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***Research Article** (Open Access)*

EFFICACY OF PROBIOTICS ON WATER QUALITY AND *VIBRIO* LOADS IN COMMERCIAL SHRIMP FARMS OF *PENAEUS VANNAMEI* AT NAKKAPALLI, ANDHRA PRADESH, INDIA

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ABSTRACT
During the course of present study the significant reduction in *Vibrio* colonies in the experimental ponds in both summer and winter crops were recorded with the application of probioitcs. The study was conducted with the application of probiotics in culture ponds of Nakkapalli, Visakhapatnam, Andhra Pradesh, India. During the culture, application of feed probiotics in combination with the immunostimulants yielded better growth, survival rate and more successful crop free of diseases. Success rate and productions were high in the ponds where probiotics were applied when compared to the control ponds. The results of the present study during both crops shows that periodic and systematic application of probiotics in shrimp culture yields better, economical and successful crops in the commercial production of *Penaeus vannamei* in coastal Andhra Pradesh of India.

Keywords: *Probiotics, Vibrio, P. vannamei*

INTRODUCTION
According to Saulnier *et al.,* (2000) for shrimps *V. harveyi* and *V. penaeicida* are considered as true pathogens. In shrimp culture ponds of Tamil Nadu, India among the bacterial diseases of shrimp, infection caused by *V. harveyi* stood first place reported as by Felix (2000). According to Selvin and Lipton (2003) occurrence of *V. alginolyticus* was always together with the WSSV infected *P. monodon.* Vaseeharan and Ramasamy (2003) reported that the possible source of vibrio in hatcheries was infected post larvae and *Artemia* nauplii with *Monodon Baculo Virus* (MBV). As opportunistic pathogen, vibrio can infect the post larvae when the count level increases to $2x10^2$ cfu/ml, which leads to mass mortality. According to Abraham and Palaniappan (2004) *V. harveyi* (94.05%) and *Vibrio orientalis* (54.1%) were observed as dominant luminescent bacterial species in shrimp hatcheries located at Tamil Nadu, India. The chief source of these bacterial species in shrimp hatchery was the fecal matter of brood stock as observed the bacterial cells of 97.30% and 2.70% in the gut content of the shrimp. Thakur *et al.,* (2004) studied about the vibrio loads of shrimp ponds and the number of colonies observed and ranged from $1.8x10^1$ to $7.8x10^4$ cfu/ml. The number of colonies increased with the culture duration and highest number was noticed on 122^{nd} day of culture. Whereas infected shrimp showed more number of vibrio colonies of $8.3x10^6$ cfu/g of hepatopancreas. He also reported for the species of vibrios such as *V. parahaemolyticus, V. alginolyticus,* V. *anguillarum* and *V. vulnificus.* Gopal *et al.,* (2005) observed vibrio bacterial species that occurred in water, sediments and shrimps from culture ponds of East and West Coast of India. They reported that more vibrio population was in culture ponds from West Coast i.e. 104 cfu/ml when compared to the culture ponds from East Coast i.e. 102 cfu/ml.
According to Abraham and Sasmal (2009) abundance of the heterotrophic population is varies in different environmental conditions, therefore heterotrophic population is influenced by environmental factors. They observed number of heterotrophs in pond water and sediment ranged from 104-106/ml and 105-107/g respectively.

Microbial management at pond bottom will arrest the propagation of diseases. It is obvious to maintain the culture successfully by proper pond preparation, stocking good viable seed with moderate stocking density, good phytoplankton bloom, good water quality, avoid feed waste and proper routine monitoring, all these factors decrease the bacterial loads and reduce the chances of disease occurrence in culture system. The purpose of the present study is to estimate the total vibrio loads from the culture ponds of Visakhapatnam, Andhra Pradesh, India.

MATERIALS AND METHODS

The present work was carried out in commercial shrimp farms located at Visakhapatnam Andhra Pradesh, India, during the year 2019. Modified extensive shrimp farms were selected for this research work. The data was recorded from both control and experimental ponds. For studies on water quality parameters, samples of pond water were collected from control and experimental ponds for 0, 30, 60, 90 and 120 days of culture. For collection of water samples 500 ml capacity polythene bottles were used and stored in ice box and brought to the laboratory within a span of two hours of sampling. Water temperature, pH, and salinity were recorded on field. The temperature was measured by using mercury centigrade thermometer with an accuracy of 0.1^0C. Temperature was expressed as degree Celsius (^{0}C). pH was measured using pH meter (Elico, Make). Water samples were collected from four corners of the culture ponds and readings were recorded.

The samples were brought to the laboratory of the Department of the Zoology, Andhra University, Visakhapatnam for further analysis, and standard methods were followed (APHA, 1995). Pond water levels were maintained at 1-1.2 meter in all the ponds and continuous aeration was provided throughout the culture period. No probiotics was used in control ponds but 10-20% water exchange was done once in 15 days upto 90 days of culture and then 70% water exchange was done once a week till harvest. In case of experimental ponds the water is treated with commercial water probiotics Pro-W@30kg/hectare for every 15 days throughout the culture period. A minimum of 5-10% water exchange was done in experimental ponds. Salinity of the pond water was measured using refractometer. For this water collected from four corners of the pond. Dissolved oxygen was measured by modified Wrinkler's iodometric method (APHA, 1995).

Microbial analysis

For studies on microbial analysis water samples were collected following the method as described by Dalmin *et al.*, (2001). Water samples were collected in 100ml sterilized PVC bottles just below the water surface. Necessary precautionary measures were taken to minimize the contamination through handling. Each water sample is serially diluted 10^{-6} using sterile distilled water as a blank which was prepared by sterilized seawater in an autoclave at 15 lbs and 121^0C for 15 minutes.

Total vibrio counts were determined following the procedure of Dalmin *et al.*, (2001). For this TCBS agar medium was used. For isolation of vibrios spread plate method was used to inoculate bacteria into agar petri plates and incubated for 20-24 hours. Total Vibrio Count (TVC) was expressed as colony forming units/ml (cfu/ml).

RESULTS AND DISCUSSION

Table 1: Total *Vibrio* Counts in culture ponds at Nakkapalli during summer crop of the year 2019

S. No.	Days of Culture	Control Pond TVC Water cfu/ml	Experimental Pond TVC Water cfu/ml
1	Before 30 days	NIL	NIL
2	30 days	$0.22\times10^2\pm0.14$	NIL
3	60 days	$0.39\times10^2\pm0.39$	$0.17\times10^2\pm0.55$
4	90 days	$2.58\times10^2\pm0.12$	$1.05\times10^2\pm0.40$
5	120 days	$2.85\times10^2\pm0.19$	$1.90\times10^2\pm0.55$

Table 2: Total *Vibrio* Counts in culture ponds at Nakkapalli during winter crop of the year 2019

S. No.	Days of Culture	Control Pond TVC Water cfu/ml	Experimental Pond TVC Water cfu/ml
1	Before 30 days	$0.19\times10^2\pm0.52$	$0.20\times10^2\pm0.52$
2	30 days	$0.21\times10^2\pm0.68$	$0.15\times10^2\pm0.19$
3	60 days	$0.20\times10^2\pm0.34$	$0.19\times10^2\pm0.23$
4	90 days	$0.39\times10^2\pm0.84$	$0.21\times10^2\pm0.45$
5	120 days	$2.97\times10^2\pm0.38$	$0.35\times10^2\pm0.73$

It is evident from the present results of summer crop of 2019 at Nakkapalli, water salinity in ppt was recorded at different time intervals of culture in control pond and experimental pond (treated with water probiotic) were ranged from 27.20±2.7 to 30.42±1.8 and 28.54±3.6 to 31.22±1.8 respectively. Mean values of pH recorded at different time intervals of culture in control and experimental ponds were ranged from 7.5±1.6 to 8.4±1.7 and 7.4±1.8 to 8.9±1.5 respectively. Similarly mean values of temperature in (^{0}C) ranged from 28.55±1.3 to 30.34±1.9^{0}C and 28.20±1.2 to 30.72±1.3^{0}C were recorded for control and experimental ponds respectively. Mean values of dissolved oxygen concentrations were recorded from culture ponds and it was ranged from 5.27±2.31 to 7.24±1.41 and 5.38±2.31 to 8.24±1.41 mg/lit for both control and experimental ponds respectively. It has been observed that values of dissolved oxygen levels in experimental ponds significantly higher than control ponds. Similarly mean values for total ammonia was varied from 0.07±0.01 to 0.21±0.03 and 0.05±0.01 to 0.17±0.05 mg/lit for both control and experimental ponds respectively. Hardness ranged from 985±11.58 mg/lit to 1207±5.36 and 776±9.63 to 1124±4.31 mg/lit respectively. Total alkalinity values found to be varied from 163±5.21 to 193±2.55 and 155±2.55 to 195±5.21 mg/lit for both control and experimental ponds respectively. Similarly mean values of total *vibrio* counts (TVC) in pond water varied from $0.22\text{x }10^2\pm0.14$ to $2.85\times10^2\pm0.19$ cfu/ml and $0.17\times10^2\pm0.55$ to $1.90\times10^2\pm0.55$ cfu/ml for both control and experimental ponds respectively **(Table 1).**
Similarly the results of water quality parameters of the shrimp farms in the winter crop of 2019 at Nakkapalli salinity ranged from 29.53±2.5 to 32.92±1.5 and 31.25±1.7 to 33.54±2.5 ppt for both control and experimental ponds respectively. pH varied from 7.3±2.2 to 8.9±2.6 and 7.5±1.3 to 8.7±1.5 respectively. In the same way temperature varied from 28.53±1.2 to 31.23±1.4 and 28.24±1.5 to 31.25±1.5 for both control and experimental ponds respectively. Dissolved oxygen content found to be varied from 5.52±1.65 to 6.43±1.58 mg/lit and 5.58±1.58 to 7.57±1.29 mg/lit for both and control ponds respectively. Similarly mean values of total ammonia varied from 0.07±0.01 to 0.23±0.02 mg/lit and 0.09±0.02 to 0.21±0.01 mg/lit for both control and experimental ponds respectively. Values of hardness ranged from 955±4.67 to 1378±5.31 mg/lit and 898±5.46 to 1232±4.26 mg/lit for both control and experimental ponds respectively. Similarly total alkalinity varied from 123±4.14 to 194±1.43 mg/lit and 176±5.19 to 205±3.28 mg/lit for both control and experimental ponds respectively. Similarly mean values of total *vibrio* counts were also varied from $0.19\times10^2\pm0.52$ to $2.97\times10^2\pm0.38$ cfu/ml and $0.15\times10^2\pm0.19$ to $0.35\times10^2\pm0.73$ cfu/ml for both control and experimental ponds respectively (Table 2).
In the present study by the application of probiotics in the experimental ponds showed positive results in the shrimp production when compared to the control ponds. The water samples of all the ponds were analyzed periodically for total *Vibrio* colonies and the findings were revealed and the total *Vibrio* colonies were increased in control ponds of both seasons during the study period. There is significant reduction in the total *Vibrio* colonies in the experimental ponds. It is evident from the obtained results from the present study that the reduction of the total *Vibrio* colonies with increased dosage of the pond probiotic and also by increase the frequency of application from 15 days duration to 7 days duration. The positive bacteria in the probiotics reduced the proliferation of the pathogenic bacteria in all the ponds where the application of probiotics is followed. At the same time due to the probiotic application the toxic gases of the pond bottom were reduced and the oxidation of the organic matter is observed in the culture ponds.

The luminous vibrio is responsible for mortalities of cultured juvenile shrimp, *P. monodon*. Usually the disease occurs during initial 45 days of culture and can cause about 50% mortality as reported by Mohney *et al.,* 1994; Lavilla-pitago *et al.,* 1998). Fernandes *et al.,* (2013) studied about the pond environment and bacterial populations. The physico-chemical parameters of the culture pond for *P. monodon* was within the optimum range were recorded. In their study abundance of total heterotrophic bacteria was remained steady state at 103 cfu/ml of the pond water. Whereas in sediments it was varies as 103 cfu/g and 104 cfu/g. Kannapiran *et al.,* (2009) recorded different heterotrophic bacterial counts in both water and sediment of the pond as $1.3x10^4$ to $25.3x10^4$ cfu/ml, $1.5x10^6$ to $26.2x10^6$ cfu/g respectively. The density of luminescent bacteria *V. harveyi* is varied between $0.6x10^4$ and $8.8x10^4$ cfu/ml in water was observed. Similarly in the present study during summer crop the mean values of total *vibrio* counts (TVC) in pond water varied from $0.22x\ 10^2 \pm 0.14$ to $2.85\times10^2\pm0.19$ cfu/ml and $0.17\times10^2\pm0.55$ to $1.90\times10^2\pm0.55$ cfu/ml for both control and experimental ponds respectively. Whereas in winter crop the mean values of total *vibrio* counts were varied from $0.19\times10^2\pm0.52$ to $2.97\times10^2\pm0.38$ cfu/ml and $0.15\times10^2\pm0.19$ to $0.35\times10^2\pm0.73$ cfu/ml for both control and experimental ponds respectively.

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