International Journal of Current Science Research
www.drbgrpublications.in

Volume: 3; Issue: 2; February-2017; pp 1069-1086. ISSN: 2454-5422

Effect of BLOOMAX™ on plankton enhancement in low saline semi-intensive pond culture system of the white leg shrimp, *Litopenaeus vannamei* (Boone, 1931)

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Abstract

Optimal and stable bloom development and water quality maintenance in the initial stage of low saline *Litopenaeus vannamei* semi intensive shrimp culture has always been a challenging issue. The research work involves the documentation on field performance of BLOOMAX™, a commercial probiotic in developing optimal phytoplankton bloom, maintaining remarkable water quality, nutrient status, phytoplankton abundance and diversity. Nutrient composition and physico-chemical parameters of water were monitored in test and control ponds and were found to be optimal for the production of shrimps in the treated ponds. However, bloom crash was observed in the control ponds. Phytoplankton diversity and abundance exhibited fourteen species of phytoplankton in BLOOMAX™ treated ponds and twelve species in control ponds. Test ponds demonstrated density of 256-357x10^4 cells ml^{-1} and control ponds with 214-279x10^4 cells ml^{-1} with frequent crash which may be due to improper stoichiometric balance and other parameters.

Keywords: BLOOMAX™, Probiotics, Phytoplankton, Bloom development, and Physico-chemical parameters.

Introduction

Aquaculture of *Litopenaeus vannamei* species in India is growing extensively. Water quality highly influences the plankton productivity as well as the growth rate and development of the (cultured) animal (Jhingran 1991). In order to accomplish successful and sustainable aquaculture, the qualitative and quantitative abundance of plankton are of scientific

importance. The phytoplankton population represents the biological wealth of a water body (Prasad and Singh, 2003). The physical and chemical parameters of the environment that house the planktons will determine the species that can grow and survive well. Every plankton species requires specific nutrients and physical conditions to enable them to grow optimally. Planktons were found growing naturally and their diversity and abundance often vary, depending on several environmental factors such as light, temperature, salinity, pH and nutrient availability (Araujo and Garcia, 2005; Alonso-Rodríguez and Paéz-Osuna, 2003). However, the plankton diversity varies from pond to pond even within similar ecological conditions (Boyd, 1982).

Abundance and diversity of specific phytoplankton in the ponds serves as a unique indicator of aquatic salinity. Reports reveal that, cell volume and diversity of phytoplankton varies with the salinity, thus indicating the regulatory role of salinity on the free floating producer community in the aquatic ecosystem (Abhijit *et al.,*2014). Salinity has direct impact on cell morphogenesis and cell elongation (Pickett-Heaps *et al.,*1990; Harold, 2002). Hildebrandt *et al.,* 2006 observed that the height of the centric diatom is reduced at increased salinity. Many researchers have published reports on various impact of salinity on phytoplankton cell volume. In regards to shrimp culture, there is always an increasing complaint from the aquaculture farmers that, the bloom development is delayed, and indecorous in low saline waters (<20 ppt). This leads to reduction in survival of the shrimp larvae at the initial stage of the culture, consequently leading to lesser production and economic loss. The commercial probiotic product, BLOOMAX™ (TIL Biosciences, Animal Health Division of Tablets (India) Limited, Chennai, India), which is a combination of four probiotic strains was used in low saline aquaculture shrimp ponds for stable and optimal bloom development and the impact of the probiotic product was monitored *in situ.*

Hence, this research reports on the effect of BLOOMAX™ on various physic-chemical parameters, phytoplankton population and diversity, survival throughout the crop and Feed Conversion Ratio (FCR) at low saline *L. vannamei* shrimp ponds.

Materials and methods

Study site

Six shrimp ponds of equal size (0.5 ha each) located in Kanchivayil, Ponneri (13.39˚ N, 80.22˚ E), Tamilnadu (Fig.1) were selected for the study during the period of March to June.

Product composition

BLOOMAX™ is a commercial probiotic product used exclusively for bloom development and water quality maintenance in shrimp aquaculture ponds. The commercial probiotic product was in the form of dry powder aseptically packed in airtight cover and presented in contamination free air tight plastic containers. It contained probiotic bacterial population of *Bacillus subtilis* ABPL 135, *Bacillus* species ABPL 026, *Bacillus* species ABPL with density of 2 x 10^{10} of each of the above strain and *Pseudomonas aeruginosa* ABPL 150 with cell density of 1 x 10^{10}.

Experimental design

Proper pond preparation was carried out in all the selected ponds. Bore water with salinity ranging from 13 to 18 ppt was pumped onto the shrimp ponds. Three test ponds were treated with BLOOMAX™ and three ponds were considered as control. 1.5 Kg of BLOOMAX™ for each 0.5 hectare pond was mixed thoroughly with same pond water and was broadcasted across the pond between 9 to 11 am on sunny days. Physico-chemical parameters of the water and plankton analysis were carried out once in week and was analysed. Survival rate and FCR were calculated at the end of the culture and economic benefit achieved by the farmer using BLOOMAX™ were analysed.

Sampling analysis

Physico-chemical analysis

The physicochemical parameters of water were analysed in the control and test ponds pre and post usage of BLOOMAX™ once in a week at three different points/spots. Water samples were collected between 07.00 and 08.00 hrs for *in situ* examination and laboratory analysis. Samples were shuttled in 250 ml polyethylene bottles to laboratories. The collected samples were transported in ice-container to the laboratory and were analysed immediately for alkalinity, hardness and nitrate. Water samples were filtered using 0.45 µm membrane filter prior to analyses in order to remove any suspended particles and materials. The aquatic salinity in the ponds were recorded *in situ* by means of a portable hand-held optical refractometer (Atago, Japan) and cross-checked in laboratory using Mohr-Knudsen method as salinity is a key factor in this research work. pH was measured using electronic pH pen (Erma, Japan), and temperature was measured using standard Celsius Thermometer. The dissolved oxygen was estimated by modified Winkler's method as described by Strickland and Parsons (Strickland and Parsons, 1972). The total hardness of the water was estimated by complexometric titration using EDTA (Vogel 1978) and alkalinity was measured as per

APHA (APHA/AWWA/WPCF 1998). Transparency was measured based on the penetration of light using a Secchi disc.

Plankton analysis

Plankton was collected using standard plankton net of 1 m length, 25 cm mouth diameter, and mesh size of 20 µm. The plankton net was towed horizontally and vertically to sample the plankton. For horizontal towing, plankton sample was collected by lowering the net horizontally into the water then pulled until the net extended and began to tow. The net was scooped through the shrimp pond water while walking slowly along the pond's bank. For vertical towing, the net was lowered into the water to approximately 1 m depth and was kept vertical and off the bottom. The net was pulled straight up through the water column. The samples were then rinsed into collection vessels.

The samples were preserved in 4% neutral formaldehyde (final concentration) in polyethylene bottles for plankton abundance and identification. Samples were observed and morphologically identified with ZEISS research microscope coupled with an image analysing system using keys and illustrations by Prescott (Prescott, 1962), Patrick and Reimer (Patrick and Reimer, 1966), Round (Round *et al.,*1990) , Tomas (Tomas, 1997), and other taxonomic literature.

Total number of phytoplankton (standing drop) present in a litre of water sample was calculated using the formula: N = nv/V

Where,

N = Total number of phytoplankton cells per litre of water filtered

n = average number of plankton cells in 1mL of plankton sample

v = volume of plankton concentrate (mL)

V = volume of total water filtered (l)

The units of standing drop are N/l or $NX10^3$ /m^3

Phytoplankton abundance and diversity in the shrimp ponds were thus determined.

Statistical analysis

The data were presented as mean ± SE. Student's t-test was applied to determine the significant difference ($P<0.001$) between the control and test ponds. All statistical calculations were performed using SPSS for Windows version 11.5 (SPSS Inc, Chicago, IL,

USA). All column charts were plotted using Origin 6.1 (Origin Lab Corporation, Massachussets, USA).

Results

It is vital to provide shrimp with a healthy environment and probiotics has a great deal of potential (Gomez-Gil *et al.,*2000) . Bloomax™ was used in test ponds for optimal and stable bloom development and control were left untreated. Figure 2 shows the weekly mean of water temperature on the shrimp ponds during 119 days of shrimp cultivation period. The results of the study showed that the water temperature at the surface fluctuated between 25 ˚C to 34˚C. The pH of the water ranged from 7.9 to 8.4 during the culture period (Fig. 3). Salinity of the water ranged from 13 to 21 ppt over the cultivation period (Fig. 4). Salinity was observed to increase with increase in temperature during the sunny days, as increased temperature may increase the condensation of water, leading to agglomeration of salt. Figure 5 shows the transparency in control and test ponds. Transparency was reported to be high on both test and control ponds at the initial days of the culture. Test ponds administered with Bloomax™ soon developed optimal and stable thus favouring the growth of the fry leading to improved survival rate. In addition, no bloom crash was reported in the test treated ponds. In contrast, control pods encountered bloom crash twice during the culture period, once in 77 days and another in 112 days. Also, the transparency level varied across week's symbolizing the unstable bloom development. Dissolved oxygen level (Fig. 6) ranged from 2.9 to 3.9 ppm in control ponds and 3.4 to 4.8 ppm in test ponds treated with Bloomax™. The DO level was observed to drop upon bloom crash.

Nitrate level in the control pond ranged from 0.12 to 0.17 ppm and 0.12 to 0.25 ppm in Bloomax™ treated test ponds (Fig. 7). Nitrite level reached a maximum of 0.18 mg/l in the control ponds with maximum report of 0.04 mg/l in the test ponds (Fig. 8). Total hardness ranged from 1150 to 1450 mg/l in control ponds and was maintained within 1200 mg/l in the test ponds (Fig. 9). Total alkalinity was reported to be on the range of 130 to 170 ppm in both test and control ponds (Fig. 10). Phytoplankton diversity and abundance exhibited fourteen species of phytoplankton in BLOOMAX™ treated ponds and twelve species in control ponds. Test ponds demonstrated density of 256-357x10^4 cells/ml and control ponds with 214-279x10^4 cells/ml (Fig. 11). Percentage survival of shrimps was calculated at the end of the culture and was 86% in test ponds and 71% in the control ponds (Fig. 12). FCR was

calculated post-harvest and was found to be 1.2 in test ponds and 1.8 in control ponds (Fig. 13).

Discussion

Pond management is essential for a productive shrimp farm. In this sense, adequate nutrient levels will allow the right biomass and structure of phytoplankton. An excessive supply of nutrients, as in the case of coastal waters, will result in an over-enrichment that eventually will promote algal blooms, primary productivity and growth of some macrophytes. Additionally, nutrients in excess will alter phytoplankton composition with a resulting change of dominant species; such changes imply the substitution of larger species for smaller ones and the replacement of diatoms by dinoflagellates (Alonso-Rodríguez and Paéz-Osuna, 2003). However, the scenario differs for shrimp culture with low saline water as the water lacks minerals in it. Hence, there is raising issue on shrimp ponds regarding optimal and stable bloom development pertaining to the biological wealth of the culture medium leading to good growth and survival of the shrimp post larvae stocked onto it.

In an aquatic environment such as in shrimp ponds, pH value and carbondioxide concentration are dependent on the photosynthetic and respiration processes. CO_2 released by shrimp during respiration will be utilized by phytoplankton for their photosynthetic process which produces oxygen as a by-product. The removal of CO_2 through photosynthesis process reduces carbonic acid concentration which will result in the rise of pH in the pond. 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 (Delgado *et al.,* 2003).

The lower salinity throughout this study was correlated to the evaporation and rainfall. This result goes hand in hand with the work reported by (Guerrero-Galván *et al.,*1999) and (Mmochi *et al.,* 2002) which found that salinity values were influenced by the evaporation during the hot season and by rainfall in the rainy season. Everett (Everett *et al.,* 2007) also reported that their study showed that rainfall dilutes the water column and lowers the salinity.

Nutrients such as nitrogen and phosphorus in the shrimp ponds were originated mainly from prepared feed (Cremen *et al.,* 2007), fertiliser used, water pumped into the pond, juveniles stocks, rainfall (Xia *et al.,* 2004) and shrimp excretion (Cremen *et al.,* 2007). Nitrification is a transformation process of ammonia (oxidation by bacteria) to nitrite and then to nitrate.

Nitrite level was observed to be high on control ponds, whereas, the level was in control throughout the cultivation period in the test ponds. On the other side, nitrate level was high on test ponds which are due to the beneficial bacterial population enhancement with the application of Bloomax. The maximum tolerable concentration of ammonium for shrimp is 0.1 mg/l (Anon, 2003). The increase in ammonium concentrations at certain times over the cultivation period could be due to a few reasons; first, when shrimp size increases, the feeding rates will increase accordingly and resulted in increase in ammonium waste. The second reason is the decomposition of organic materials by microbes and fertilisation practices (Guerrero-Galván *et al.,*1999).

Transparency was high in the control ponds during the initial period and was in control over the culture period. However, bloom crash was observed twice during the culture duration, which consequently lead to higher transparency and less DO in the shrimp pond. As a consequence of improper bloom and less available dissolved oxygen, mortality and stunt growth was reported in the control ponds. Algal blooms can produce hypoxia or anoxia with resulting shrimp (Alonso-Rodríguez and Paéz-Osuna, 2003). Nitrates to ammonia reduction process were induced by decreased level of available oxygen level, which is thus toxic to the shrimps. Decreased oxygen level also interferes in the metabolic performances of the shrimp and can reduce growth and moulting, consequently leading to increased mortality (Edward Gnana Jothi *et al.,* 2016). These events are highly expensive for shrimp farming. Anoxic episodes can change community structure inside and outside the shrimp ponds because sessile and non-sessile communities are affected. As a result of the changes in biota, nutrient release from sediments is enhanced (Alonso-Rodríguez and Paéz-Osuna, 2003). In contrast, the test ponds treated with Bloomax™ exhibited stable and optimal bloom over the culture period leading to good growth and survival of the shrimps. Also, due to the presence of optimal bloom, required dissolved oxygen was present in the test ponds, which nullified the usage of oxygen releasing chemicals in the shrimp ponds, thus reducing the production cost for the farmers.

This study showed that Bacillariophyta, Cyanophyta and Chlorophyta constituted the major composition of the microalgal population in the shrimp pond. Initially, the ponds were dominated by Cyanophyta (*Anabaena* spp., *Oscillatoria* spp.), followed by Chlorophyta (*Chlorella* sp.). When shrimps were introduced into the pond, diatoms started to occur and the diatoms became the dominant species during shrimp culture period. These results were in agreement with Asma Liyanna Shaari *et al.,* 2011 findings. According to Smith 1993,

accumulated sediments in shrimp ponds consisted mainly of silica and Smith 1994 stressed that amorphous silica is an important component of pond sediment and that the activity of diatoms is fundamental to the silica cycle in shrimp ponds. Hence, an increase in silica content in shrimp pond was suspected in promoting diatom growth which in turn will depress the growth of Cyanophyta especially *Oscillatoria spp* (Yusoff *et al.,* 2002).

The high nitrate concentrations may be responsible for the dominance of diatoms. Vanni and Findlay 1990 and Cremen *et al.,*2007 agreed that high phosphate concentrations usually encouraged the growth of Cyanophyta, whereas high nitrate concentration encourages diatoms growth. Cremen *et al.,* 2007 revealed that high ammonium and nitrite levels result in high N:P ratio that will promote diatom blooms. In addition, Smith 1983 reported that some shrimp ponds with high nitrogen loading rates could cause the absence or rare occurrence of Cyanophyta. Pond water with high microalgae density turns into green colour and this is called green water. Green water comprises a high biomass of microalgae mainly blue green and green algae which increase chlorophyll *a* concentrations in the shrimp pond. After the introduction of shrimps, light penetration into the shrimp pond decreased due to heavy particulate matter from shrimp excretion and uneaten feed (Asma Liyana Shaari *et al*., 2011).

Conclusion

The study revealed the fact that the commercial probiotic product, Bloomax™ from the house of TIL Biosciences [Animal Health Division of Tablets (India) Limited, Chennai, India] exhibited a promising effect on the water quality, phytoplankton abundance and diversity with the regulation of optimal and stable bloom in *L. vannamei* shrimp culture carried out in low saline water. Regular usage of the product at regular intervals has also minimized the production cost of the shrimps for the farmers with good growth and survival rate, thereby decreased FCR. Hence, it is confirmed that, this product must be the product of choice for shrimp farmers.

Acknowledgement

The authors are grateful to the management of TIL Biosciences, Animal health division of Tablets (India) Limited, Chennai, Tamilnadu, India for the financial support and encouragement and Mr. Ganesan, *L. vannmei* shrimp farmer, Kanchivayil, Ponneri for granting permission to carry out the study.

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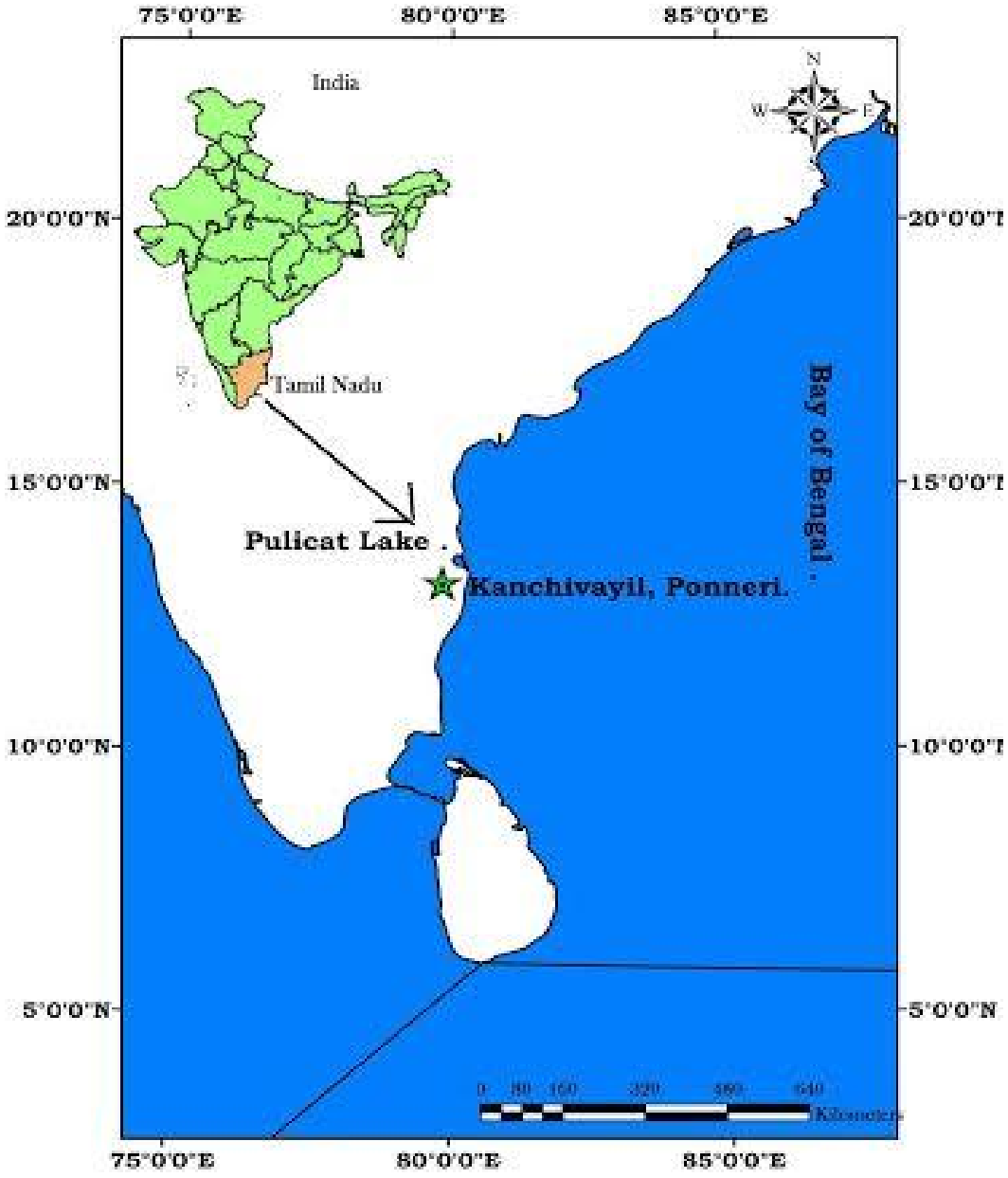

Fig. 1: Map showing the study area Kanchivayil, Ponneri, Tamilnadu, India

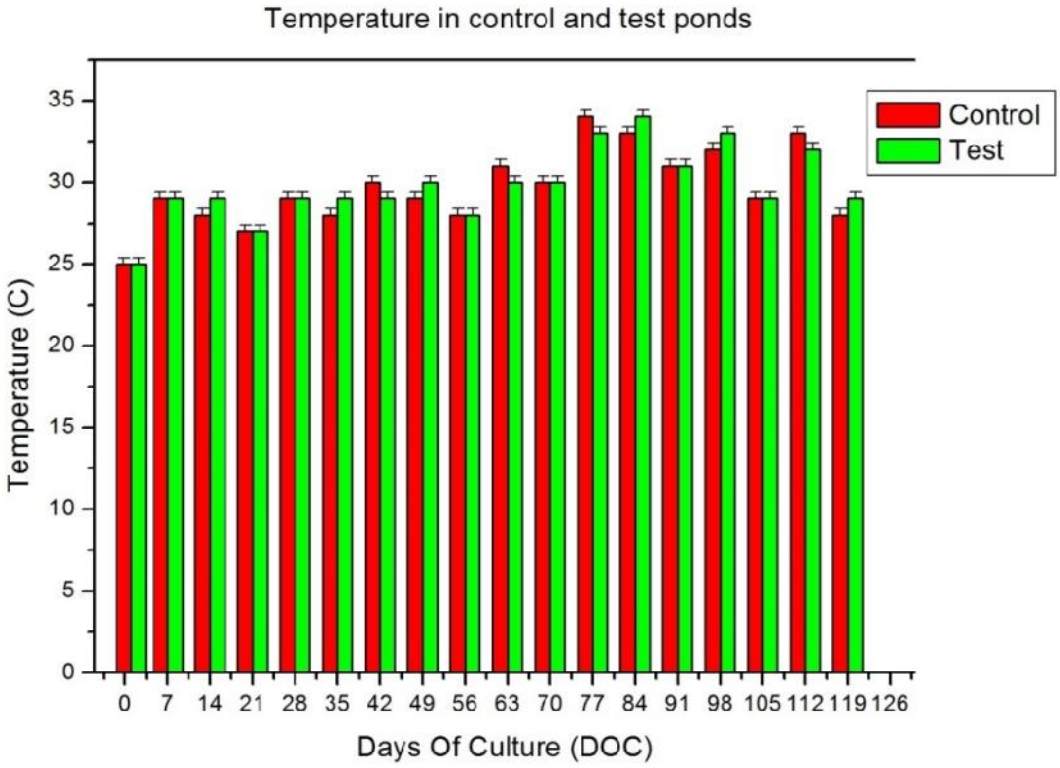

Fig. 2: Temperature in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

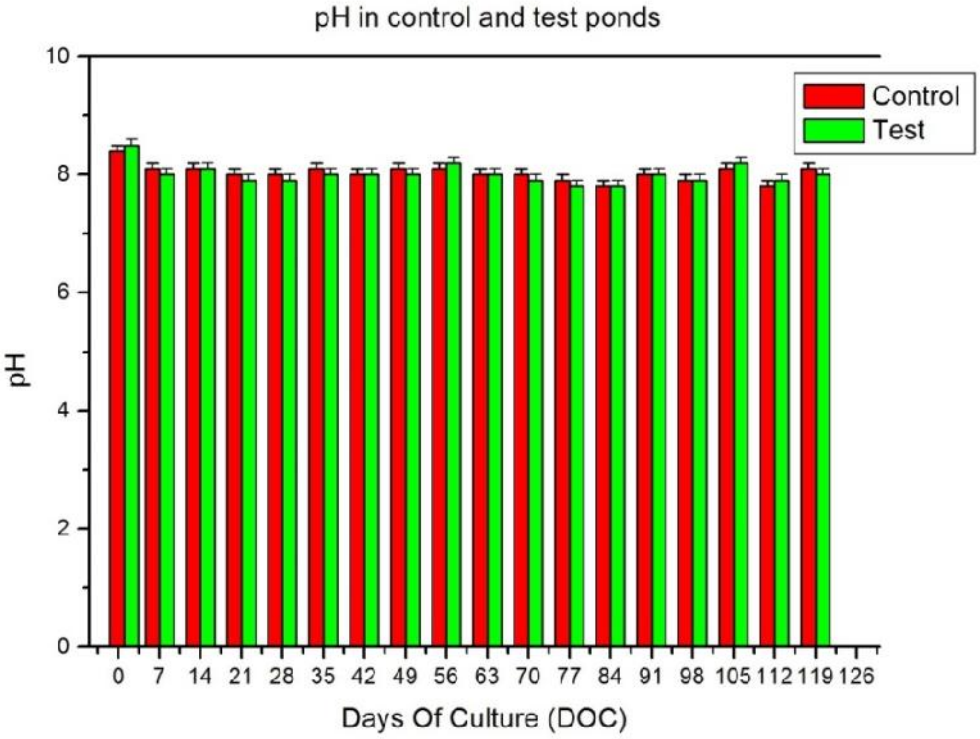

Fig. 3: pH in control and test ponds for a period of 119 days at regular intervals of 7 days

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

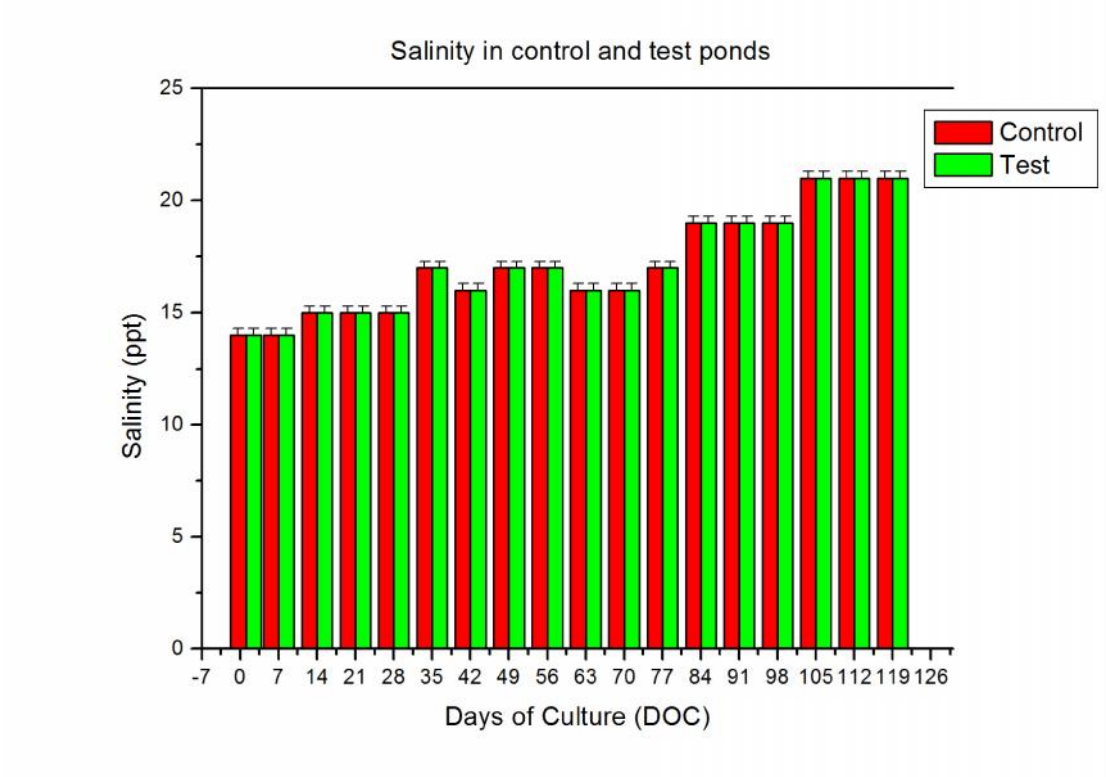

Fig. 4: Salinity in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

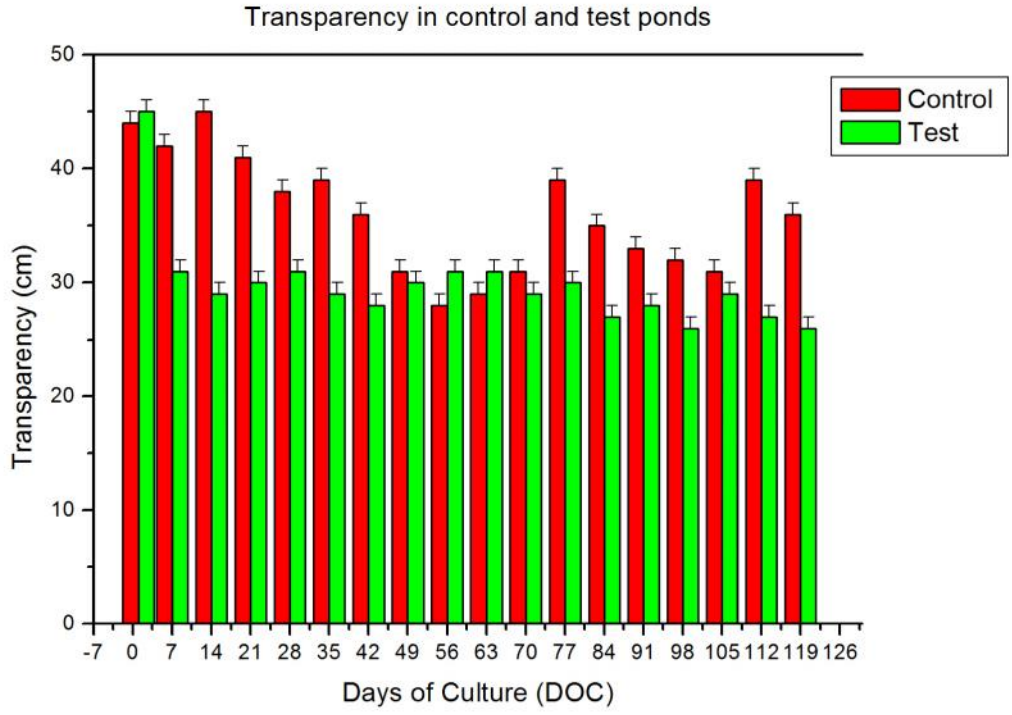

Fig. 5: Transparency in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

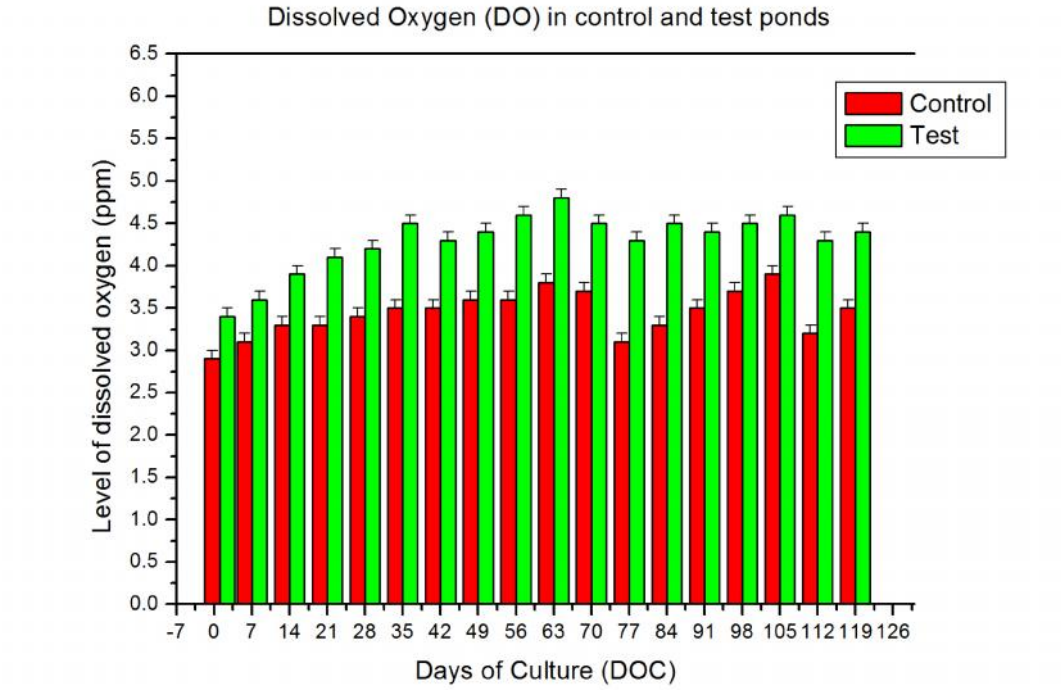

Fig. 6: Dissolved Oxygen (DO) level in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

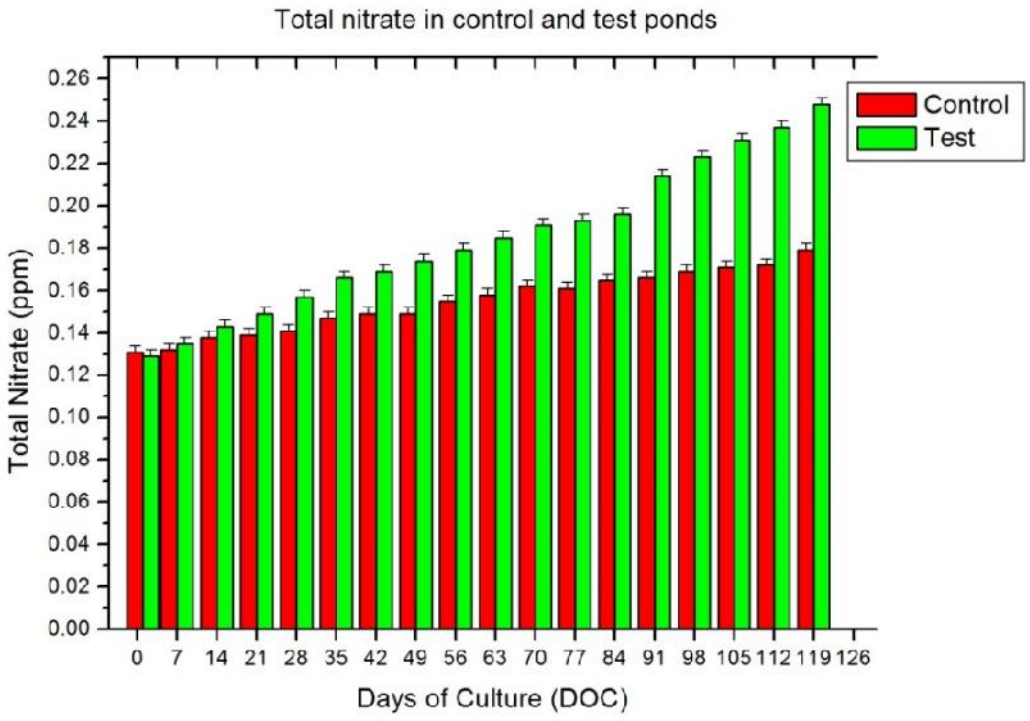

Fig. 7: Total nitrate level in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

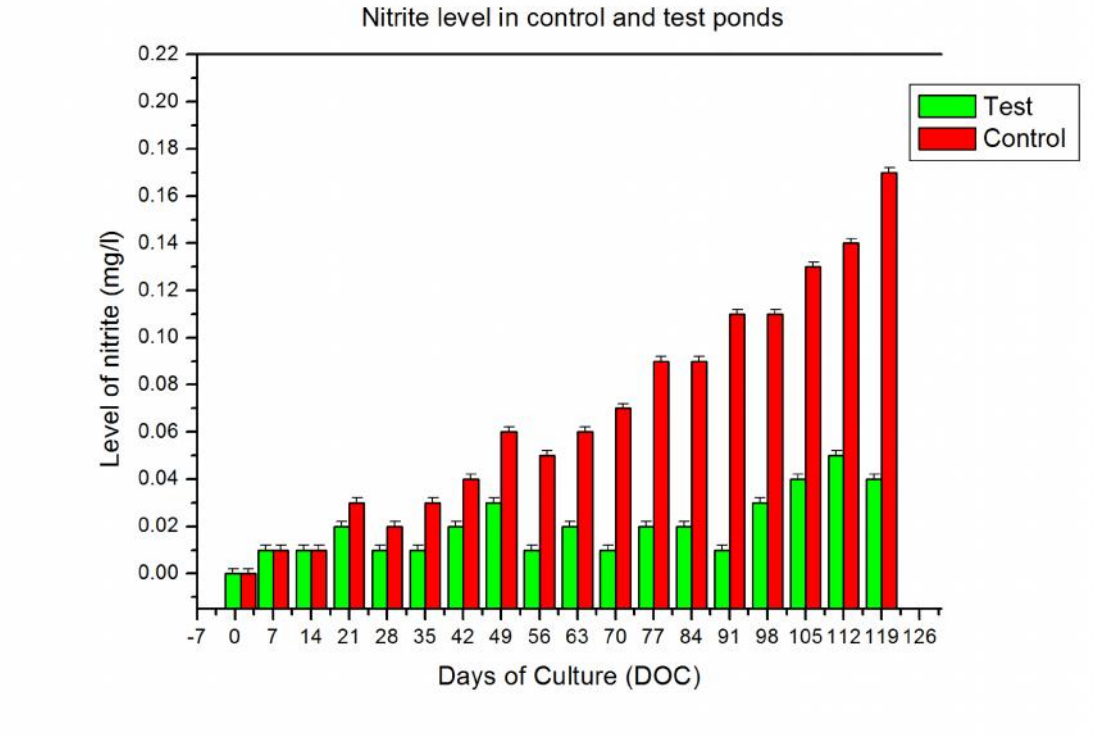

Fig. 8: Level of nitrite in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

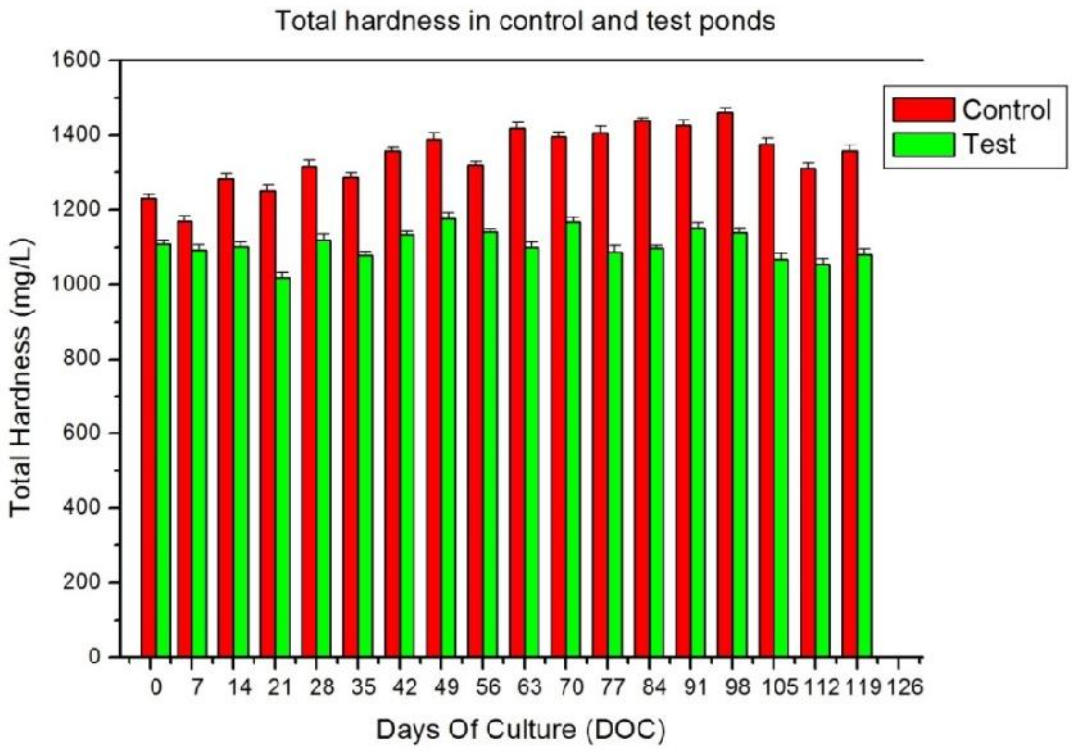

Fig. 9: Total hardness in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

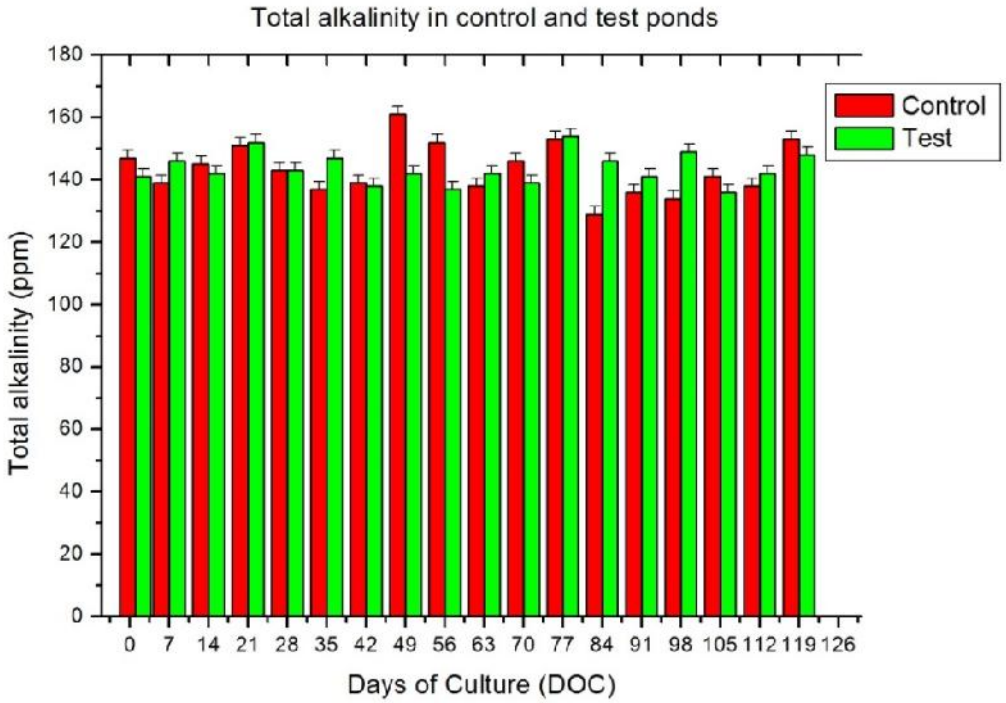

Fig. 10: Total alkalinity in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

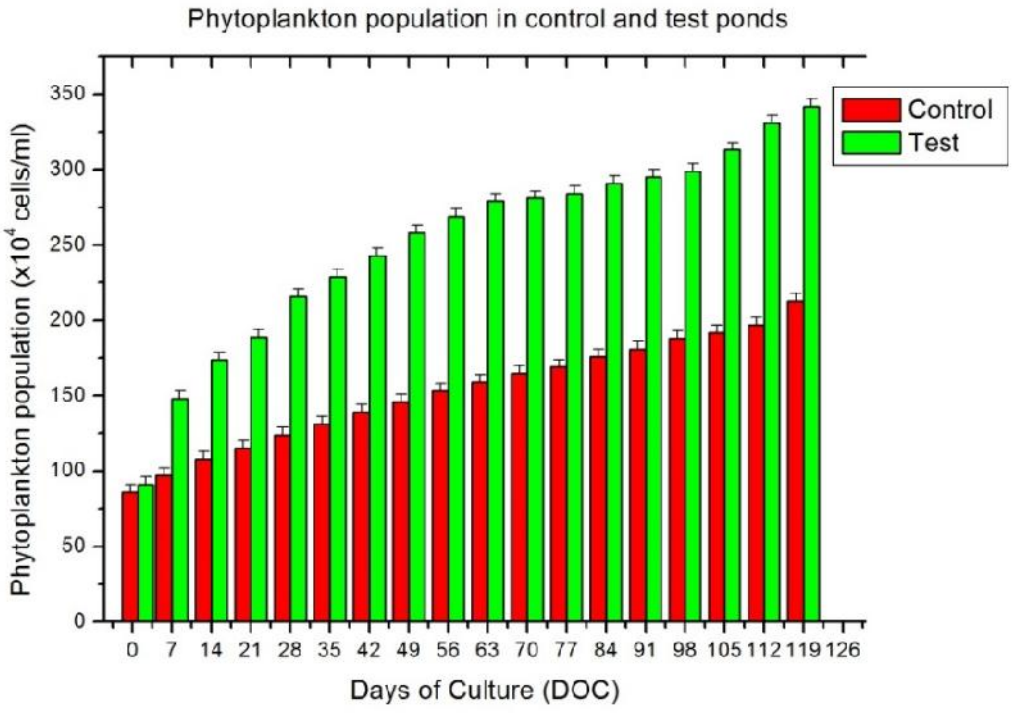

Fig. 11: Phytoplankton abundance in control and test ponds for a period of 119 days at regular intervals of 7 days
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

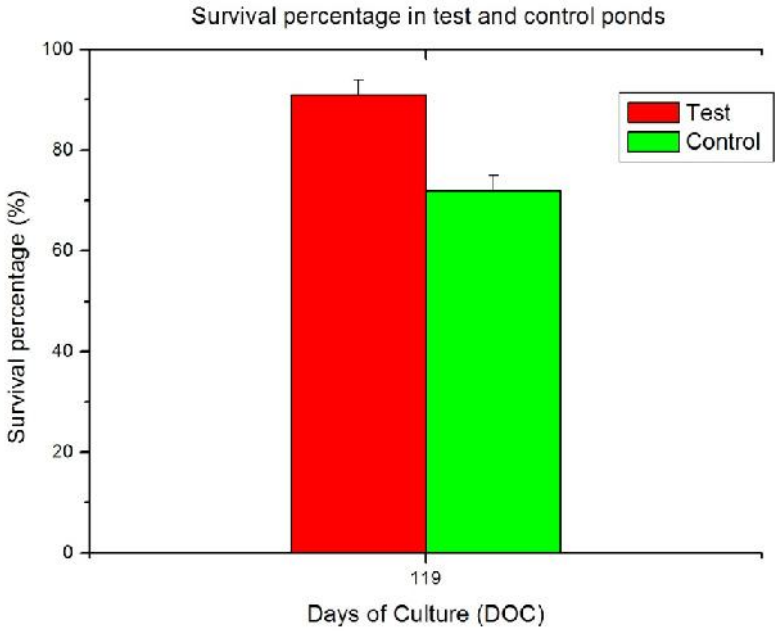

Fig. 12: Percentage survival of shrimps in control and test ponds
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

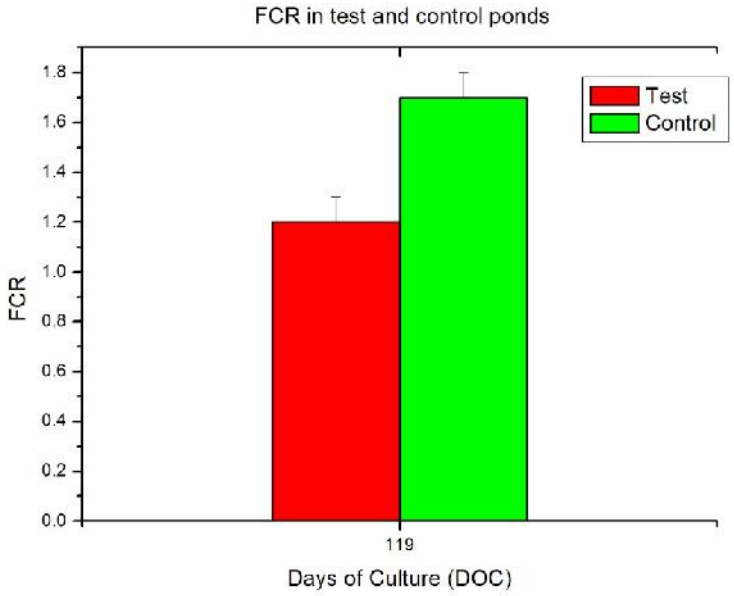

Fig. 13: FCR in control and test ponds
*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)