IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
Research Paper , UGC CARE Listed (Group -I) Journal Volume10, Iss10, 2021

Research Article

Application of Probitoics on Water Quality Parameters of Penaeus vannamei from the Culture Ponds of Ampalam Andhra Pradesh, India

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Abstract

Water quality is one of the important factors in the shrimp culture activity. In shrimp culture ponds water quality is influenced by both environmental and management practices. In this context the water quality parameters and Vibrio loads both in control and experimental ponds were analyzed over a period of two years in *Penaeus vannamei* culture farms located at Ampalam of Srikakulam District, Andhra Pradesh, India, over a period of two consecutive years i.e 2018-2019. As most of the shrimp culture farming shifted from *Penaeus monodon* to *P. vannamei* culture system, limited studies are available on the water quality, and vibrio loads in *P. vannamei* culture system. It is evident from the study findings that the water quality parameters in the experimental ponds in the culture of *P. vannamei* showed that the pathogenic bacterial loads are totally in control in the ponds where the probiotics were administered and in those control ponds where there is no probiotic application, the pathogenic bacterial loads were increased during the entire culture period in both seasons of the study period.

Keywords: Water Quality, *P. vannamei,* Probiotics.

Introduction

Penaeus vannamei is mainly cultured at the Western hemisphere. It is a native species to the Eastern Pacific coast of Mexico and Central and South of America (Briggs *et al.*, 2004). *P. vannamei*, is also called white leg shrimp falls under order of Decapoda. It consists of head, thorax, six abdominal segments and a tail (Rudloe and Rudloe, 2009). Compared to other penaeid shrimp species, *P. vannamei* is highly resistant to disease and that is the main reason why it is being the most cultured nowadays. In addition, its ability to tolerate wide range of water quality parameters (Rosenberry, 1999) such as salinity, temperature, pH and dissolved oxygen help to increase reputation of *P. vannamei* throughout the year. This species can tolerate wide range of salinity from 0.5-45 PSU. It can survive comfortably at 7-34 PSU, yet it still can grow well at salinities around 10-15 PSU. It is not only euryhaline but it also eurythermal which means it can tolerate large range of temperature. The best temperature it can live is between 23ºC to 30ºC. It also can mature at temperature of 15ºC and up to 33ºC, but at slower growth rate. It is highly resistant to temperature helps in preventing from getting disease (Briggs *et al.*, 2004). Presently, many farmers transformed from culturing of *Penaeus monodon* to *P. vannamei* because of its fast growth rate (Yaemsooksawat *et al.*, 2009). *P. vannamei* can grow as fast as the *P. monodon* and it can achieve 20 g in 44 days which is the maximum size for the cultured *P. vannamei* under intensive culture conditions (Briggs *et al.*, 2004). The uniform growth rate gives benefit to the culture industry (Ronnback, 2001). This species also required low level of protein for feed compared to the *P. monodon* and it helps the shrimp farmer to reduce the feeding cost. *P. vannamei* is also easier to reproduce compared than *P. monodon* (Ronnback, 2001). Keeping in view of the above reason in the present study an attempt has been made to evaluate the water quality parameters in *P. vannamei* cultured ponds at Ampalam during the years 2018 to 2019.

Material and Methods

The present work is carried out in commercial shrimp farms located at Ampalam of Srikakulam District, Andhra Pradesh, India, over a period of two consecutive years i.e 2018-2019. Modified extensive shrimp farms were selected for this research work. The data was recorded from both control and experimental ponds in summer and winter crops. Before performing the experiments the selected ponds were allowed to sun dry and crack the soil to remove fish and crab larvae as well other obnoxious gases. The crab netting was practiced for the ponds to stop entry of virus carrier species. Four paddle wheel aerators were installed in the four corners of the study ponds. For efficient plankton growth rice bran, fish meal, molasses, yeast, dolomite, nutrilake, soda mix was added in the probiotics. The PCR screened seeds of *P. vannamei* procured from the commercial hatchery located in Visakhapatnam were used.

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 probioits 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-2 (*Bacillus* species mixture @30kg/hectare for every 15 days throughout the culture period. A minimum of 5-10% water exchange was done in experimental ponds. The applications of different commercial probiotics of feed, soil and water were applied in the culture ponds (Super PS, Super biotic, Pond Plus, Pro-Tect) along with the immunostimulant 1,3 β-Glucan, a commercial brand β-ADVANTAGE.

Salinity of the pond water was measured using refractometer. For this water collected from four corners of the pond for the salinity determination. Dissolved oxygen was measured by modified Wrinklers iodometric method (APHA, 1995). Alkalinity was measured following modified titrimetric method (APHA, 1995). For the determination of total ammonia spectrophotometric method was followed (APHA, 1995) for this spectrophotometer (Systronic UV-VIS, Spetrometer 108) was used. The concentration of total ammonia was computed based on standard graph and results were expressed as mg/lit. Hardness of the water was estimated by titrimetric method (APHA, 1995).

Microbial analysis

For studies on microbial analysis water samples were collected following the method described by Dalmin *et al.,* (2001). For this 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)**.**

Statistical analysis

One-way ANOVA was carried out to check the effect of days on the water quality parameters, in control and experimental farms of the winter crop and summer crops at Ampalam during the years 2018 to 2019. These analyses were done by using IBM SPSS Version 22.0. Bar graphs were drawn by using mean values and SD of water quality parameters in MS Excel 2016. All values were represented as Mean ± SD.

Results

The results of water quality parameters in control and experimental ponds over a period of two years i.e. 2018 to 2019 was presented in figures.

It is evident from the present results of summer crop of 2018 at Ampalam, 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 17.89±1.2 to 20.22±1.6 and 17.98±1.1 to 20.57±1.0 respectively. Mean values of pH recorded at different time intervals of culture in control and experimental ponds were ranged from 7.59±0.28 to 8.60±0.32 and 7.90±0.24 to 8.47±0.44 respectively. Similarly mean values of temperature in (^{0}C) ranged from 31.14±1.2 to 32.66±1.3^{0}C and 31.16±1.3 to 32.76±1.0^{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 5.46±0.46 to 7.92±0.47 and 5.59±0.57 to 7.25±0.70mg/lit for both control and experimental ponds respectively. Similarly mean values for total ammonia was varied from 0.12±0.03 to 0.23±0.05 and 0.09±0.02 to 0.21±0.03mg/lit for both control and experimental ponds respectively. Hardness ranged from 978±3.48mg/lit to 1250±6.38 and 887±3.56 to 1158±5.67mg/lit for both control and experimental ponds respectively. Total alkalinity values found to be varied from 178±1.08 to 194±1.26 and 169±1.34 to 201±2.11mg/lit for both control and experimental ponds respectively. Similarly mean values of total *vibrio* counts (TVC) in pond water varied from 0.43×10^2±0.12 to 1.42×10^2±0.27cfu/ml and 0.54×10^2±0.14 to 1.28×10^2±0.33cfu/ml respectively (Figures 1-8).

Similarly the results of water quality parameters of the shrimp farms in the winter crop of 2018 at Ampalam salinity ranged from 15.29±1.2 to 18.82±1.3 and 16.79±1.4 to 18.01±1.2 ppt for both control and experimental ponds respectively. pH varied from 7.12±0.24 to 8.69±0.57 and 7.55±0.47 to 8.63±0.69 respectively. In the same way temperature varied from 25.77±1.7 to 27.48±1.8^{0}C and 29.26±1.6 to 31.05±1.3^{0}C for both control and experimental ponds respectively. Dissolved oxygen content found to be varied from 5.72±0.17 to 6.50±0.26 mg/lit and 6.57±0.34 to 8.04±0.61 mg/lit for both and control ponds respectively.

Similarly mean values of total ammonia varied from 0.10±0.05 to 0.23±0.03 mg/lit and 0.09±0.06 to 0.22±0.03mg/lit for both control and experimental ponds respectively. Values of hardness ranged from 897±3.89 to 1104±6.47mg/lit and 769±4.87 to 1089±6.01mg/lit for both control and experimental ponds respectively. Similarly total alkalinity varied from 162±1.40 to 197±2.15mg/lit and 156±1.22 to 201±2.48mg/lit for both control and experimental ponds respectively. Mean values of total *vibrio* counts were also varied from 0.86×10^2 ±0.12 to 1.41×10^2±0.31cfu/ml and 0.72×10^2±0.19 to 1.41×10^2±0.14cfu/ml for both control and experimental ponds respectively (Figures 1-8).

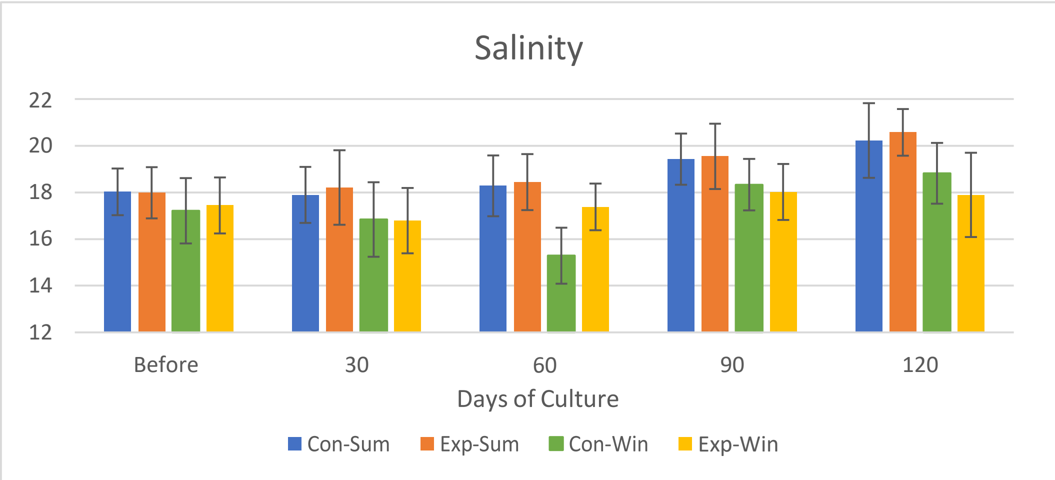

Figure 1: Salinity of the control & experimental ponds in summer & winter crops at Ampalam during the year 2018.

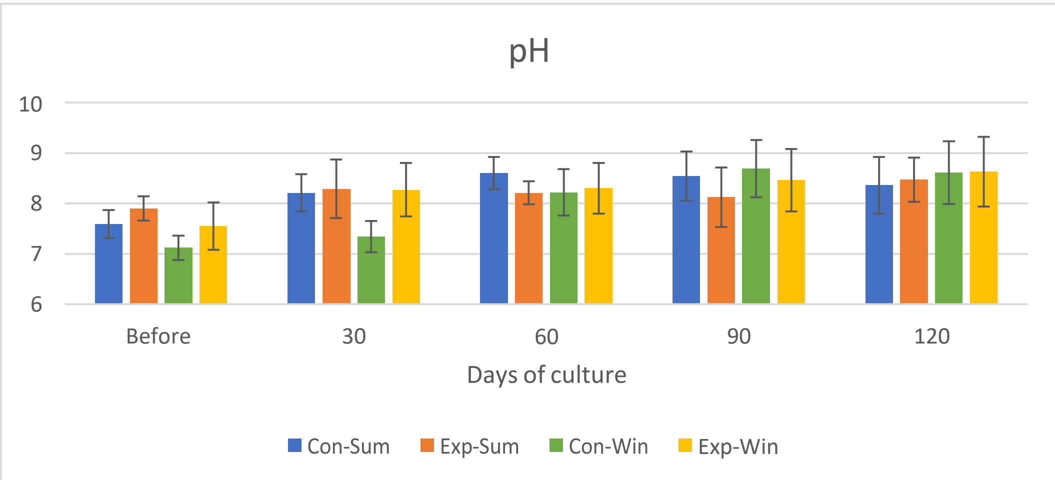

Figure 2: pH of the control & experimental ponds in summer & winter crops at Ampalam during the year 2018.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 10, Iss 10, 2021

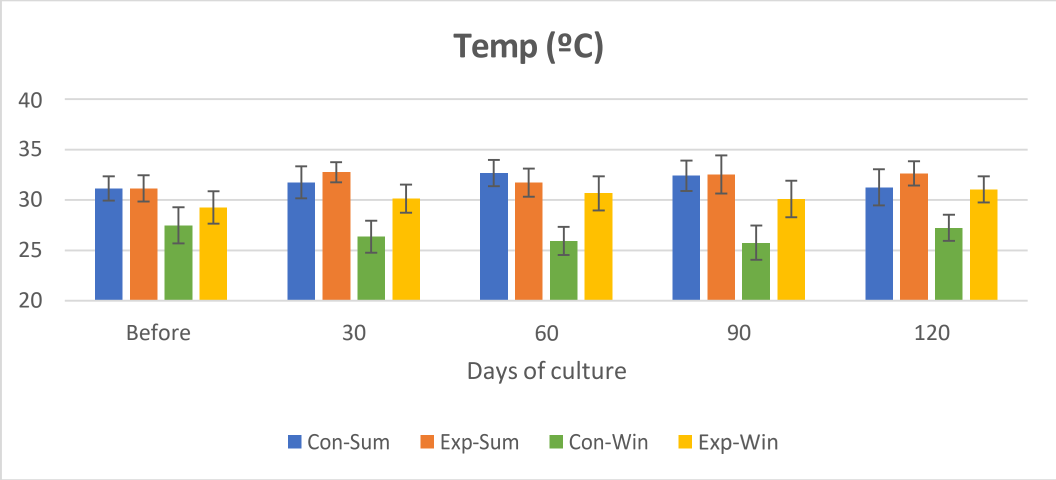

Figure 3: Temperature of the control & experimental ponds in summer & winter crops at Ampalam during the year 2018.

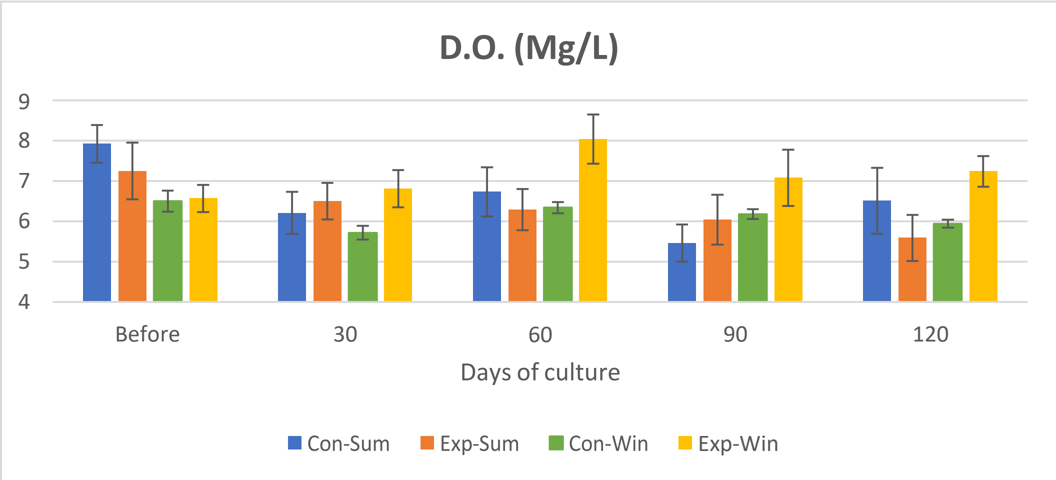

Figure 4: Dissolved oxygen of the control & experimental ponds in summer & winter crops at Ampalam during the year 2018.

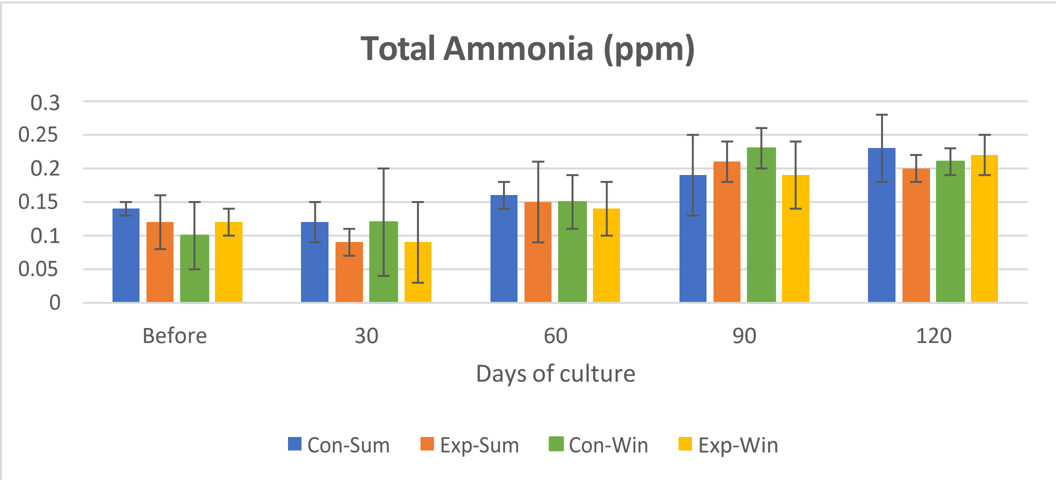

Figure 5: Total Ammonia (ppm) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2018.

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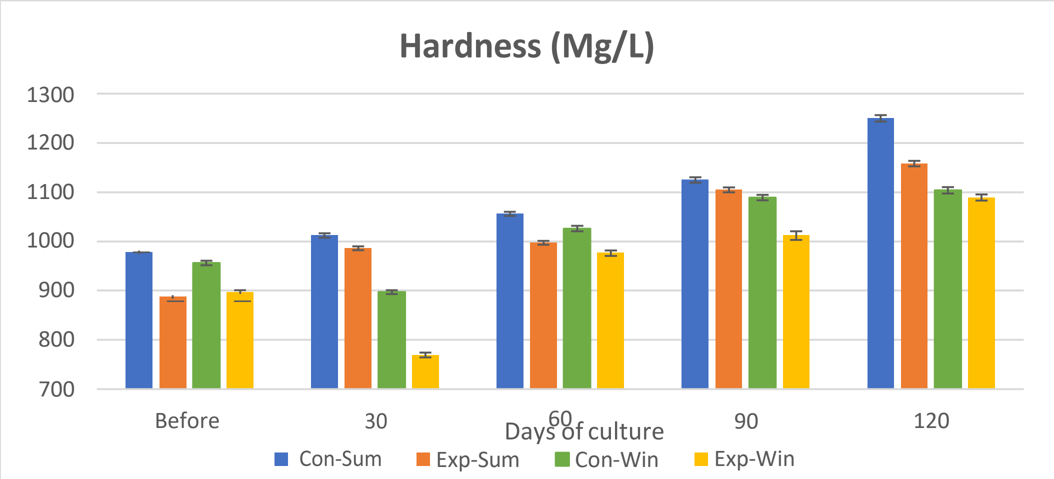

Figure 6: Hardness (Mg/L) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2018.

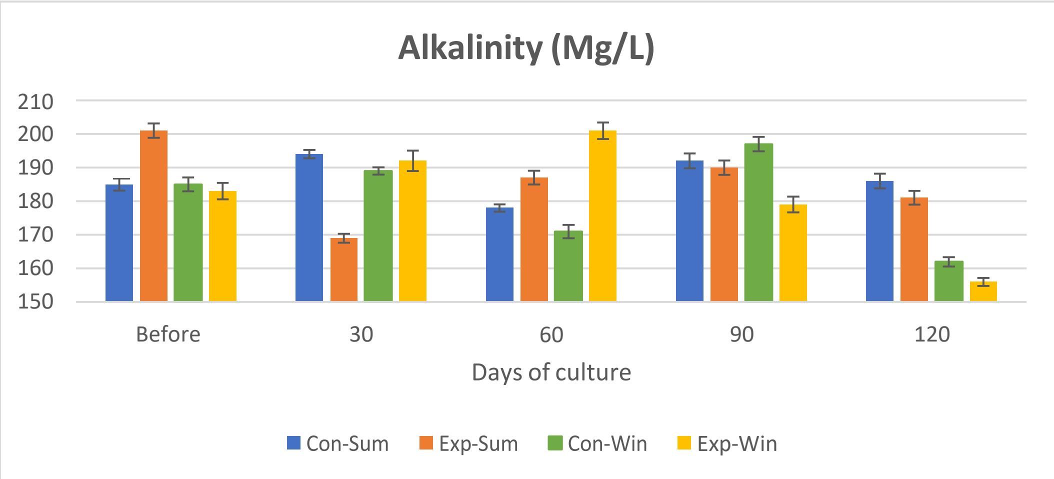

Figure 7: Alkalinity (Mg/L) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2018.

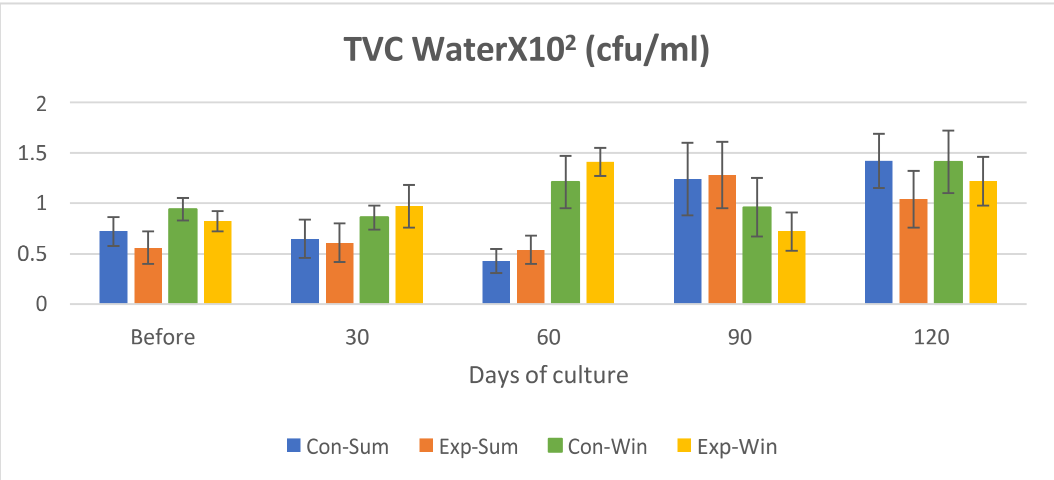

Figure 8: TVC Water (cfu/ml) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2018.

It is evident from the present results of summer crop of 2019 at Ampalam, 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 18.26±1.4 to 20.37±1.3 and 18.49±1.5 to 20.78±1.8 respectively. Mean values of pH recorded at different time intervals of culture in control and experimental ponds were ranged from 7.42±0.59 to 8.74±0.98 and 8.57±0.81 to 8.80±0.58 respectively. Similarly mean values of temperature in (^{0}C) ranged from 27.82±1.5 to 30.42±1.3^{0}C and 29.57±1.7 to 31.71±1.9^{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 5.64±0.61 to 7.56±0.63 and 5.89±0.64 to 7.68±0.59mg/lit for both control and experimental ponds respectively. Similarly mean values for total ammonia was varied from 0.14±0.07 to 0.25±0.03 and 0.16±0.09 to 0.23±0.03mg/lit for both control and experimental ponds respectively. Hardness ranged from 769±4.34mg/lit to 1073±5.62 and 789±3.17 to 1061±5.82mg/lit for both control and experimental ponds respectively. Total alkalinity values found to be varied from 168±1.82 to 201±1.69 and 164±2.04 to 194±2.09mg/lit for both control and experimental ponds respectively. Similarly mean values of total *vibrio* counts (TVC) in pond water varied from 0.86x10^2±0.19 to 1.76x10^2±0.26cfu/ml and 0.87x10^2±0.16 to 1.29x10^2±0.19cfu/ml respectively (Figures 9-16). Similarly the results of water quality parameters of the shrimp farms in the winter crop of 2019 at Ampalam salinity ranged from 14.89±1.3 to 17.61±1.1 and 14.93±1.0 to 17.34±1.3ppt for both control and experimental ponds respectively. pH varied from 7.46±0.57 to 8.60±0.96 and 7.44±0.41 to 8.62±0.67 respectively. In the same way temperature varied from 26.80±1.4 to 28.17±1.6^{0}C and 26.16±1.7 to 27.15±1.4^{0}C for both control and experimental ponds respectively. Dissolved oxygen content found to be varied from 5.77±0.88 to 7.11±0.37mg/lit and 5.25±0.69 to 7.94±0.61mg/lit for both and control ponds respectively. Similarly mean values of total ammonia varied from 0.14±0.03 to 0.21±0.05mg/lit and 0.12±0.02 to 0.21±0.04mg/lit for both control and experimental ponds respectively. Values of hardness ranged from 799±3.34 to 1198±6.75mg/lit and 726±4.61 to 1202±6.02mg/lit for both control and experimental ponds respectively. Similarly total alkalinity varied from 167±2.26 to 198±1.72mg/lit and 166±3.20 to 201±2.72mg/lit for both control and experimental ponds respectively. Mean values of total *vibrio* counts were also varied from 0.62x10^2±0.21 to 1.44x10^2±0.16cfu/ml and 0.69x10^2±0.16 to 1.42x10^2±0.20cfu/ml for both control and experimental ponds respectively (Figures 9-16).

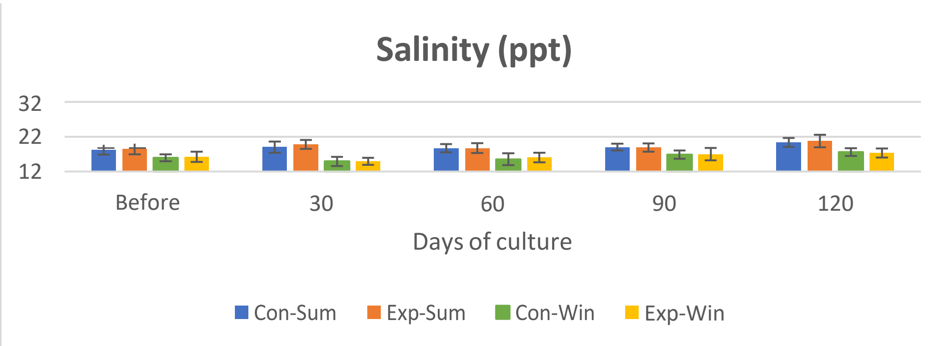

Figure 9. Salinity of the control & experimental ponds in summer & winter crops at Ampalam during the year 2019.

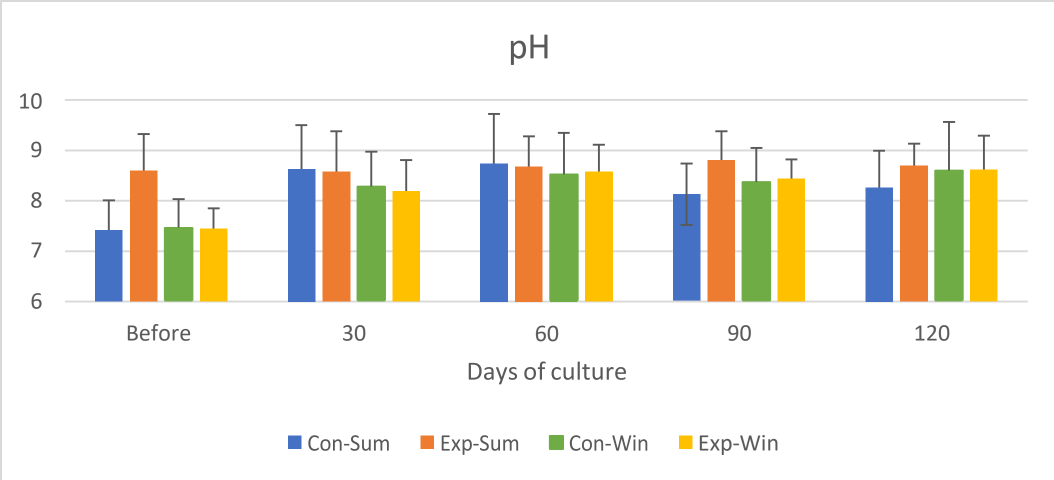

Figure 10. pH of the control & experimental ponds in summer & winter crops at Ampalam during the year 2019.

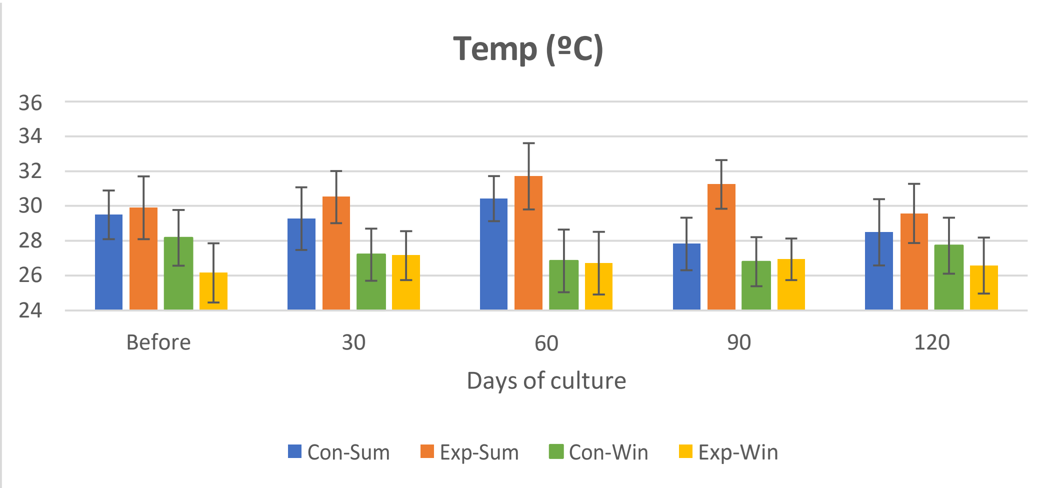

Figure 11. Temperature of the control & experimental ponds in summer & winter crops at Ampalam during the year 2019**.**

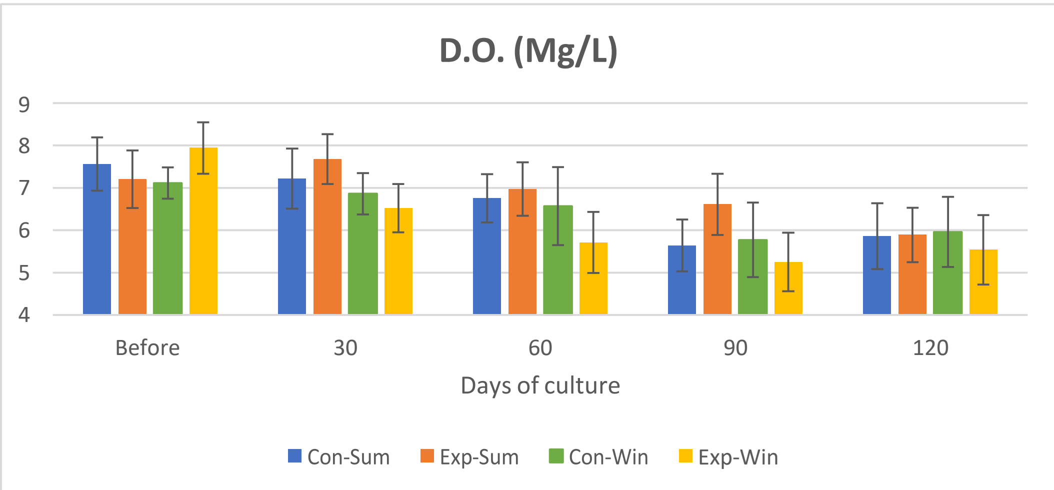

Figure 12. D.O. (Mg/L) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2019.

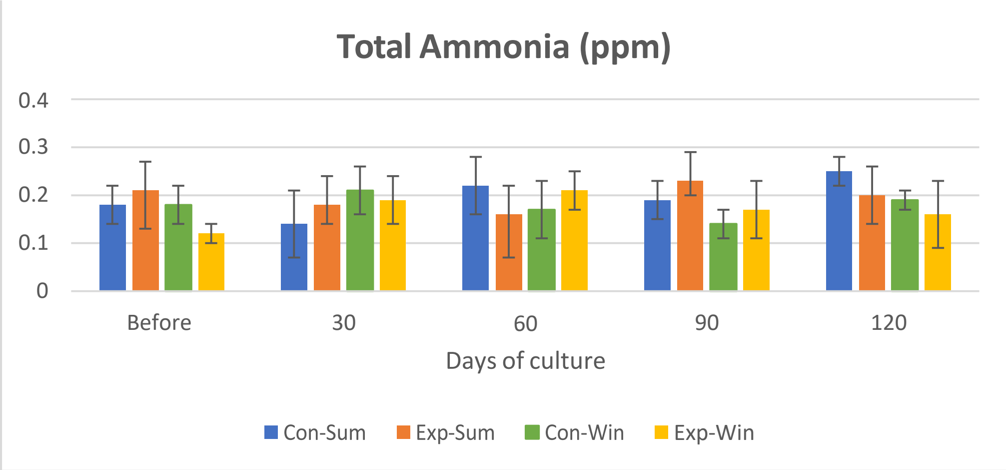

Figure 13. Total Ammonia (ppm) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2019.

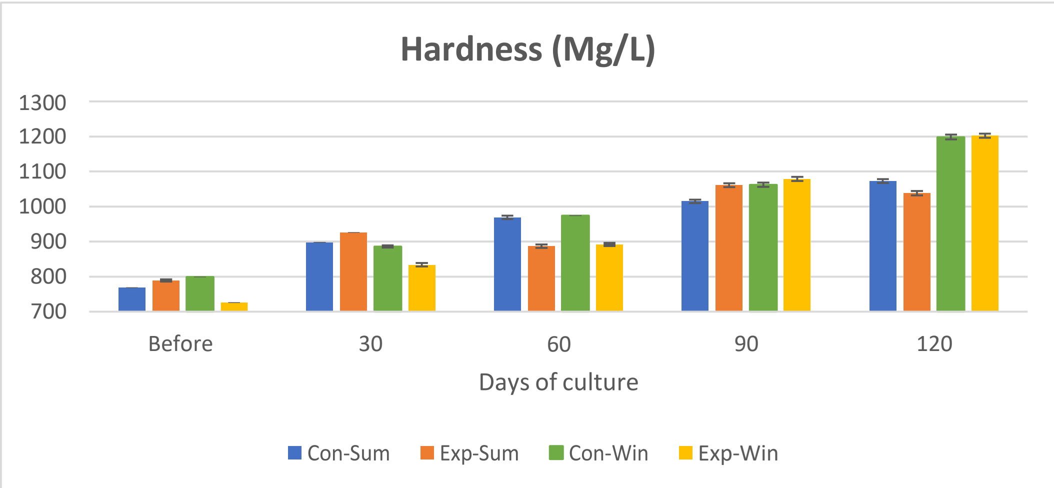

Figure 14. Hardness (Mg/L) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2019.

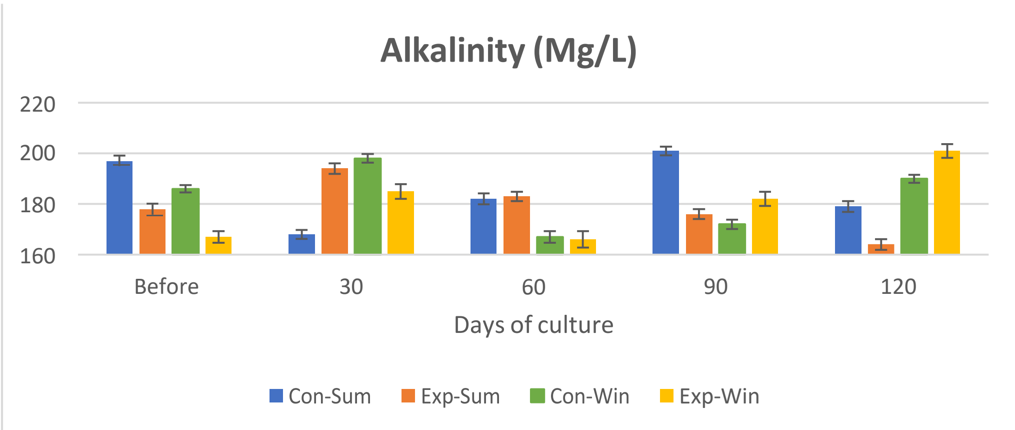

Figure 15. Alkalinity (Mg/L) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2019.

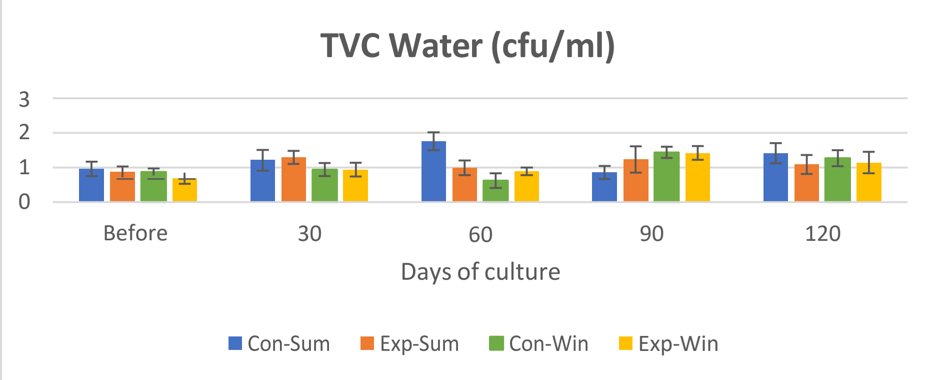

Figure 16. TVC WaterX10^2 (cfu/ml) of the control & experimental ponds in summer & winter crops at Ampalam during the year 2019.

Discussion

According to Abraham and Sasmal (2009) uniform sized shrimps can produced when shrimps were cultured at different salinities of 4-9 ppt, 9-15 ppt, 15-26 ppt waters. This can be achieved by adopting shrimps to medium and low saline waters. The optimal salinity range for white shrimp is between 15-30 ppt (Haliman and Adijaya, 2005). In the present study the recorded salinity values were in the range of 14 to 21 for the probiotics treated and control ponds.

The optimum pH range for shrimp cultivation is 7.3 to 8.5 and tolerance range is 6.5 to 9 (Suprapto, 2005). The pH of the *P. vannamei* rearing medium supplemented with various types of biofloc varies between 7.7 and 8.2 (Martinez-Porchas *et al.,* 2020). In the present study the recorded pH values were in the range of 7.1 to 8.8 for the probiotics treated and control ponds. The ideal pH range of 7.5 to 8.5 for *P. monodon* culture was reported by Soundarapandian and Gunalan (2008). Ramanathan *et al*., (2005) recorded the maximum growth and production when the range of pH in between 6.8 to 8.7 for *P. monodon.*

Das and Saksena (2001) reported that farmed shrimp shows best growth in a temperature range of 24°C to 32°C. Several finfish and shellfish can tolerate water temperature upto a maximum of 33°C (Santhanam and Ramadhas, 2001). Kumlu *et al.,* (2000) reported about survival and growth of *Penaeus semisulcatus* with the best salinity and temperature combination for the culture was 30 ppt and 30^0C. The optimal level of temperature for the best growth and high survival was 30°C, when salinity was maintained between 30 and 35 ppt. According to Raj *et al.,* (2012) higher survival rate in *P. monodon* was observed when shrimp exposed at 32^0C (37%) and 36^0C (14%) under controlled conditions. White shrimp daily growth coefficient (K) observed by Castillo-Sorianto *et al.,* (2013) in all the culture ponds was higher as compared to *P. monodon* (K) grown at 25^0C as shrimp growth rate increases with increase in temperature. In the present study the recorded temperature values were in the range of 25^0C to 32^0C for the probiotics treated and control ponds. The ideal temperature range of 26-33^0C for *P. monodon* culture was reported by Soundarapandian and Gunalan (2008). Ramanathan *et al*., (2005) recorded the maximum growth and production when the temperature range of 28-30^0C for *P. monodon.*

In intensive shrimp farming, the dissolved oxygen is greater than 3.5 ml/lit was quiet suitable for the shrimps as observed by Musig (1980) and Boyd (1990). According to Chen (1985) the critical concentration of the dissolved oxygen in shrimp culture pond is 3.7 ppm. According to Flegel *et al.,* (1995) low dissolved oxygen condition can reduce the immune system and caused for the diseases and mortality of shrimp. Boyd (1989) stated that reduction of the dissolved oxygen levels caused by the prevalence of high temperature and high organic matter in the pond bottom of the culture systems. The optimal range of dissolved oxygen was 3.5-7.5 mg/L as reported by Adiwijaya *et al.,* (2003). In the present findings the recorded average values of dissolved oxygen are greater than 5.2 mg/lit. Dissolved oxygen concentrations in white shrimp rearing media containing various types of biofloc ranged between 5.9 and 6.3 mg/L (Martinez-Porchas *et al.,* 2020). Dissolved oxygen levels were 6.11-6.48 mg/L in white shrimp rearing at various densities (Rodríguez-Olague *et al.,* 2021).

Jiang *et al.,* (1999) observed the safe level of ammonia in *P. vannamei* culture was of 2.6 mg/lit. In the present investigation the total ammonia concentrations were within the normal limits (0.09 to 0.26 ppm and correlated with similar studies of Boyd and Zimmerman, (2000). According to Gonzalez-Felix *et al.,* (2007) in culture ponds of *P. vannamei* the peak concentration of ammonia was 5.8 mg/lit when shrimp fed with 40% protein feed. Similarly 4 mg/lit was observed when shrimp fed with 35% protein feed, which resulted to survival rates of 14.9% and 24.6% respectively.

Bhatnagar *et al.,* (2004) recommended that the desirable ammonia concentration for shrimp culture was considered as 0.01-0.05 ppm. In polyculture systems of *P. vannamei* and milkfish *Chanos chanos* the desirable ammonia concentration of 0.013-0.14 was observed by Jaspe *et al.,* (2011). In the present study a marginal increase in ammonia concentrations were observed throughout the culture period as ranged from 0.09-0.26 ppm. Similar results of 0.01-0.14 mg/lit was observed by Sayeed *et al.,* (2009) for both control and experimental ponds respectively. In *P. vannamei* shrimp culture ponds the ammonia-N levels of 0.13 mg/lit was considered as safe level as reported by Karuppasamy *et al.,* (2013).

Total alkalinity is the measure of total concentration of titrible bases. Alkalinity of water is caused by the presence of bicarbonates, carbonates, hydroxides (HCO_3^-, HCO_3^-, OH^-). Alkalinity in the pond should be maintained at greater than 100 ppm for good production. Sea water has an average alkalinity of 116 ppm as $CaCO_3$. Maintaining of a total alkalinity of 100-200 ppm favors to less fluctuations in pH and good productivity as reported by Ponnuchamy (1997). Similar studies also conducted by Matias *et al.,* (2002) and Wang *et al.,* (2005). Total hardness of the pond water mainly depends upon calcium and magnesium ions.

Vibrio species are most commonly existing pathogens, leading to severe disease conditions, which affect the reduction in growth process and sporadic mortalities in penaeid shrimp species as stated by (Lavilla-Pitago *et al.,* 1998). Otta *et al.,* (1999) stated that, *Vibrio harveyi* is mainly responsible for luminous disease. This species is routinely found in coastal, marine, and estuarine waters, in close association with the surface of exoskeletal structures and in gut of marine and estuarine organisms as well as shrimp culture pond water and sediments (Otta *et al.,* 1999). *V. harveyi* is also responsible for *Vibriosis* in *Penaeus monodon*, *Penaeus japanicus* and *Pinctada maxima* (Lavilla-Pitago *et al.,* 1990). In Indian aquaculture practices numerous studies were conducted to evaluate the efficiency of antagonistic properties of commercially available probiotics they are Top-25, Epicin, MIC-sanolife, Hi-5, Superbiotic, Korag bios, Inve-Pro-W, Biomin, and Bactocell-100. The products mainly consist of *Lactobacillus* spp, *Bacillus* spp, *Pseudonomonas* spp, *Pediococcus* spp as microbial ingredients. In the present study feed probiotic Pro-2 was applied along with the immunostimulant 1, 3 β-Glucan, a commercial brand β-ADVANTAGE for both summer and winter crops. The feed probiotic applied at the rate of 5g/kg and 10g/kg with 5g/kg immunostimulant in the experimental ponds at two different study areas. The application of feed probiotic and immunostimulant was followed every day for both the seasons i.e. summer and winter during study period i.e. 2018 to 2019. It is evident from the present findings that in the experimental ponds the incidence of Vibrio loads were reduced and this might be due the enhanced the activity of immune parameters such as total haemocytic as well phagocytic activity of the shrimps.

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