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Effect of soil probiotics in the shrimp culture

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Abstract. The role of probiotic bacteria in small culture is studied but commercial level is not that much reported especially in giant tiger shrimp, *P. monodon*. Hence the beneficial effect of probiotics on the commercial culture of Indian major candidate shrimp, *P. monodon* is studied in a grow out pond. The farm is located on the Northern bank of Uppanar estuary in Maravakadu. An effort was made to evaluate the differences between regular and irregular application of soil probiotics in the *P. monodon* shrimp farming. Subsequently the performance of using probiotics frequently and the total average yield attained was 1900 Kg /0.5ha. With maximum growth increment of 34.70g, survival of 90.31%, achieved at the DOC 130. Level of ammonia found nil for the entire culture period in the regularly probiotics used ponds. Contrarily, in irregular treated ponds the total yield achieved only 1150 Kg /0.5 ha. With the maximum growth of 27.29 g, survival of 70.68% at the DOC 130 respectively. The level of ammonia found 0.3 mg /L.
Keywords: pobiotic, bcteria, *P. monodon,* growth, culture and shrimp farming.

Introduction. Global production doubled during the last decade, making in the fastest growing animal food production sector in the world (FAO, 2002). Ponds are the most commonly used aquaculture production system accounting for about 40% of the world production which offers numbers of opportunities to contribute to poverty alleviation, employment and community development, and reduction of over exploitation of natural resources and food security in tropical and sub-tropical regions (Ramanathan et al 2005). Aquaculture has become one of the major industries earning more foreign exchange because of its excellent taste, universal appeal and high market and export value. India is rich in natural shrimp resources and nearly 52 species of shrimp are contributed in fishing (Swaminathan 1980). Of these, 8 shrimp species are economically important and successful culture is practiced for two species viz., *Penaeus monodon* and *P. indicus.* Shrimp farming is one of the fastest growing sectors in many parts of the world and also one of the most controversial.

The potential brackish water area available in the coastal regions of the country for shrimp culture is estimated between 1.2 to 1.4 million ha. Presently an area of about 1, 50,000 ha are under culture with an average production of about 1, 20,000 tones per year (Nazeer 2005). The use of probiotic in the culture of aquatic organisms is increasing with the demand for more environment-friendly aquaculture practices (Balca'zar 2003). It seems likely that the use of probiotics will gradually increase and that success of aquaculture in future may be synonymous with the success of probiotics, which, if validated through rigorous scientific investigation and used wisely, may prove to be a boon for the aquaculture industry.

Material and Method. The role of probiotics bacteria in small culture is studied but commercial level is not that much reported especially in giant tiger shrimp, *P. monodon*. Hence the beneficial effect of probiotics on the commercial culture of Indian major candidate shrimp, *P. monodon* is very much need of the hour. Therefore, the present study was aimed to examine the effect of probiotic called Thionil a soil probiotic from Poseidon Biotech-Chennai on the shrimp *P. monodon* culture was studied. The following parameters were completed both experimental (A section) (proper application of soil

probiotics) and control (B section) (improper application of soil probiotics) ponds and to evaluate water quality parameters, growth rate, microbial dynamics and survival rate.

Study area: the farm is located on the Northern bank of Uppanar estuary in Maravakadu and is situated about 28 km away from Pattukottai. The southern side of the farm is elevated to a height of 3.5 mts from Uppanar estuary. The total area covered is 3.2 ha of which casing six ponds of each about 0.5 ha with the water spread is 3.0 ha that depicted in. The *P. monodon* (PL16 pass the PCR test and stress test) seeds were purchased from Oceanic shrimp hatchery, Marakkanam and were transported in oxygenated double-layered polythene bags with crushed ice packs between inner and outer covers of the bag and packed in a carton. The stocking density per pond was 12/m2 (60,000 PL's / pond).

Application of probiotics: in section-A ponds viz., A1, A2, A3 were treated with soil probiotics namely Thionil, Manufactured by Poseidon Biotech, Chennai and in the ponds of Section-B i.e., B1, B2, B3 there was irregular application of soil probiotics were applied to study the variation between the experimental and control ponds, the application procedure is clearly presented table 4. While administering the probiotics into the ponds a general procedure is followed as, Bottom soil quality management 1.5 kg of Thionil soil probiotic was mixed in 50 kg of dry sand and broadcasted through out the pond during 08:00 hrs.

Table 1

Application of probiotics during culture

*DOC**	*Ponds (A1, A2, A3) (Soil probiotic) (Kg)*	*Ponds (B1, B2, B3) (Soil probiotic) (Kg)*
Before stocking	1.5	-
1	1.5	1.0
15	1.5	-
30	1.5	-
45	1.5	2
55	2	-
65	2	1
75	2	-
85	2	2
95	2	1
105	2	
115	2	2
125	2	-

Water quality management: the water quality parameters were recorded in both regularly and irregularly treated probiotic ponds respectively. The water level was measured by using a standard scale with cm marking. The water salinity was measured by using a hand refractometer (Erma-Japan).The pH of the pond water was measured by using electronic pH pen manufactured by Hanna Instrumental Company, Japan. Water temperature was measured in the experimental ponds itself using a standard centigrade thermometer. The dissolved oxygen was estimated by using digital dissolved oxygen meter. Transparency was measured in terms of light penetration using a secchi disc. In the first 3-4 weeks of culture, water exchange was not performed. Water was exchanged five days once or depends upon the water and shrimp quality. The purpose of water exchange is to maintain water quality and also to stimulate moulting in shrimps, resulting in acceleration of growth and production. To analyze the microbial level in the on going ponds the water and sediment samples were collected separately from different parts of the ponds in sterile conical flask and were mixed to make a single sample. This procedure was repeated for every pond and the final samples were brought to the laboratory immediately and were analyzed for microbial counts. It was then transferred to a sterile conical flask (150 ml) containing 99ml of sterile diluents and serial dilution was performed to get $10^{-}1$, $10^{-}2$, $10^{-}3$, $10^{-}4$, and $10^{-}5$ suspension samples. To enumerate Total Heterotrophic Bacteria (THB), Zobell marine agar medium to enumerate pathogenic bacteria in the culture ponds viz., *Vibrio sp.* Feed management plays a vital role in the

shrimp culture. In the first month after stocking, feeding rates were based on estimated survival and feeding tables, and distributed four times per day. After 30th DOC, daily rations were adjusted using feed trays and increased to five times per day there after.

Sampling: the first sampling was taken after 40th DOC and number of individuals and the average body weights (ABW) were recorded in each sampling. Five hauls were made in each pond that are healthiness, survival rate, ABW, ADG of the shrimps estimated. Sampling was regularly performed in every ten days until harvest.

Results and Discussion. Water quality parameters in the experimental ponds are presented in table. 6. The highest salinity was recorded at the maximum of 25 ppt in the month of July 2007 and minimum of 15 ppt in Nov 2007 respectively. The pH was recorded in the experimental ponds during the period from July to Nov 2007. The water pH varied from 7.6 to 8.4 that are depicted. The dissolved oxygen was recorded at the maximum of 4.5 ppm in July and minimum of 3.5 ppm in Nov 2007 respectively. The level of ammonia was recorded at the maximum of 0.30 mg/L in the month of Nov 2007 and minimum of 0.05 mg/L in Aug 2007.

Table 2

Water quality parameters in the culture ponds

Parameters	*Section-A*	*Section-B*
Salinity (ppt)	15-25	15- 25
pH	7.6 - 8.3	8.0 - 8.4
Dissolved oxygen (ppm)	4.0 - 4.5	3.5 - 4.5
Temperature (°C)	25 - 30	25 - 30
Transparency (cm)	30 - 45	25 - 42
Ammonia (mg/L)	Nil	0.05 - 0.30

Rate of survival was recorded in the regularly used soil probiotics was 90.31%, contrarily in the ponds of irregularly used soil probiotics was recorded as 70.68%. Maximum growth was observed in the ponds treated with regular application of soil probiotics that was monitored during each sampling moreover, comparatively 20% greater average weight gain was noticed in the shrimps that were treated regularly which are showed Table 3.

Table 3

ABW of *P.monodon* reared in ponds with regular and irregular application of soil probiotics

PONDS	DOC									
	40	50	60	70	80	90	100	110	120	130
	ABW(g)									
Section – A	6.5	8.5	11.4	14.5	18	21.28	24.59	27.1	30.36	34.7
Section – B	6.1	8.0	10.96	12.27	15	17.46	20.13	22.42	24.3	27.19

The bacterial population changed during every sampling. Moreover, bacterial population found more in the sediment sample of both the regular and irregular applied ponds. Albeit, at the end of culture the bacterial population found declined in irregularly treated ponds. Level of CFU especially *Vibrio sp.* in the regularly treated ponds were found declined significantly. However the level of CFU in all the irregularly treated probiotic ponds there were no declinement was noticed which are depicted in Tables 4-5.

Table 4

Average yellow colony population in ponds with regular and irregular application of soil probiotics

PONDS	DOC											
	10	20	30	40	50	60	70	80	90	100	110	120
Section – A	90	100	110	130	160	200	220	270	250	220	200	210
Section – B	90	90	100	100	90	110	130	110	100	90	80	60

Table 5

Average green colony population in ponds with regular and irregular application of soil probiotics

PONDS	DOC											
	10	20	30	40	50	60	70	80	90	100	110	120
Section – A	40	50	40	40	60	50	60	40	50	40	50	40
Section – B	50	60	90	90	130	160	200	180	200	200	180	150

The present investigation carried to evaluate the performance of regular and irregular application of soil probiotics Thionil in the culture ponds and the results revealed that the growth and survival were found highly appreciable only in regularly applied ponds than in the irregular use. Results of the present study closely resemble and comparable with the study of Dalmin et al (2001). Colonization of the gastrointestinal tract of animals by probiotics is only after birth, and before the definitive installation of a very competitive indigenous micribita. Addition of some commercial preparations as probiotics are reported to maintaining water quality parameters thereby improving growth rate, weight gain and survival rate with an attractive FCR in farmed organisms as the probiotic microbes like lactobacilli control enteric pathogenic microorganism through "competitive exclusion" process (Fuller & Turvey 1971).

Concentration of ammonia was nil for the probiotic treated ponds than the irregularly applied ponds. Similarly the level of chlorophyll was observed maximum in regularly treated probiotic ponds than in contrary culture ponds. So it can be recommended that the soil probiotic can be very well utilized for the shrimp ponds to get reasonable growth and survival.

Application of microbial supplement in the probiotic ponds hindered the growth of *Vibrio sp.*, like *V. alginolyticus* and *V. harveyi* because of the colonization of the beneficial microbes like *Bacillus sp., Pseudomonas sp., Lactobacillus sp*., and *Saccharomuces sp.,* in the shrimp gut. Since the shrimp in the infrequently treated ponds were dominated with

green colony, which caused Vibriosis can be attributed as the reason for low survival in the infrequently applied ponds when compared with the frequently indulgence ponds. This was evident from the presence of higher load of green colony, in the water and sediment of improper probiotic ponds than in the probiotic used ponds.

Even though the probiotics are helping in maintaining the microbial load in the soil ambiance the shrimps are bottom dwelling organisms, the depth and volume of water in a pond has certain physical and biological consequences. The role of the microorganism in aquaculture ponds was reviewed, focusing on the decomposition of organic matter and its influence on pond dynamics. Decomposition of organic matter was studied in lab-scale 2-l microcosms with daily feed additive simulating feeding levels applied in intensively managed ponds. Towards the end of a culture cycle in intensively fed ponds, the shrimp feed input rapidly becomes the main factor affecting water quality. At very importantly the efficacy of soil probiotics on shrimp farming is closely associated with the methane measurement is one among the duplicate anaerobic treatments and in very small amounts (De la Pena et al 1995).

Important water quality parameters monitored during the study were, temperature, salinity, oxygen, pH, and ammonia levels. The volumes of water behave like a buffer, which prevents weather fluctuations from influencing the environment in which shrimp lives. The ideal water depth is between 0.8 to 1.5 mt depending upon the stage of culture. It is recommended that a minimum depth of 1mt be maintained at operational level. In the present study 100 cm water level was maintained in all ponds throughout the culture period. The stocking density between 10-20PLs /m2 is ideal for successful shrimp farms (Ramanathan 2005). The shrimp seeds were stocked at the stocking density of 12/ m2 in all ponds.

Salinity is the prime parameters to control growth and survival of shrimps. Evethough *P. monodon* is euryhaline animals it is comfortable when exposed to optimum salinity. At high salinity the shrimps will grow slowly but they are healthy and resistance to diseases. If the salinity is low the shell will be weak and prone to diseases. The salinity of the present study was maintained 15-25 ppt in all ponds. Soundarapandian and Gunalan (2008) recommended a salinity range of 10-35 ppt was ideal for *P. monodon* culture. Chen (1980) opined that salinity ranges of 15-20 ppt are optimal for culture of *P. monodon*. Soundarapandian and Gunalan (2008) stated that *P. monodon* adapted quite well in freshwater conditions also because of its wide range of salinity tolerance.

pH is one of the vital environmental characteristics, which decides the survival, and growth of shrimp under culture; it also affects the metabolism and other physiological process of shrimps. The optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production (Ramanathan et al 2005). The role of pH which was ranged from 7.6 to 8.4 these were closely resembles the finding in the present investigation of both the experimental treatments (8.11 to 8.67 in low saline ponds).

Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. Oxygen affects the solubility and availability of many nutrients. Low levels of dissolved oxygen can cause damages in oxidation state of substances from the oxidized to the reduced form. Lack of dissolved oxygen can be directly harmful to shrimps and cause a substantial increase in the level of toxic metabolites. Low-level of oxygen tension hampers metabolic performances in shrimp and can reduce growth and moulting and cause mortality (Gilles Le Molluae 2001). The dissolved oxygen in all the culture ponds in the present study was ranging between 3.2 to 4.2 ppm.

Water temperature is probably the most important environmental variables in shrimp cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival. In general, a sudden change of temperature affects the shrimp immune system. The optimum range of temperature for the black tiger shrimp is between 28 to 30^{o}C (Ramanathan et al 2005). The temperature in the present study was25 to 30^{o}C and the low temperature 25^{o}C was observed due to cloudy weather. The optimum range of temperature of *P. monodon* was between 26 to 33^{o}C (Soundarapandian & Gunalan 2008) and temperature range of 28 to 33^{o}C supports normal growth (MPEDA) as observed in the present study.

Ravi et al (1998) described the benefits of probiotics in maintaining water quality and enhancing growth rate in India Whit Prawn, *P. indicus*. Shrimp aquaculture production in much of the world is depressed by disease, particularly caused by luminous *Vibrio* and/or viruses. Abundance of luminous *Vibrio* strains decreased in ponds and tanks where specially selected, probiotic strains of *Bacillus* species were added. A farm on Negros, in the Philippines, which had been devastated by luminous *Vibrio* disease while using heavy doses of antibiotic in feed, But they achieved survival of 80-100% of shrimp in all ponds treated with oral probiotics (Moriarty 1998).

Ruangpan & Kitao (1991) reported in their study that the high abundance of luminescent *Vibrio* is consistent with occurrence of disease and poor or zero harvest results. *V. harveyi*, a pathogen of *P. monodon* that causes severe losses. The farm, which used the *Bacillus* bacteria, had either a very low abundance or a complete absence of luminous *Vibrio* in pond water and very good harvest result. This consistent and high productivity occurred, even though the proportion of luminescent *Vibrio* in the pond water was high in the sea water source, and the abundance of total green colony in the pond water was higher than in the water source. Furthermore, luminescent *Vibrio* were completely absent at all stages of grow out from the pond sediment in the presence of *Bacillus* species.

Conclusions. An effort was made to evaluate the differences between regular and irregular application of soil probiotics in the *P. monodon* shrimp farming. Subsequently the performance of using probiotics frequently and the total average yield attained was 1900 Kg /0.5ha. With maximum growth increment of 34.70g, survival of 90.31%, achieved at the DOC 130. Level of ammonia found nil for the entire culture period in the regularly probiotics used ponds. Contrarily, in irregular treated ponds the total yield achieved only 1150 Kg/0.5 ha. With the maximum growth of 27.29 g, survival of 70.68% at the DOC 130 respectively. The level of ammonia found 0.3 mg/L.

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