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Bacillus Probiotic Strains of Ecotoxnil® as Eco-friendly and Efficient Bio-decomposing Agent in Curbing Sludge and Toxic Gases from *Litopenaeus vannamei* (Boone, 1931) Shrimp Culture Ponds

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Abstract
The study was conducted to check the efficacy of probiotic, Ecotoxnil in maintaining the water quality and as a bio-decomposer reducing the sludge and toxic gases like ammonia and nitrite in the *Litopenaeus vannamei* shrimp aquaculture pond. Shrimp pond affected with sludge formation and toxic gases like ammonia and nitrite which reported mortality was selected. Agricultural lime was applied at the rate of 50kg/h for 3 days for buffering the water and increasing the pH followed by feed holiday for one day prior to the application of the probiotics. Water quality parameters including pH, temperature, salinity, total alkalinity, and concentration of toxic gases like ammonia, nitrite and relationship between sludge depth with ammonia and nitrite were recorded. Values of pH, temperature, salinity, and total alkalinity ranged from 7.713±0.3 to 8.224±0.3, 28.467±0.7˚C to 29.902±0.6˚C, 14.012±2.1 psu to 15.413±2.1 psu, and 146.367±1.6 to 219.008±1.6 ppm respectively. Values of toxic gases like ammonia and nitrite ranged from 0.642±0.01ppm, which upon application of Ecotoxnil was observed to come down to 0.002±0.0 ppm and 0.349±0.01 ppm which upon application of Ecotoxnil has reached the lowest concentration of 0.001±0.0 ppm respectively. The depth of sludge in the pond was decreased to 3.212±1.1cm from 10.821±1.1cm post application of Ecotoxnil. Simple linear regression pattern constructed between sludge depth as independent variable and concentration of ammonia as dependent variable expressed an R^2 value of 0.9011 with $p<0.05$ and simple linear regression pattern constructed between sludge depth as independent variable and concentration of nitrite as dependent variable expressed an R^2 value of 0.9108 with $p<0.05$. From the data obtained, it was evident that the application of Ecotoxnil has significant impact on the reduction of toxic gases like ammonia, nitrite and minimising bottom sludge in the pond.

Keywords: Probiotic, Ecotoxnil, Sludge, Toxic gases, bio-decomposing agent

1. Introduction

The UN FAO estimates that half of the world's seafood demand will be met by aquaculture in 2020, as wild capture fisheries are overexploited and are in decline. Shrimp culture is widespread throughout the tropical world [1]. Albeit the global shrimp industry is experiencing a dramatic growth, shrimp aquaculture pond bottom management practices are still ambiguous and are considered to be trivial. Shrimp aquaculture farmers and technicians attempt to measure water quality variables frequently and maintain them in optimal ranges. Astonishingly, little efforts were emphasized in maintaining pond soil condition. Interaction between soil and water plays a noteworthy role on water quality and hence emphasis should be laid on pond soil condition. Augmented feeding in *Litopenaeus vannamei* shrimp farming has become a practice in farming recently. This may consequently lead to higher organic load deposition in the pond bottom, polluting the pond soil and depreciating the dissolved oxygen level in pond soil surface layer. Anaerobic conditions thus build in the pond bottom favours the amplification of anaerobic bacteria capable of producing toxic metabolites which may affect the shrimp survival and health status at one hand and making the pond prone to infections by other pathogens contrariwise thus weakening the entire culture system [2].

However, in many countries farmers now realize that the prophylactic application of antibiotics is deleterious and so are using probiotics to maintain health of the animals and indeed make their industry more sustainable and profitable [3, 4].

Profitability and the sustainability of shrimp aquaculture are improved by microbial biotechnology [5]. Therefore, farmers realize that usage of probiotics will help improve the health of the animal and indeed make their industry sustainable and profitable [4]. Decreasing organic waste in the pond can be achieved by modifying the microbial communities in shrimp ponds and in the intestinal tracts of the shrimp. This biological technology comprises of manipulating physico-chemical factors to alter microbial species composition, rates of metabolic activity and adding appropriate numbers of selected *Bacillus* species to carry out particular functions at faster rates than those occurring under existing conditions [6].

The major role of microbes in pollution abatement is the decomposition of waste organic matter to carbon dioxide and nitrogen gas. The rate determining step in the degradation of organic matter is the hydrolysis of macromolecules by extracellular enzymes [7]. Cleavage of aromatic ring structures and the double bonds in polyunsaturated fatty acids require molecular oxygen, which is added to these compounds by oxygenase enzymes, which occur in many different species of bacteria [8, 9]. Several different *Bacillus* species, including *B. subtilis* produce oxygenases, and thus are potentially important as candidates for large scale production for bioremediation [10]. The exo-enzymes of *Bacillus* species assist in speeding up the degradation rates and the degree of degradation and thus lower sludge volumes [11, 12].

With greater emphasis on rising demands and ambiguous culture practices, shrimp aquaculture pond management plays a pivotal role in developing sustainable and successful shrimp production. In order to develop a wealthy biological ecosystem, probiotic bacteria are of great deal. In this study, selected *Bacillus* probiotic strains of Ecotoxnil from TIL Biosciences (The animal health division of Tablets (India) Limited), Chennai was tested for its efficacy as bio-decomposing agent in curbing sludge and toxic gases in the *Litopenaeus vannamei* shrimp aquaculture ponds.

2. Materials and methods

2.1 Study site

Four shrimp ponds of equal size (1 acre each) located in Muarmalla (16.674˚ N, 82.167˚ E), East Godavari district, Andhra Pradesh, (Fig. 1) were selected for the study during the period of March to June 2017.

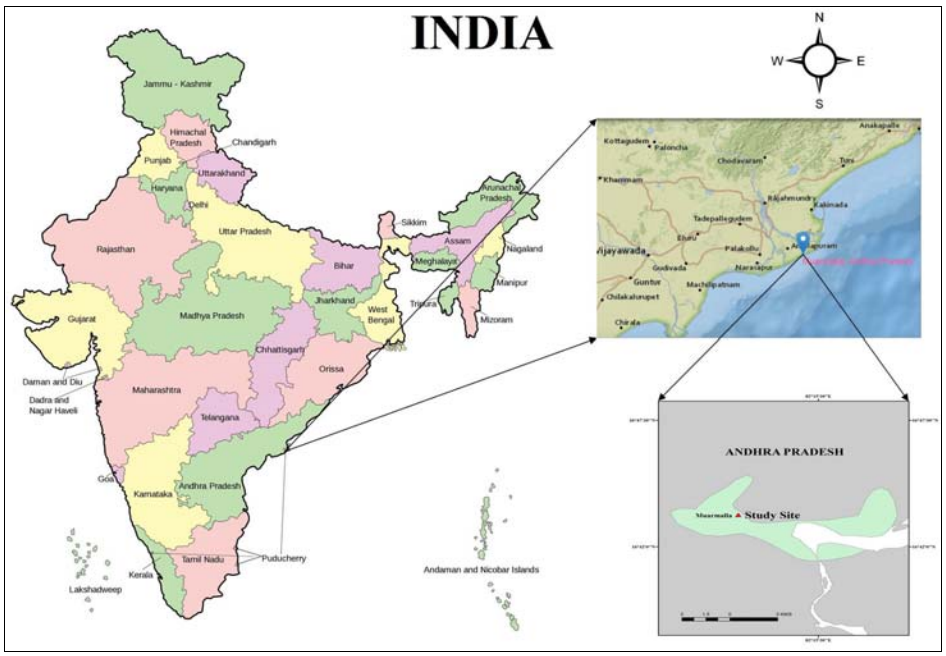

Fig 1: Map showing the study area Muarmalla, East Godavari district, Andhra Pradesh

2.2 Product composition

Ecotoxnil is a commercial soil probiotic product from the house of Tablets (India) Limited, Chennai, certified by Coastal Aquaculture Authority (CAA) of India (CAA Reg. No. CAA/F16/PRO/00007), Government of India. The composition of Ecotoxnil per 1000gms is presented in table 1.

Table 1: Composition of Ecotoxnil

Composition of Ecotoxnil per 1000 gms		
Bacillus sp. ABPL 142	5×10^9	gms
Bacillus sp. ABPL 144	5×10^9	gms
Bacillus thermodenitrificans ABPL 140	5×10^9	gms

2.3 Experimental design

Shrimp pond was properly prepared prior to stocking. Bore water with salinity ranging from 14 – 17 psu was pumped onto the shrimp ponds. Post larvae (PL) at the stage of 11 were subjected to laboratory analysis for monitoring the health status of the PLs including White Spot Syndrome Virus (WSSV), *Enterocytozoon hepatopenaei* (EHP), and *Vibrio* infection. With results being negative for all the aforementioned tests, PLs were stocked at a density of 40/m^2 with the water depth of 1.5m and pond area of 1 hectare each. Water exchange was not entertained in the ponds. Physico-chemical parameters of the water were carried out once in week and was analysed. Ecotoxnil probiotic was not applied onto the ponds until the pond developed sludge and toxic gases which were in turn confirmed by visual observation of turbidity and in the elevation of toxic gases concentration in the pond. The pond has also exhibited drop in feeding. Ecotoxnil probiotic was then applied on to the pond and was examined for the level of sludge in the pond bottom, concentration of ammonia and nitrite. Examination of physico-chemical parameters of the water were carried out for ten days continuously since Ecotoxnil was applied and the shift in the parameters were noted.

2.4 Sampling analysis
2.4.1 Physico-chemical analysis
The physicochemical parameters of water were analysed in the ponds pre and post usage of Ecotoxnil at four different points/spots. Water samples were collected between 07.00 and 08.00 hrs for *in situ* examination and laboratory analysis. Collected samples were examined for temperature, pH, and salinity on the spot. The salinity in the ponds was recorded *in situ* by means of a portable hand-held optical refractometer (Atago, Japan) and cross-checked in laboratory using Mohr-Knudsen method. pH was measured using electronic pH pen (Erma, Japan), temperature was measured using standard Celsius thermometer. Bottom water was collected for estimating the concentration of ammonia and nitrite. Samples collected were shuttled in 250 ml polyethylene bottles to laboratories in ice-container to the laboratory and alkalinity, ammonia and nitrite was measured as per APHA[13]. Transparency was measured based on the penetration of light using a Secchi disc. The amount of sludge in the pond bottom was measured using a manually designed sludge depth meter. A filter paper was fixed over a steel measuring scale using a double sided tape at one side and on the other side; another steel scale of same measurement was attached to it using a double sided tape. The lower tip of the scale was covered using a cello fine tape to avoid mechanical damage to the filter paper while inserting the scale into the sludge (Fig. 2). This sludge depth meter was manually inserted onto the centre bottom of the pond. The depth of the sludge was calculated by the mark matching the colour of the sludge.

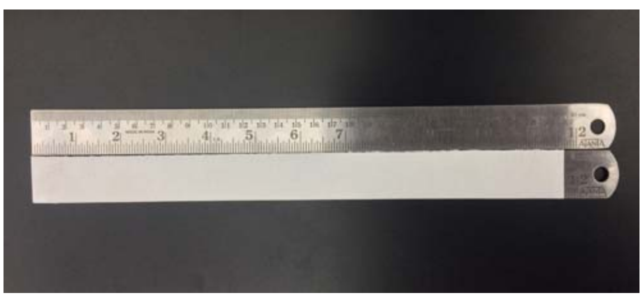

Fig 2: Manually designed sludge depth meter

2.5. Product response parameters
Condition of the pond was monitored including turbidity, water quality parameters, intensity of the bloom and concentration of toxic gases in the pond. Prescribed dosage of Ecotoxnil, 500gm/acre was brewed with 3kg of country jaggery for 3 to 4 hours in the morning and the mixture was mixed with wet sand to facilitate the mixture to reach the bottom of the pond in less time and applied over the surface of the pond. Condition of the pond was then monitored post application for the changes.

2.6. Statistical analysis
The data were presented as mean ± SE. All statistical calculations were performed using SPSS for Windows version 11.5 (SPSS Inc, Chicago, IL, USA). Linear regression was calculated between sludge depth and ammonia and sludge depth and nitrite with $p<0.05$. All column charts were plotted using Origin 6.1(OriginLab Corporation, Massachussets, USA).

3. Results
The ponds were monitored for the changes in physicochemical parameters, turbidity and concentration of toxic gases. Figure 3 shows the larger view of the ponds pre (Fig. 3a) and post (Fig. 3b) usage of Ecotoxnil probiotic, in which ponds with turbidity and clear waters were captured respectively. Figure 4 shows the closer view of the ponds in which turbidity was observed before the application of Ecotoxnil (Fig. 4a) and turbidity controlled pond with clear water (Fig.4b).

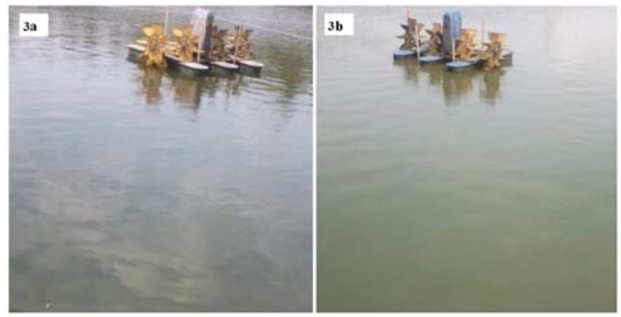

Fig 3: Enlarged view of the pond with sludge (3a) and view of the pond post application of Ecotoxnil with better water quality (3b)

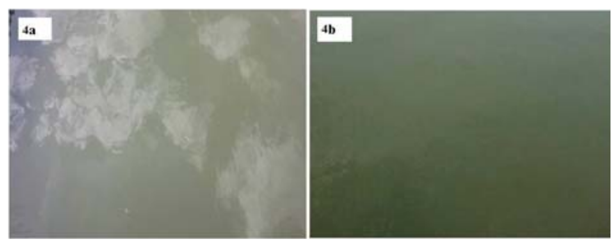

Fig 4: Closer view of the pond water exhibiting traces of sludge in the surface water post aeration (4a) and clearer view of the pond water post application of Ecotoxnil with improved water quality

pH being a significant parameter in shrimp aquaculture pond was properly monitored during the 10 days of experimental period and it varied from 7.713±0.3 to 8.224±0.3 (Fig. 5). Temperature in the ponds ranged from 28.467±0.7˚C to 29.902±0.6˚C (table 2) (Fig. 6). Salinity in the pond extended from 14.012±2.1 psu to 15.413±2.1 psu (Fig. 7). Concentration of carbonate ranged from 6.613±0.6 ppm to 10.354±0.6 ppm. Concentration of bicarbonate in the pond ranged from 135.754±2.7 ppm to 208.654±2.7 ppm (Fig. 8). Total alkalinity in the pond was observed to be diversified from 146.367±1.6 to 219.008±1.6 ppm (table 3).
Concentration of toxic gases plays a terrific role in impacting the growth of the shrimp, microbial dynamics of the pond, bottom and water quality negatively. Concentration of ammonia in the pond was observed to reach an all-time high value of 0.642±0.01ppm, which upon application of Ecotoxnil was observed to come down to 0.002±0.0 ppm (Fig. 9). The highest concentration of nitrite was marked as 0.349±0.01 ppm which upon application of Ecotoxnil has reached the lowest concentration of 0.001±0.0 ppm in 5 days post application (Fig. 10). The depth of sludge in the pond was decreased to 3.212±1.1cm from 10.821±1.1cm (Fig. 11) post application of Ecotoxnil (table 4).

Table 2: Effect of Ecotoxnil on pH, temperature, and salinity

DOC	pH	Temperature (˚C)	Salinity (psu)	Remarks
65	7.727 ± 0.3	28.793 ± 0.4	14.863 ± 1.7	pH was checked
66	7.713 ± 0.3	28.467 ± 0.7	14.012 ± 2.1	50kg/h agricultural lime was applied
67	7.914 ± 0.3	29.778 ± 1.2	14.117 ± 2.3	50kg/h agricultural lime was applied
68	8.112 ± 0.3	29.846 ± 0.9	14.567 ± 2.3	No feed was given for one full day and 50kg/h agricultural lime was applied
69	8.224 ± 0.3	29.517 ± 1.3	14.657 ± 2.1	500g/acre of Ecotoxnil was applied with wet sand
70	8.117 ± 0.3	29.723 ± 1.1	14.898 ± 2.2	70% of feed was given
71	8.183 ± 0.3	29.717 ± 1.2	15.413 ± 2.1	70% of feed was given
72	8. 164 ± 0.3	29.902 ± 0.6	15.023 ± 1.6	70% of feed was given
73	8. 104 ± 0.3	29.846 ± 0.7	15.322 ± 1.9	100% of feed was given
74	8. 097 ± 0.3	29.736 ± 0.5	15.189 ± 3.1	100% of feed was given

*Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

Table 3: Effect of Ecotoxnil on total alkalinity

DOC	Carbonate (ppm)	Bicarbonate (ppm)	Total alkalinity (ppm)	Remarks
65	6.613 ± 0.6	135.754 ± 2.7	142.367 ± 1.6	pH was checked
66	6.981 ± 0.6	139.629 ± 2.7	146.61 ± 1.6	50kg/h agricultural lime was applied
67	7.823 ± 0.6	157.821 ± 2.7	165.644 ± 1.6	50kg/h agricultural lime was applied
68	8.627 ± 0.6	173.278 ± 2.7	181.905 ± 1.6	No feed was given for one full day and 50kg/h agricultural lime was applied
69	10.354 ± 0.6	208.654 ± 2.7	219.008 ± 1.6	500g/acre of Ecotoxnil was applied with wet sand
70	9.934 ± 0.6	199.231 ± 2.7	209.165 ± 1.6	70% of feed was given
71	9.867 ± 0.6	197.896 ± 2.7	207.763 ± 1.6	70% of feed was given
72	9.659 ± 0.6	194.567 ± 2.7	204.226 ± 1.6	70% of feed was given
73	9.321 ± 0.6	189.786 ± 2.7	199.107 ± 1.6	100% of feed was given
74	9.089 ± 0.6	181. 298 ± 2.7	190.387 ± 1.6	100% of feed was given

*Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

Table 4: Effect of Ecotoxnil on ammonia, nitrite and sludge decomposition

DOC	Ammonia (ppm)	Nitrite (ppm)	Sludge depth (cms)	Remarks
65	0.613 ± 0.01	0.324 ± 0.01	10.723 ± 1.1	pH was checked
66	0.631 ± 0.01	0.343 ± 0.01	10.819 ± 1.1	50kg/h agricultural lime was applied
67	0.642 ± 0.01	0.349 ± 0.01	10.818 ± 1.1	50kg/h agricultural lime was applied
68	0.639 ± 0.01	0.338 ± 0.01	10.819 ± 1.1	No feed was given for one full day and 50kg/h agricultural lime was applied
69	0.627 ± 0.01	0.329 ± 0.01	10.821 ± 1.1	500g/acre of Ecotoxnil was applied with wet sand
70	0.476 ± 0.01	0.211 ± 0.01	10.176 ± 1.1	70% of feed was given
71	0.107 ± 0.01	0.097 ± 0.01	7.987 ± 1.1	70% of feed was given
72	0.089 ± 0.01	0.052 ± 0.01	5.543 ± 1.1	70% of feed was given
73	0.004 ± 0.00	0.007 ± 0.00	4.876 ± 1.1	100% of feed was given
74	0.002 ± 0.00	0.001 ± 0.00	3.212 ± 1.1	100% of feed was given

*Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

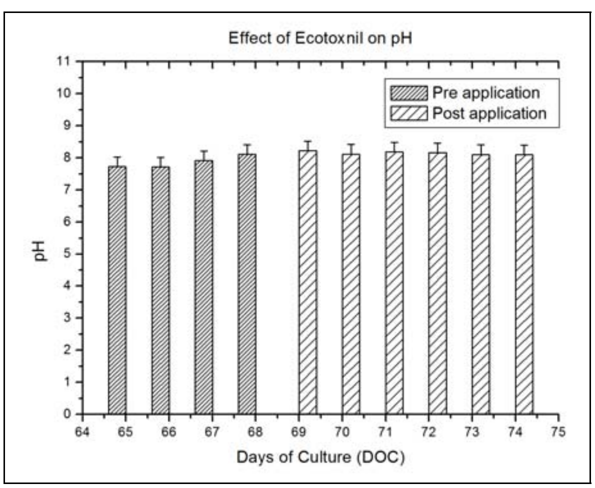

Fig 5: Impact of liming and Ecotoxnil on pH in the sludge affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

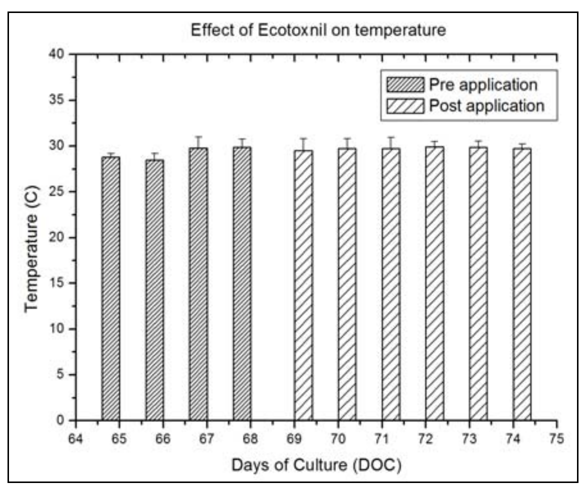

Fig 6: Impact of Ecotoxnil on temperature in the sludge affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

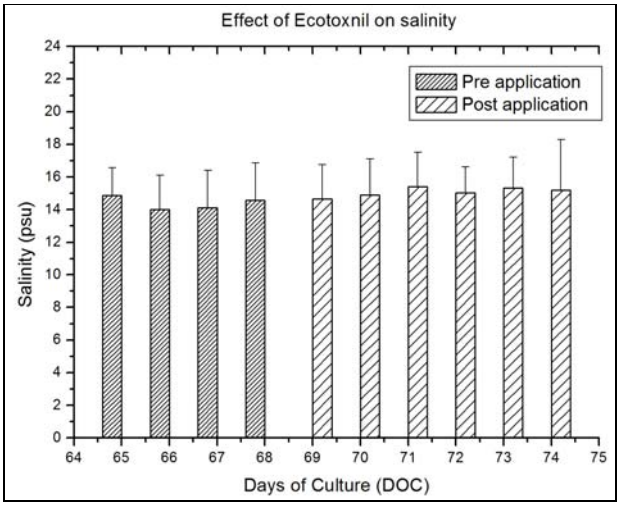

Fig 7: Impact of Ecotoxnil on salinity in the sludge affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

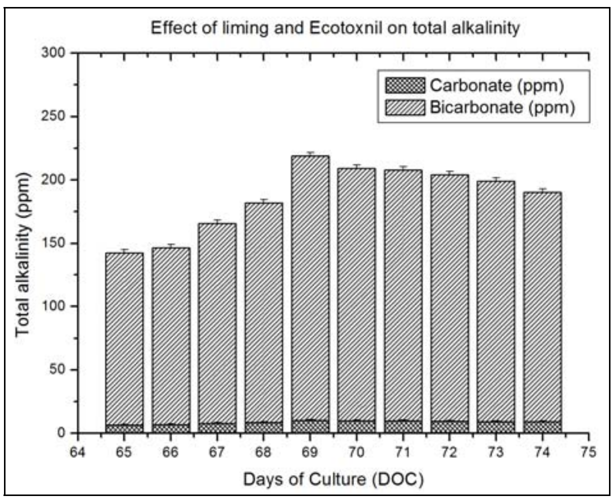

Fig 8: Impact of Ecotoxnil on total alkalinity in the sludge affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

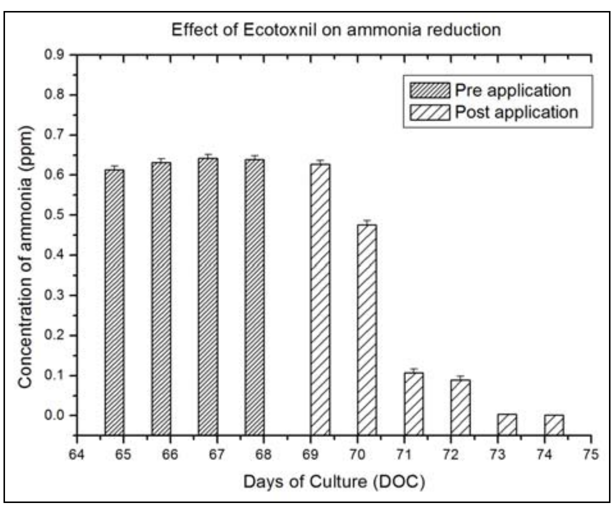

Fig 9: Impact of Ecotoxnil on the control of ammonia in the sludge affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

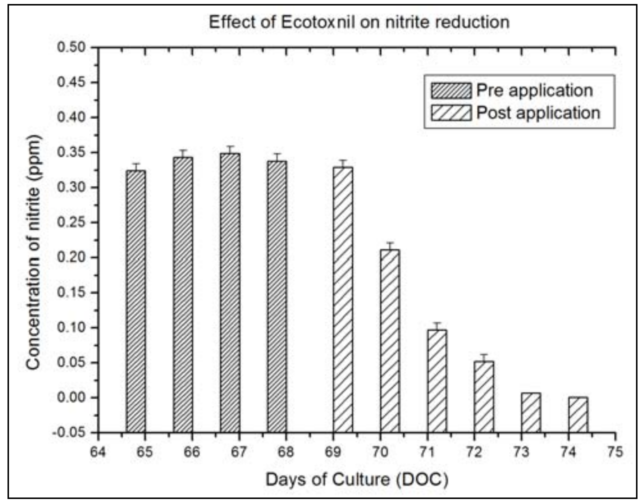

Fig 10: Impact of Ecotoxnil on the control of nitrite in the sludge affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

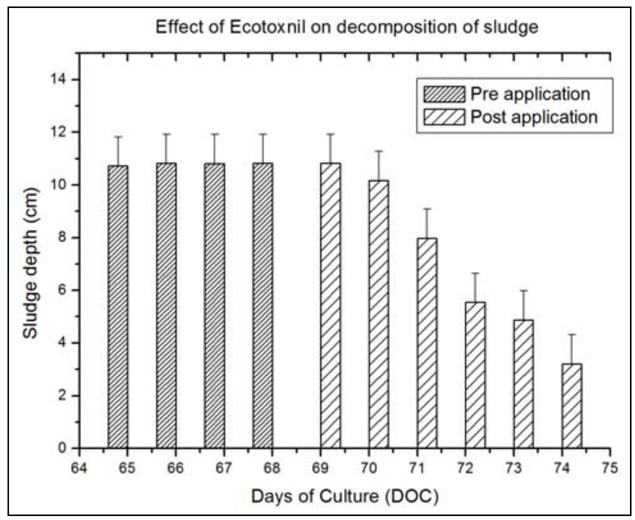

Fig 11: Impact of Ecotoxnil on the effective control of sludge in the affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

Concentration of ammonia and nitrite was observed to be directly related with the depth of the sludge in the shrimp pond (Fig. 12). It was noticed that the concentration of ammonia and nitrite decreased with depth of the sludge post application of Ecotoxnil probiotic in the pond. Simple linear regression pattern constructed between sludge depth as independent variable and concentration of ammonia as dependent variable expressed an R^2 value of 0.9011 with $p<0.05$ (Fig. 13) and simple linear regression pattern constructed between sludge depth as independent variable and concentration of nitrite as dependent variable expressed an R^2 value of 0.9108 with $p<0.05$ (Fig. 14).

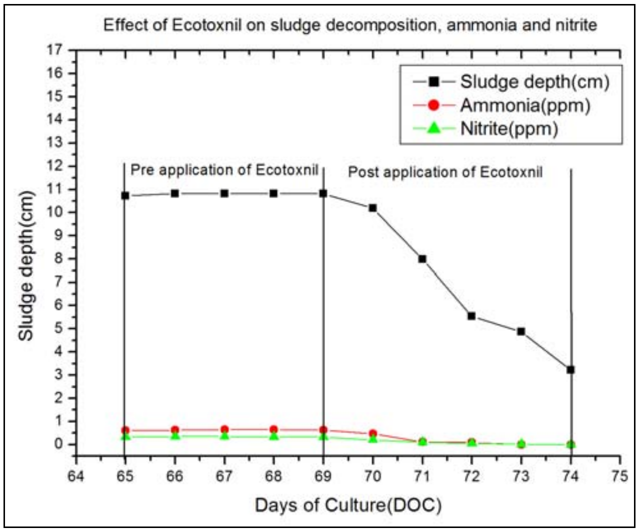

Fig 12: Impact of Ecotoxnil on the control of ammonia, nitrite and sludge in the affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

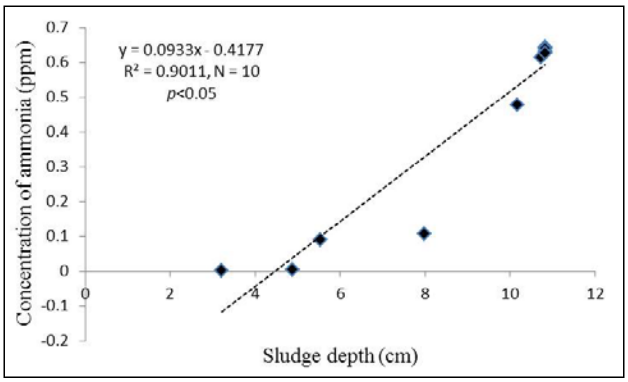

Fig 13: Simple linear regression pattern exhibiting the relationship between ammonia and sludge and impact of liming and Ecotoxnil on the control of ammonia in the sludge affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

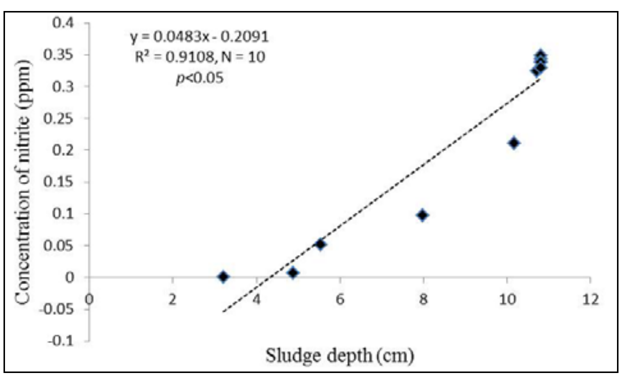

Fig 14: Simple linear regression pattern exhibiting the relationship between nitrite and sludge and impact of liming and Ecotoxnil on the control of nitrite in the sludge affected pond. *Results are presented as means with standard errors of four shrimp ponds (mean ± SE; n = 4)

4. Discussion

Pollution of the coastal environments by aquaculture wastes affects not only the wild capture fisheries, but also the long term sustainability of the aquaculture industry itself, as has been the case for the shrimp production in Asia and the Americas [14, 15]. Microbial remediation and related technologies are now readily accepted by users in the field of aquaculture, as an obvious cost-benefit is seen at harvest. The maintenance of good water quality and the control of disease are closely linked to managing the communities of microbes including microalgae. The application of microbial products in aquaculture tanks and ponds enables farmers to minimise the amounts of organic matter released from their operations [16, 5].

The major impact of the waste material is a build-up in organic matter on the sediment, which stimulates bacterial activity to the point where oxygen is respired at a greater rate than it can diffuse into the sediment. The resulting anoxic sediment surface affects the faunal community composition. The rate of organic matter degradation is slow at low temperatures and thus pollution problems are usually worse in cool temperate climates than in the tropics; also, rates vary with sediment type and biogeochemistry, with pollution effects being worse on muddy sediment and less on sandy sediment [17].

The selection of microbes—usually bacteria—for bioremediation should be based on several criteria [3]. Obviously, the microbes should be able to live and function under the environmental conditions of interest, so would normally be native to that or a similar environment. In aquaculture, the selected bacteria must not be pathogenic to humans, who could be infected after handling or eating the shrimp or fish. The selected bacteria must not carry transmissible resistance genes against clinically important antibiotics. They must not produce toxins that affect humans, shrimp or fish. They should, obviously, have appropriate functional properties for degrading the organic wastes, including the secretion of exo-enzymes for a wide range of organic polymers. Technological properties that they require include being cost-effective to manufacture under industrial conditions and having long term stability and shelf life.

The *Bacillus* group of Gram positive bacteria, which produce spores that survive heat and desiccation, meet these criteria for use in commercial bioremediation products. Gram negative bacteria do not form spores and thus do not survive well when dried; this makes them expensive to use on a large scale in bioremediation. All *Bacilli* grow aerobically, and some are facultative anaerobes, which use nitrate or change to a fermentative metabolism when oxygen is absent. All produce a wide range of extracellular enzymes and thus in consortia, can digest a wide range of polymeric organic compounds [18].

Physicochemical parameters of the pond water play a significant role in proper management. Unfortunately when the parameters changes due to environmental factors and their consequential process, certain measures has to be taken to bring back the parameters to the optimal level. pH remains as one of the basic physicochemical parameter linked with various other chemical and biochemical process in the pond. In aquaculture practices, agricultural lime is considered for application in order to increase the pH in the pond and to buffer the water. On the other side, gypsum is considered to be the component of choice to decrease the pH under hyper pH condition. Efficacy of the probiotic was observed to be high when used under optimal pH in the pond. So when the experimental ponds were monitored for the physicochemical parameters, the pH was observed to be low (7.713±0.3) and hence 50kg/h per day of agricultural lime was applied on the pond by evening for three days. When the pH reached 8.1, no feed were given to the animal for one full day and the probiotic Ecotoxnil 500g/acre was applied with wet sand on the following day. It was then followed by 70% feeding for three days and full feeding from the fourth day. Ramanathan *et al*. [19], reported that, the optimum range of pH required for maximum growth and production of penaeid species is 6.8 to 8.7.

Temperature is yet other significant 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. In culture pond the optimum temperature range is 25-30°C and temperature beyond this range is lethal [20] to shrimps. Rengipat *et al*. [21], mentioned that the use of *Bacillu*s species provided disease protection by activating both the cellular and humoral immune defense mechanism in tiger shrimp (*Penaeus monodon*). In the current studies, the temperature ranged between 28.467 ± 0.7 to 29.902 ± 0.6 ˚C. Temperature almost remained stable throughout the study period favouring the growth of the animal.

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 Edward [22]. The normal growth of shrimp can be achieved between 15 and 20 ppt [23-26]. Boyd [27] (1989) also stated that the ideal salinity for shrimps from 15-25 ppt and high or low salinity affects the moulting frequency. Salinity in the current study ranged from 14.012 ± 2.1 psu to 15.413 ± 2.1 psu (Fig. 7). Though salinity is one among the key factor which could influence the growth of the shrimp, it is well balanced by the topping up of water to manage the loss of water through seepage and condensation, thereby balancing the salinity of the water irrespective of the temperature.

The total concentration of bases in water expressed in milligrams per liter of equivalent calcium carbonate ($CaCO_3$) is the total alkalinity. But in most pond waters, bicarbonate and carbonate are found in greater concentration than other bases. The total concentration of all divalent cations in water expressed in terms of milligrams per liter of calcium carbonate is the total hardness. Calcium and magnesium are the dominant divalent cations in nearly all pond waters. As a general rule, hardness like alkalinity is derived from the dissolution of limestone. When limestone dissolves, it gives equal amounts of hardness and alkalinity. In most waters, total hardness and total alkalinity concentrations are approximately equal [28]. Total alkalinity in the ponds was well maintained with the application of lime in any form as required. In recent days the physico-chemical parameters of the pond are mostly adjusted manually by the application of certain components by the aquaculturists.

The pH of the culture medium is directly related with metabolism and other physiological process of shrimps. Low pH increases the toxicity of nitrite to cultured organism [29] and the toxic form of sulphide [30] and high pH increases the unionized ammonia [31]. Also, the lowering of pH value at the bottom of the pond was mainly due to higher sludge accumulation. With the increase in the amount of sludge, the pH of pond will decrease due to increase in CO_2 concentration as a result of respiration process which occurs in various microorganisms as well as shrimps [32]. Protein catabolism in crustaceans ends up with ammonia and can account for 40-90% of nitrogen excretion [33] and nitrite is an intermediate product of nitrification. However, ammonia is more toxic than nitrite. Generally, ammonia exists in water both in ionized and unionized forms. Among these two, ionized ammonia is more toxic than unionized form. Ammonia concentration depends on pH, temperature and to lesser extent salinity. Effect of probiotics on managing the toxic gases were well noticed from the result obtained. Chen and Sheu [34] (1990) observed that the safe level of total ammonia for adolescent of *Penaeus monodon* was 4.3 ppm. Previously, Boyd [35] (1982) stated that pond seldom contains more than 2 or 3 ppm of total ammonia nitrogen. The safe level of nitrite was 1.2 ppm for *P. monodon* [36].

Shrimp excretes faecal waste in the pond. In addition, unused feed also settles in the bottom of the pond. Faecal waste and unused feed remains as the source of organic matter in the shrimp pond. When this organic matter accumulates, levels of toxic compounds increase, and water quality subsequently deteriorates. Thus, excess amounts of organic matter and toxic compounds need to be removed. Probiotic bacteria can aid this process. The use of microorganisms like bacteria to remove waste products is called bioremediation. A surplus of organic matter provides excellent growth conditions for opportunistic pathogens and sets the stage for infectious disease. Beneficial bacteria such as *Bacillus* species mineralize organic matter and thereby help to reduce the burden. Nitrogen compounds such as nitrite, nitrate and ammonium ions originate from decomposing waste and animal excretions. When these compounds exceed certain levels they are toxic. Ammonium ions interfere with neuronal processes, and prolonged exposure to elevated nitrite levels causes slow suffocation, especially when oxygen is limited. Although unproblematic at low concentrations, prolonged exposure to nitrate can cause weight loss and render animals susceptible to infectious disease. To avoid such complications, beneficial bacteria are introduced to culture systems. These species perform nitrification and or denitrification and thereby lower ammonium, nitrate and nitrite levels in the water. Bacterial nitrification is the oxidation of ammonium to nitrate via hydroxyl amine and nitrite. Denitrification is the reduction of nitrate to nitrous oxide and finally to nitrogen gas, which returns into the atmosphere. Nitrite is an intermediate in both processes. To efficiently remove all three compounds, a mix of bacteria capable of nitrification and/or denitrification is advantageous.

Sulfur compounds like hydrogen sulfide are another problem. Hydrogen sulfide is generated during anaerobic degradation of organic matter at the bottoms of ponds. Hydrogen sulfide interferes with aerobic respiration and thus leads to the suffocation of animals. Black sludge, which occurs when hydrogen sulfide and iron react to form iron sulfide, indicates the presence of hydrogen sulphide [37]. In the present study, relationship between sludge formation with ammonia and hydrogen sulphide formation were studied. Results obtained analysed with statistical data clearly ascertain the increase in the concentration of ammonia and hydrogen sulphide with increase in the depth of the sludge. Hydrogen sulfide is used by several bacterial species and converts the compounds to non-toxic sulfate and nitrogen gas [37]. Hydrogen sulfide, which can form in pond bottom sediment, is toxic to aquatic animals because it interferes with reoxidation of cytochrome a3 in respiration. There are three forms of sulfide (H2S, HS- and S2-), and they exist in a pH- and temperature-dependent equilibrium. As pH increases, the proportion of hydrogen sulfide declines, and that of HS- rises until the two forms have roughly equal proportions at pH 7. At greater pH, HS is the dominant form, and there is no S2- until the pH is above 11. Hydrogen sulfide is toxic to aquatic animals because it interferes with reoxidation of cytochrome a3 in respiration. This effect is caused almost entirely by H2S, while HS is essentially non-toxic. Even if it is toxic, S2- is not an issue, because it does not occur at pH values found in aquaculture systems. The main practices for lessening the risk of hydrogen sulfide toxicity are conservative feeding to avoid wasted feed on pond bottoms, plenty of aeration to prevent low dissolved-oxygen levels and provide a flow of oxygenated water across the soil-water interface, and liming to prevent acidic sediment and water [2]. Also, appealing results obtained with the usage of Ecotoxnil probiotics were evidenced.

In the ponds treated with Ecotoxnil, the animals and water quality were in good condition with notable decrease in the concentration of obnoxious gases like ammonia, hydrogen sulphide, sludge formation and less incidence of bacterial infection and gill problem. Furthermore, Ecotoxnil has also influenced the phytoplankton density and diversity to a greater extent. Also, the production and survival were remarkable in the ponds with greater profit to the farmer. Priyadarshini *et al*. [38] 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. Ecotoxnil has conferred all the aforementioned properties to the shrimps in the ponds and thus paved the way for greater production, superior water quality and pond management with effective and attractive feed conversion ratio.

5. Conclusion

To conclude, Ecotoxnil 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, and obnoxious gases like ammonia, hydrogen sulphide, enabling proliferation of beneficial phyto and zoo planktons, degradation of metabolic organic and inorganic waste in the pond bottom, reduction of black soil and sludge, 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 a sustainable and eco-friendly aquaculture with better yield and lesser FCR especially at higher saline waters.

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7. References

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