to 37 ppt in the control and test ponds. However, there is no significant difference ($P < 0.001$) was observed between the two experimental groups (Figure 4).

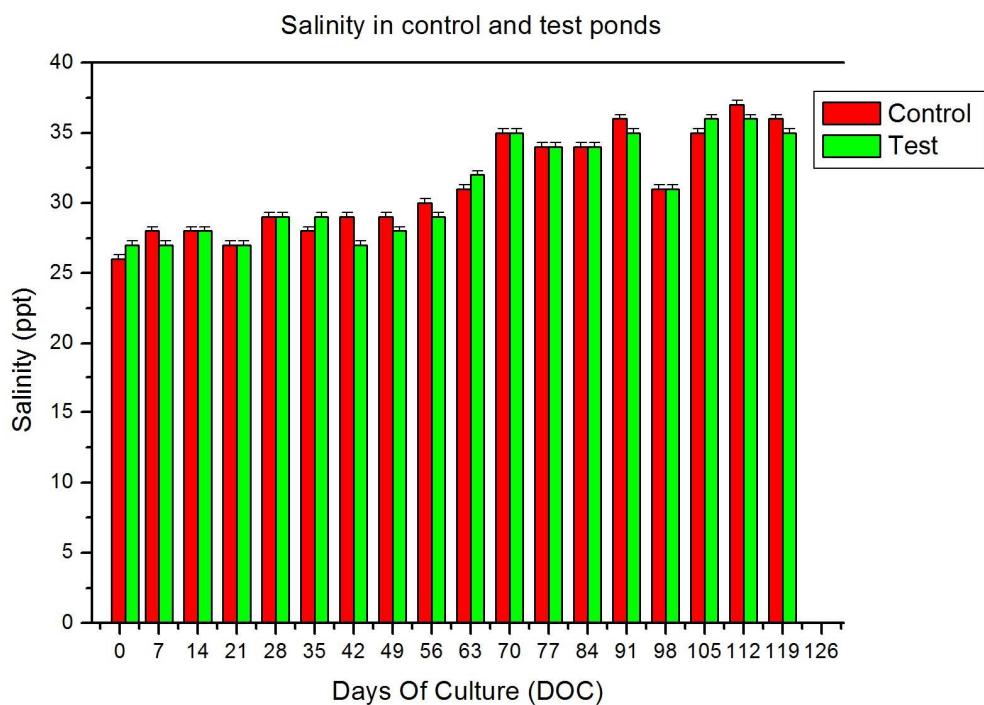


Figure 4: Values of salinity recorded on control and test ponds for a period of 119 days at regular intervals of 7 days
 *Results are presented as means with standard errors of three replicates for each experimental group (mean \pm SE; $n = 3$)

Dissolved oxygen is yet another critical factor required for the respiration of shrimps, in maintaining a balance between the biological system and chemical environment and maintaining the hygiene in the culture ponds. Decreased oxygen level induces the reduction of nitrates to ammonia, which is toxic to the shrimps. It also impedes metabolic performances in shrimp and can reduce growth and moulting which leads to increased mortality [14]. Thus, it is important to maintain aeration in the culture ponds by setting up required aerators. During our study, mechanical aeration was temporarily stopped at regular intervals during daytime with sunlight, as the photosynthesis will be higher in the presence of sunlight by the phytoplankton, thus leading to increased oxygen level than the control ponds. The dissolved oxygen level was observed to be in the range of 4 to 4.4 ppm in the control and test groups with no significant difference ($P < 0.001$) between the two groups (Figure 5). Though the oxygen level in the control and test ponds were not significantly different, ponds treated with Ecotrax® in test ponds had better plankton bloom and hence increased oxygen level favouring the growth of animals and restricting the use of oxygen releasing chemicals. Thus favouring the aquaculture farmers by helping their economy through cutting the expenditure spent on chemicals.

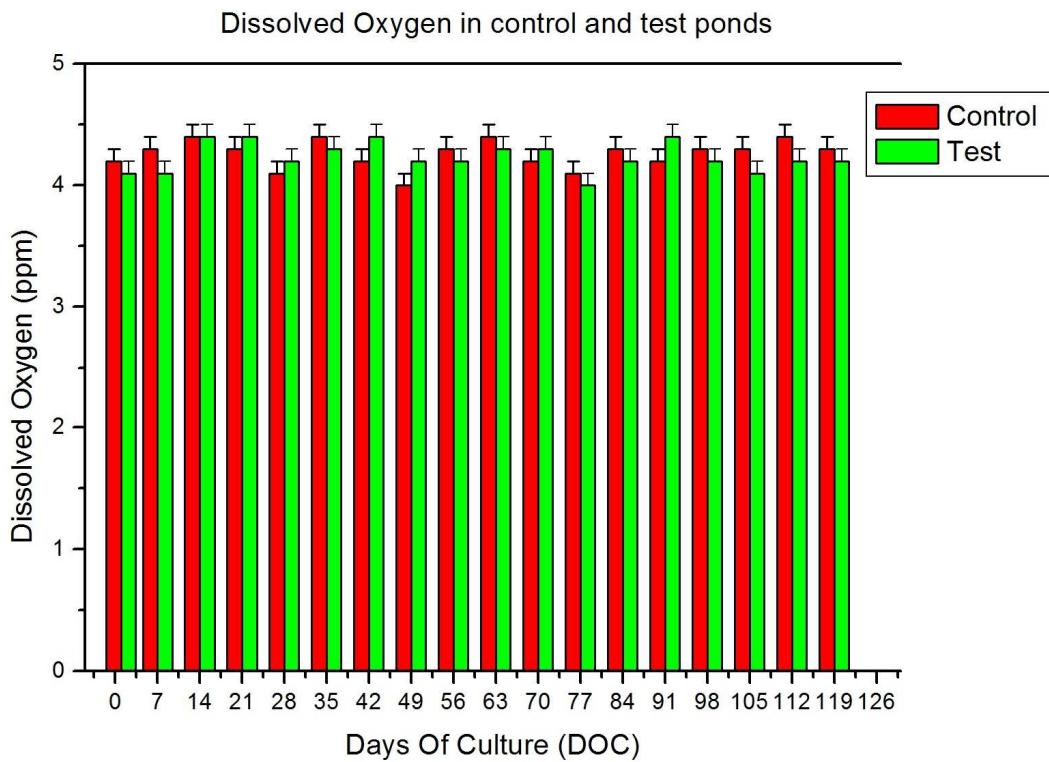


Figure 5: Values of dissolved oxygen recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean \pm SE; n = 3)

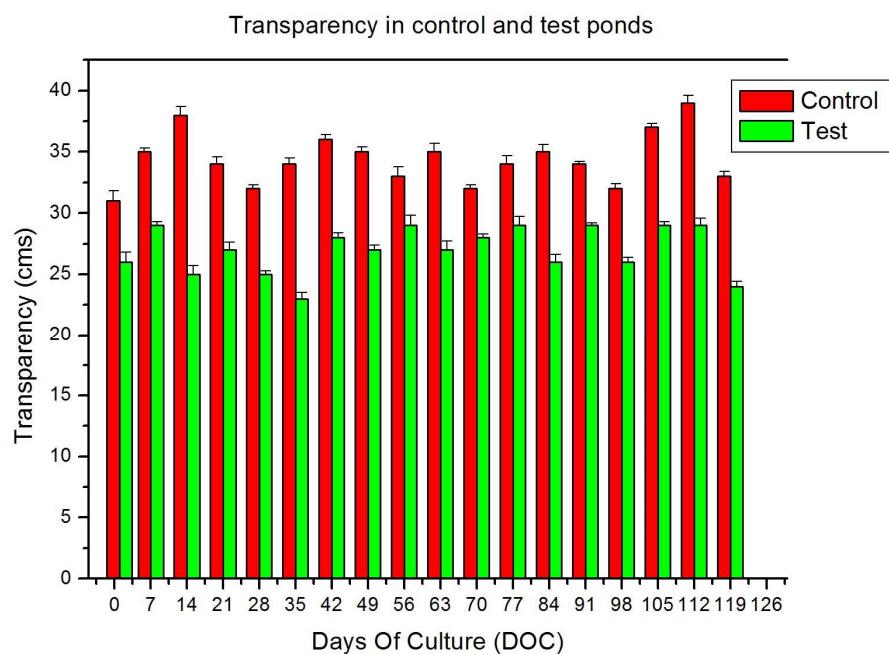


Figure 6: Values of transparency recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean \pm SE; n = 3)

Transparency is a factor exemplifying the plankton bloom. Phytoplankton plays a significant role in the pond ecosystem and minimizes the water quality fluctuations thereby enriches the water quality and suppress the bacterial growth [2]. Higher the transparency, lower the plankton growth. Probiotic bacteria develop healthier bloom by nourishing the water with the enzymes produced by the bacteria and enable the proliferation of beneficial phyto and zooplankton, which forms as the natural feed for the freshly stocked post larvae. Also, it reduces the incidence and development of filamentous algae. Transparency in the control group ranged from 31 to 39 cm. Nevertheless,

transparency in test ponds ranged from 23 to 29 cm. The means of the two experimental groups were notable and the variation was observed to be statistically significant ($P < 0.001$) by Student's *t*-test between the control and test groups with the P value of 1.1185E-12 (Figure 6). In our study Ecotrax® applied in the test ponds reported less transparent than the control ponds with significant differences. The visual appearance of the pond water in Ecotrax® applied ponds were observed to be hygienic with healthier animals during sampling with no infections as well.

Water hardness is caused by the presence of dissolved bicarbonate minerals like calcium bicarbonate and magnesium bicarbonate. Water hardness is determined by the concentration of multivalent cations in the water. Multivalent cations are positively charged metal complexes with a charge greater than 1+. Common cations found in the hard water include Ca^{2+} and Mg^{2+} . The total water hardness is the sum of the molar concentration of Ca^{2+} and Mg^{2+} in mol/L. Agricultural lime was also used to soften the water through lime softening and probiotics enriched in Ecotrax® has found to enhance the process of lime softening and thus decreased hardness of water in the test ponds. Total hardness estimated using complexometric titration method showed statistically significant difference ($P < 0.001$) between the means of two experimental groups with P value of 3.92364E-13 (Figure 7). Total hardness ranged from 1170 to 1461 mg/L in the control ponds and from 1017 to 1191 mg/L in the test ponds, thus exhibiting significant difference ($P < 0.001$). Total hardness in the test and control ponds was observed to be significantly different with lesser hardness in the test ponds treated with Ecotrax®.

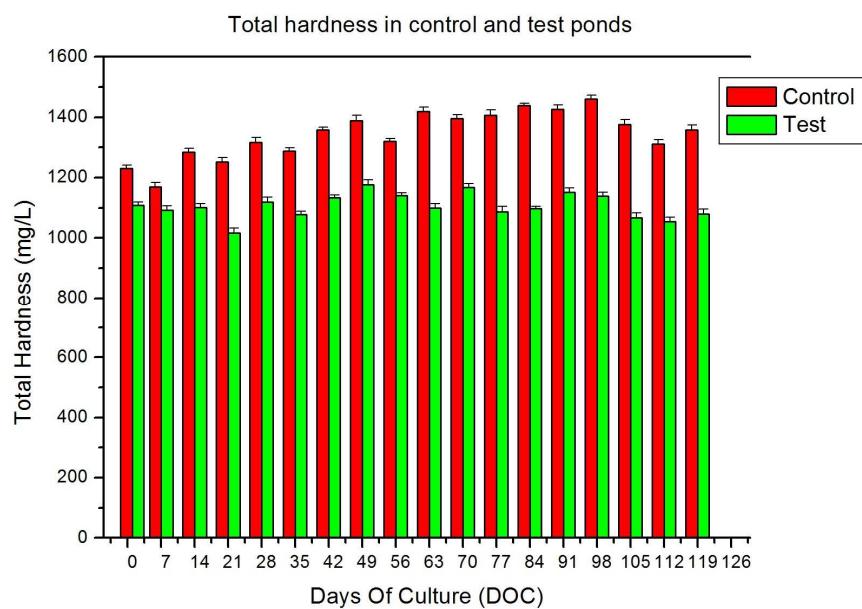


Figure 7: Values of hardness recorded on control and test ponds for a period of 119 days at regular intervals of 7 days
 *Results are presented as means with standard errors of three replicates for each experimental group (mean \pm SE; $n = 3$)

The growth, survival and feed transformation were calculated post harvesting and is presented in the Table 6.

Table 6: The values obtained on growth, survival and feed conversion in control and test ponds

S. No.	Particulars	Values ^a		
		Control Ponds	Test ponds	P value
1.	Stocking Density / m ²	30	30	-
2.	DOC	119	119	-
3.	Initial weight (mg)	4.27 \pm 0.3	4.27 \pm 0.3	0.56144
4.	Final weight (gm)	26.31 \pm 0.3	33.33 \pm 0.3	2.81084E-7*
5.	ABW (gm)	26.31 \pm 0.3	33.33 \pm 0.3	2.81084E-7*
6.	Total production (kg)	6674.11 \pm 1.2	9318.33 \pm 1.7	2.13583E-10*
7.	Survival nos.	253672 \pm 45	279833 \pm 51	4.19236E-12*
8.	Survival (%)	84.55 \pm 0.2	93.27 \pm 0.2	2.15988E-6*
9.	Count	38 \pm 5	30 \pm 2	-
10.	Feed consumption (kgs)	10603 \pm 10	11781 \pm 10	0.5526
11.	FCR	1.58 \pm 0.4	1.26 \pm 0.1	1.57227E-4*

^a Values are means with standard errors of three replicates for each experimental group (mean \pm SE; $n = 3$)

* Significance at $P < 0.001$ as determined by Student's *t*-test

Comparatively, the average body weight of the shrimp is significantly higher ($P < 0.001$) in test ponds (33.33 ± 0.3 gm) administered with Ecotrax® than that of the control ponds (26.31 ± 0.3 gm). Total production of the shrimps (biomass) was significantly higher ($P < 0.001$) as well with 9318.33 ± 1.7 kg harvested from the test ponds, in contrast to 6674.11 ± 1.2 kg in the control ponds. The survival percentage in test ponds were 93.27 ± 0.2 % with survival numbers of 279833 ± 51 . Whereas, the survival percentage in the control ponds were 84.55 ± 0.2 % with 253672 ± 45 animals survived out of 300000 seeds stocked and significant difference ($P < 0.001$) was observed statistically. Feed consumption in test ponds was comparatively higher than the control ponds and feed conversion ratio (FCR) was 1.26 ± 0.1 in test ponds and 1.58 ± 0.4 in control ponds and significant difference ($P < 0.001$) was observed (Figure 8). Paul Raj, [15], reported that the average Indian cultured food conversion ratios were varied from 1.5 to 1.75.

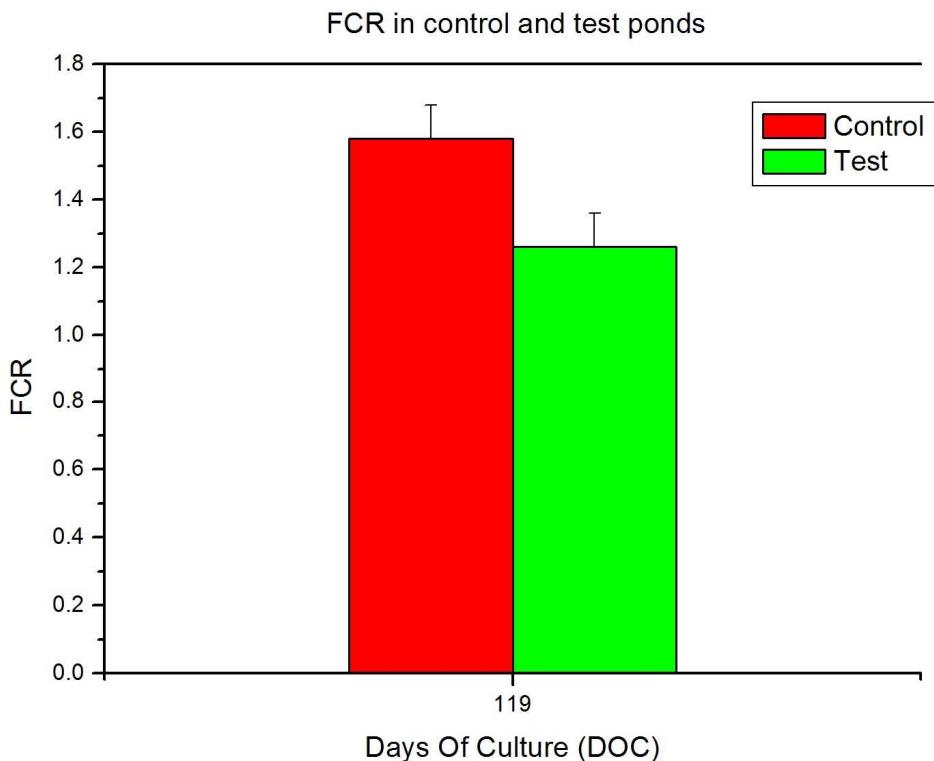


Figure 8: Values of FCR calculated on control and test ponds post harvesting after a period of 119 days.

*Results are presented as means with standard errors of three replicates for each experimental group (mean \pm SE; $n = 3$)

Effective feed management strategy plays a key role in generating profit for the farmers and plays a significant role in the pond and animal maintenance as well. Feed management could be highly maintained with thorough monitoring of check trays and effectiveness in applying the logic by correlating the feed consumption with other parameters, past sampling data, physicochemical parameters, topography of the pond, history of production in the pond, application of chemicals, minerals and probiotics. It has to be kept in mind that, the feed has to be managed properly in days of special importance like new moon day and eighth and ninth day following the new moon day and half-moon day where maximum shrimps undergo moulting. Periodic sampling also plays a vital role in shrimp culture. By conducting weekly samplings, growth and health status of the shrimps could be effectively monitored, which eventually helps in efficient feed management. Upon every weekly sampling, the growth and average body weight of the animals in the probiotics, Ecotrax® treated test ponds were found to be higher than the control ponds. This growth and average body weight increase in shrimps treated with probiotics is in line with a study conducted by Soundarapandian *et al.*, [11].

In the present study, control ponds were found to be infested with bacterial infections and an incidence of luminous bacteria was visually observed during night time. This eventually led to unhealthy animals and increased mortality. Frequent black gill problem was also observed in the control ponds. In addition, the incidence of the dissolved oxygen problem was also encountered in the control ponds during nights and was controlled applying oxygen releasing chemicals. However, mortality in the control ponds remained inevitable. Whereas in the test ponds treated with the probiotic consortium, Ecotrax®, the animals and water quality were in good condition and no incidence of bacterial infection, gill problem and dissolved oxygen deficiency was observed. Furthermore, the production and survival were remarkable in the test ponds with greater profit to the farmer. Priyadarshini *et al.*, [16], reported that,

the benefits of probiotics include improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, increased immune response, better water quality management and improved degradation of metabolic organic and inorganic waste materials. The commercial probiotic consortium, Ecotrax® has conferred all the aforementioned properties to the shrimps in the test ponds and thus paved the way for greater production, superior water quality and pond management with effective and attractive Feed conversion ratio.

CONCLUSION

To conclude, the commercial probiotic consortium, Ecotrax® has been observed to be highly effective in water quality management by developing healthier algal bloom by nourishing the water with the enzymes produced by the component bacteria, reducing the incidence and development of filamentous algae, enabling proliferation of beneficial phyto and zoo planktons, degradation of metabolic organic and inorganic waste in the pond bottom, elimination of pathogenic bacteria, improved feeding and overall health and production of the cultured shrimps. This could be a potential bio-fertilizer and bio-control additive of choice to aquaculture farmers for better yield and lesser FCR.

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REFERENCES

- [1] R. Karthik, A. J. Hussain, and R. Muthezhilan, *Biosci. Biotechnol. Res. Asia.*, **2014**, 11, 297-305.
- [2] D. Yuvaraj, and R. Karthik, *J. Fish. Aquat. Sci.*, **2015**, 10(6), 445-463.
- [3] D. Moriarty. *Aquaculture*, **1997**, 151, 333–349.
- [4] D. Iribarren, P. Daga, M. T. Moreira, and Feijoog, *Aquacult. Int.*, **2012**, 20(4), 779-789.
- [5] L. Verschueren, G. Rombaut, P. Sorgeloos, and W. Verstraete, *Microbiol. Mol. Biol. Rev.*, **2000**, 64, 655– 671.
- [6] A. Irianto, and B. Austin, *J. Fish. Dis.*, **2002**, 25, 633-642.
- [7] J. D. H. Strickland, T. R. Parsons; *A Practical Handbook of Seawater Analysis*, 2nd Edn., Fisheries Research Board of Canada, **1972**, 167 – 310.
- [8] A. I. Vogel. *A text book of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis*. 4th Edn., The English Language Book Society and Langman.Co; **1978**.
- [9] B. Gomez-Gil, A. Roque, and J. F. Turnbull. *Aquaculture*, **2000**, 191, 259–270.
- [10] Ali Farzanfar, *FEMS Immunol. Med. Microbiol.*, **2006**, 48, 149-158.
- [11] P. Soundarapandian, and S. Sankar. *Int. J. Zoo. Res.*, **2008**, 4(1), 35-41.
- [12] S. Rengipat, S. Rukpratanporn, S. Piyatiratitivorakul, and P. Menasaveta, *Aquaculture*, **2000**, 191, 271-288.
- [13] N. Ramanathan, P. Padmavathy, T. Francis, S .Athithian, and Selvaranjitham. *Manual on polyculture of tiger shrimp and carps in freshwater*. Tamil Nadu Veterinary and Animal Sciences University, Fisheries College and Research Institute, Thoothukudi; **2005**, p. 161.
- [14] G. L. Molluae, *The Advocate*, **2001**, 18.
- [15] P. B. B. Raj, *Fish World*, **1999**, 13-17.
- [16] P. Priyadarshini, B. Deivasigamani, T. Rajasekar, G. Edward Gnana Jothi, S. Kumaran, M. Sakthivel, and S. Balamurugan, *Drug Invention Today*, **2013**, 5, 55-59.