Sub-Project ID: 031

## Program Based Research Grant (PBRG)

# Sub-project Completion Report on

# Development of *in-situ* Breeding Technology of Prawn (*Macrobrachium rosenbergii*) and Adoption of Sustainable Eco-Friendly Culture of Prawn and Shrimp (*Penaeus monodon*)

**Sub-project Duration**
**31 May 2018 to 16 January 2022**

**Coordinating Organization**

**Bangladesh Fisheries Research Institute**
**Mymensingh-2201**

**Project Implementation Unit (PIU)**
**National Agricultural Technology Program-Phase II Project**
**Bangladesh Agricultural Research Council**
**Farmgate, Dhaka-1215**

**December 2021**

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# Development of *in-situ* Breeding Technology of Prawn (*Macrobrachium rosenbergii*) and Adoption of Sustainable Eco-Friendly Culture of Prawn and Shrimp (*Penaeus monodon*)

## Implementing Organization


**Shrimp Research Station**
Bangladesh Fisheries Research Institute
Bagerhat-9300

**Fisheries and Marine Resource Technology Discipline**
Khulna University
Khulna-9208



**Project Implementation Unit (PIU)**
**National Agricultural Technology Program-Phase II Project**
**Bangladesh Agricultural Research Council**
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**December 2021**

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**Edited by**
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# Abbreviation and Acronyms

| | |
|---|---|
| % N | Percentage of Nitrogen |
| AHNPD | Acute Hepatopancreatic Necrosis Disease |
| BARC | Bangladesh Agricultural Research Council |
| BFRI | Bangladesh Fisheries Research Institute |
| BOD | Biochemical Oxygen Demand |
| BP | Baculovirus Penaei |
| CaO | Calcium Oxide |
| CBSF | Cluster Based Shrimp Farming |
| CFU | Colony-forming Unit |
| Co-PI | Co-Principal Investigator |
| DHC | Differential Hemocyte Count |
| DNA | Deoxyribonucleic Acid |
| DO | Dissolved Oxygen |
| EC | Electric Conductivity |
| EDTA | Ethylenediamine Tetra Acetic Acid |
| EHP | Enterocitozoon Hepatopenaei |
| ELISA | Enzyme-linked Immunosorbent Assay |
| EMS | Early Mortality Syndrome |
| FAO | Food and Agricultural Organization |
| FMRT | Fisheries and Marine Resource Technology |
| g | Gram |
| GAV | Gill-associated Virus |
| GoB | Government of Bangladesh |
| ha | Hectare |
| HCl | Hydrochloric Acid |
| IFAD | International Fund for Agricultural Development |
| IHHN | Infectious Hypodermal & Hematopoietic Necrosis |
| IMNV | Infectious Myonecrosis Virus |
| K | Potassium |
| Kg | Kilogram |
| $KMnO_4$ | Pottasium Per Manganet |
| KU | Khulna University |
| L-DOPA | L-dihydroxyphenylalanine |
| LoA | Letter of Agreement |
| LRT | Larvae Rearing Tanks |
| $m^2$ | Square Meter |
| MBV | Monodon Baculovirus |
| MrNV | Macrobrachium Noda Virus |
| MRS | De Man, Rogosa and Sharpe agar |
| MrTV | MacrobrachiumTaihu Virus |
| N | Nitrogen |
| NARI | National Agricultural Research Institute |
| NATP-II | National Agricultural Technology Program: Phase II |
| $NH_3$ | Ammonia |
| $NH_3$-N | Ammonia-nitrogen |
| NHP | Necrotising Hepatopancreatics |
| $NO_2^-$-N | Nitrite-nitrogen |
| $NO_3$-N | Nitrate-nitrogen |
| $^oC$ | Degree Celsius |
| OIE | Office of International Epizooties |
| OM | Organic Matter |
| P | Phosphate |
| PBRG | Program Based Research Grant |
| PBS | Phosphate Buffer Saline |
| PCR | Polymerase Chain Reaction |
| PCR | Project Completion Report |
| PI | Principal Investigator |
| PIU | Project Implementation Unit |
| PL | Post Larvae |
| Pro-PO | Pro-Phenoloxidase |
| RAPD | Randomly Amplified Polymorphic DNA |
| RNA | Ribonucleic Acid |
| S | Sulfur |
| SDS | Sodium Dodecyl Sulfate |
| SOD | Superoxide Dismutase |
| SRDI | Soil Resource Development Institute |
| SRS | Shrimp Research Station |
| THC | Total Haemocyte Count |
| TK | Taka |
| TSP | Triple Super Phosphate |
| TSV | Taura syndrome virus |
| WB | World Bank |
| WSSV | White Spot Syndrome Virus |
| XSV | Extra Small Virus |

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# Executive Summary

In Bangladesh, Shrimp sector, playing a significant role in foreign exchange earnings, employment generation and poverty reduction. Since the last decades the sector has been suffering from various issues and problems related to culture and production. In shrimp (*P. monodon*) culture, various diseases, white spot disease (WSD) in particular, has become a serious constraint. In the recent years, infestation of various diseases including WSD and newly reported AHPND (Acute Hepatopancreatic Necrosis Disease) affected shrimp production immensely. These are hindering our export earnings to be flourished from this sector. The giant freshwater prawn (*Macrobrachium rosenbergii*), is playing a significant role in the economy, employment opportunity and poverty elevation in Bangladesh.

Considering the situation, the first experiment has been designed to evaluate the impact of cluster farming to cope with disease outbreak. Under this experiment a sum of around 3.25 acre gher from five different owners has been taken through lease process near Chitoli, Boitpur, which merged to form a cluster with well-designed water inlet and outlet facility. Growth, water quality parameters and disease screening information of the culture period was found satisfactory. No disease outbreak recorded throughout the culture period, which was one of the great achievements of this study. Besides, from the traditional gross production of shrimp is around 250-300 kg/ha, with this management intervention, production maximized more than two times (735.6 kg/ha at the stocking density of 3 PL/$m^2$ and 854.4 kg/ha at the stocking density of 6 PL/$m^2$) compared to the traditional farming. Once getting significant production of 854.4 kg/ha at the stocking density of 6 PL/$m^2$ with no aeration, in the second year, emphasize given on to optimize maximum stocking density to increase per ha production. Therefore, instead 3 and 6 PL/$m^2$, treatment designed to 6 and 9 PL/$m^2$ to take into consideration in this higher stocking can be sustained without aeration. Due to corona pandemic and lockdown situation, stocking of PL became delay. With no aeration and higher stocking density of 9 PL/$m^2$ found not suitable Due to lower salinity overall survival found 15% less than the first-year survival. For the same reason, production dropped to 694 kg/ha with the stocking density of 6 PL/$m^2$. Even though the average production dropped to 694 kg/ha in the second year compared to 854.4 kg/ha with the stocking density of 6 PL/$m^2$, is still more than double than the average traditional production of shrimp i.e., 300kg/ha. In the second study effort given to develop a detailed checklist of the available shrimp pathogen in the farming system of Bangladesh. So far, more than 20 viruses have been reported to infect marine shrimp worldwide. Among them seven pathogens of marine shrimp and prawn are currently listed by the World Organization for Animal Health (OIE) as causing modifiable aquatic animal diseases are also considered to be potential threats in recent shrimp/prawn aquaculture in Bangladesh. For the identification of OIE Listed pathogen for Shrimp and Prawn, PCR protocol were optimized. Pathogen specific primers were synthesized from the gene bank. Then PCR protocol was optimized for the pathogens at the shrimp health management laboratory of Shrimp Research Station, Bagerhat. Samples were collected from Khulna Satkhira and Bagerhat region. All the samples tested with PCR for all the OIE listed pathogens and three pathogens viz., WSSV, EMS and EHP found positive. Among these pathogens EHP is the first reported pathogens in *Macrobrachium rosenbergii* but also it is the first report in the world and deserves serious attention to improve management practices to help secured production of shrimp and prawn in the future. It also explains sudden mortality issue of marketable size Prawn in the southern region of Bangladesh.

Shrimp being export commodity, use of medicines and antibiotics are strictly prohibited. Therefore, to cope with disease outbreak sustainable solutions is mandatory. Along with improve management facility, boost up shrimp immunity is considered to be more effective against therapeutics. Under this project, attenuated/ killed bacteria were considered as immunogenic agent to boost shrimp immunity and administrated with feed for three weeks. Immunity status was measure with total hemocyte count as in crustaceans, hemocytes act as the major defense mechanism. The feed mixed with attenuated bacteria gave almost double count of hemocytes (1200000 cell/ml) compared to the control group (600000

cell/ml). No doubt this is a tremendous increase to the count of hemocytes and further challenge experiments can give a clear idea for the effectiveness of immunity against disease outbreak and mass mortality. All male prawn pl has a great demand among the farmers because of fastest growth rate. It can be achieved by ablation of androgenic gland of known age (50-60 days old PL) prawn pl. for this prawn pl production activity was started in the prawn hatchery of the station. After getting required aged PL, several batches of PL (500 PLs) operated for micro surgery. Only 2% PL survived but unfortunately all found male when checked. The poor survival rate was due to the stress under the microscope and as male prawn is generally sturdy, so the survived prawn was all-natural male. In short, microsurgical ablation of androgenic gland is a complicated task and required subsequent trials. One-year trial is not sufficient for such kind of sophisticated research where timing and perfection is mandatory and it comes by practice. It would be better to have an extension of the project to provide another year of time to master the microsurgical approaches, if succeed, could be a great opportunity for the prawn industry of the country.

Baseline surveyed of this study found that in the southwest Bangladesh mostly traditional prawn-carp poly-culture and rice-prawn rotation were practiced where stocking density ranged 10000 to 30000 post larvae/ha and production ranged from 350-625 kg/ha (2020). This study was conducted with the aim of enhancing freshwater prawn through eco-friendly approach applying probiotics in prawn culture. In first year, considering availability and used (%) 6 commercial probiotics were selected for trial to justify their effect on prawn growth and production performance. Considering prawn production/ha the commercial probiotics are denoted as P1, P2, P3, P4, P5, P6 and production were found 857.70, 841.20, 808.56, 805.44, 796.63 and 789.84 kg/ha respectively. All the probiotic treated pond showed higher production than the without probiotic 661.64 kg/ha. In 2nd year research was done to intensify freshwater prawn culture increasing the stocking density in non-aerated and aerated culture systems applying probiotics. Three probiotics (P1, P2 and P3) were selected based on the production performance of first year experiment. The stocking density in non-aerated ponds was 2, 4 and 6 $m^2$, and in aerated ponds were 4, 8 and 12 $m^2$. In case of non-aerated system, production using probiotic-1 (P1) were 895.68, 1274.28 and 1292.71 kg/ha at the stoking of 2, 4 and 6 $m^2$ respectively. Stocking density 4/$m^2$ could be suggested for probiotic based non-aeration culture system where net benefit could be earned 4,00,818tk/ha. Similarly at aerated culture system the production were 1494.66, 1905.09 and 2061.70 kg/ha respectively at 4, 8 and 12$m^2$ respectively at same probiotics (P1). Stocking density 8/$m^2$ could be suggested for probiotic based with aeration culture system considering net benefit 5,88,804 tk/ha. All three probiotics treated prawn showed higher digestive enzyme protease, amylase and lipase than the without probiotic. The immune enzyme Pro-Phenoloxidase (Pro-PO) and Superoxide Dismutase (SOD) concentration were higher in probiotic treated prawn than without probiotic, which reflected the higher immune response. Disease challenge test was done applying pathogenic bacteria *Vibrio* spp. in both with and without probiotics and colony of harmful *Vibrio* spp. was found less in probiotics treated pond and prawn survival (%) was higher in probiotic applied pond. The application of carbon enriched ingredients (molasses + rice polish) showed higher growth and production (1,108.57 kg/ha) than the control (751.80 kg/ha). The net benefit 3,61,142 Taka/ha could be earn applying carbohydrate in prawn culture. Locally available carbohydrate enriched ingredients could increase beneficial bacteria in pond system. Therefore it could be used as substitute of commercial probiotics.

**Keywords:** *Macrobrachium rosenbergii*, Probiotics, Shrimp disease, Cluster-farming

# PBRG Sub-project Completion Report (PCR)

## A. Sub-project Description

1. **Title of the PBRG sub-project: Development of *in-situ* Breeding Technology of Prawn (*Macrobrachium rosenbergii*) and Adoption of Sustainable Eco-Friendly Culture of Prawn and Shrimp (*Penaeus monodon*)**

2. **Implementing organization(s):** Bangladesh Fisheries Research_Institute, Mymensingh-2201, Shrimp Research Station, Bangladesh Fisheries Research Institute, Bagerhat-9300, Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna

3. **Name and full address with phone, cell and E-mail of Coordinator, Associate Coordinator and PI/Co-PI(s):**


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## 4. Sub-project budget (Tk.):

**4.1.** Total Tk. 249,4500.00 ((Two crore forty nine lac four thousand five hundred )

| | | |
|---|---|---|
| **A. Coordination component** | : Tk. | 1153200.00 |
| **B. Component 1 (BARC)** | : Tk. | 1342000.00 |
| **C. Component 2 (BFRI)** | : Tk. | 14910000.00 |
| **D. Component 4 (KU)** | : Tk. | 7540000.00 |

**4.2. Latest Revised (if any):** Not applicable

## 5. Duration of the sub-project:

5.1 Start date (based on LoA signed): 31 May 2018
5.2 End date: 16 January 2022

## 6. Background of the sub-project

**A**. The fisheries sub-sector of Bangladesh as an important component of agricultural activity considers as the most potential source of economic and employment generation and a vital

source of animal protein provider, as well. The sector is highly diverse in resource and species type. In the recent years there has been a steady and rapid growth of aquaculture and fish food production, income generation and livelihood improvement of fishers, however, there still prevails/exists fish production gap in the country. And this gap has been widening every year because of higher population growth rate. The scientist community and the policy makers of the country indicate the weakness in research capacity of the institutes and research extension linkage in technology generation and transfer process is the two most vital and responsible causes for this. Thus, to address the situation through establishing strong research support and linkage, as NATP-II thoughts, all research and extension institutes need to make strong footing with team building holistic research culture to achieve desired output. With this consideration, as an effective approach, the program-based research grant of NATP-II is particularly aimed to support coordinated research program amongst NARI to jointly combating national agricultural problems and strengthening the research and research management capacities of the institutes. Therefore, under the principal objective of NATP-II, the fisheries division component shall have to play the role to ensure smooth and efficient implementation of sub-project activities to achieve the desired project output through coordination of activities and strong and effective monitoring of research progress under an additional increased research support against each institute.

**B.** In Bangladesh, Shrimp sector, playing a significant role in foreign exchange earnings, employment generation and poverty reduction. Since the last decades the sector has been suffering from various issues and problems related to culture and production. The two main production areas of shrimp are located in the Southwestern part composed of Khulna, Satkhira and Bagerhat districts; and the other one is located in the Southeastern part of the country composed of Chattogram and Cox's bazaar districts (Rahman, 1998).

Among shellfish species, freshwater prawns, particularly, the Giant Freshwater prawn *M. rosenbergii*, are one of the most economically important farmed species in the world and is commercially cultured in many tropical, sub-tropical countries like Bangladesh, India, Thailand, Vietnam, China, etc. It is the second largest foreign currency earning sector of Bangladesh. Aquaculture of the species has been established for more than 30 years and is expanding worldwide with approximately 23.5% increment in the world annual production between 1998 and 2010 (FAO, 2010).

Faced with increasing disease problems in penaeid shrimp culture, farmers turned to fresh-water prawn farming. Freshwater prawns were considered relatively less susceptible to diseases. However, with intensification of culture and increased world trade of the farmed species, emerging diseases are beginning to constitute an increasingly serious health problem in freshwater prawn culture.

Some work, however, in a scattered way have been carried out by different organizations to address the problem seems insufficient to be able to have an insight to the reasons. Freshwater prawns (*M. rosenbergii*) having more potentiality over giant tiger shrimp (*P. monodon*) with its flexibility to be cultured throughout the country. However, since 2011, unfortunately the prawn hatcheries in Bangladesh have been suffering from mass larval mortality and delayed molting issues causing loss of millions of Taka to the hatchery owner.

On the other hand, the culture technologies of this species are still varying. The differential growth between sexes is of great concern in *M. rosenbergii* culture where in males grow much faster than females (Holthuis, 1980). Culture of all-male prawn gave significantly higher yield with shorter culture period than the mixed-sexes and the all-female culture (Sagi et al. 1986; Cohen et al. 1988). Moreover the manual sexing was not justified by the relatively small increase of income. The androgenic gland of crustaceans is the only source of hormone that controls sex differentiation to maleness and the development of male characters. Therefore, the alternative biotechnological method has been explored for producing an all-male stock of freshwater prawn, among which the neo-female technology is promising (reviewed by Sagi and Aflalo, 2005). The ablation of the androgenic gland at early stage of development caused sex reversal to females (neo-female) (Nagamine et al. 1980) while the implantation of the androgenic gland in female *M. rosenbergii* resulted in sex reversal to males (neo-male) (Malecha et al.1992). Sagi and Cohen (1990) based on two crosses (a total of 567 offspring) showed that the mating of the neo-female with the normal male resulted in 99.1% and 100% male offspring. Therefore, the present study will conduct to explore the possibility of using the neo-female technology to produce all-male offspring in indigenous strain of freshwater prawn in Bangladesh.

For sustainable management and improvement of the species, study of its population structure is necessary. To address the issue, Khan et al. (2014) characterized genetic diversity in three river populations of *M. rosenbergii* using microsatellite DNA markers. While they used wild populations, in this study genetic variation between wild and hatchery populations of *M. rosenbergii* had been compared by randomly amplified polymorphic DNA (RAPD) analysis. Comparison of genetic diversity between wild and hatchery populations would enable us to understand population structure of the species. Among different DNA markers, RAPD is easy and simple to work. It functions based on amplification of discrete regions of the genome by polymerase chain reaction with short random primers. This marker has extensive use in estimation of genetic variability and relatedness in many organisms (Hadrys et.al., 1992). The present study, therefore, used RAPD analysis in estimation of genetic variation in both wild and hatchery-derived gher populations of *M. rosenbergii* and to compare the level of genetic variation between the two stocks.

Besides the culture of giant freshwater prawn, in shrimp (*P. monodon*) culture, various diseases, white spot syndrome virus (WSSV) in particular, has become a serious constraint, thereby, farmed shrimp production reduced to 87,972 mt. ton in 2009-10 from 1,02,854 mt. ton in 2008-09. Still now the production rate however rose slightly, due to horizontal spreading and substitution by Giant Freshwater Prawn, *M. rosenbergii*. Moreover, not all the farms are registered yet, therefore, tracing back a lot either infected or affected by impurities is still beyond our means. These are hindering our export earnings to be flourished from this sector.

Unarguably, the main problem is the infestation of different types of diseases some are known while some are not yet been reported in Bangladesh. So far, more than 20 viruses have been reported to infect marine shrimp worldwide. Many have not been associated with clinical signs of disease and some have been observed only by electron microscopy and are poorly characterized. Among them seven pathogens of marine shrimp and prawn are

currently listed by the World Organization for Animal Health (OIE) as causing modifiable aquatic animal diseases and two are under study for listing (OIE 2017), viz., White spot syndrome virus (WSSV), Yellow head virus (YHV), Taura syndrome virus (TSV), Infectious myonecrosis virus (IMNV), Necrotising hepatopancreatics (NHP), Infectious hypodermal and hematopoietic necrosis (IHHN), White tail disease (MrNV & XSV) and Acute hepatopancreatic necrosis disease (AHNPD). Apart from the list, Enterocitozoon hepatopenaei(EHP), Gill-associated virus (GAV) and M. Taihu Virus (MrTV) are also considered to be potential threats in recent shrimp aquaculture in Bangladesh.

Among the known disease, *P. monodon* facing devastating crop loss due to White Spot Disease (WSD) outbreak in Bangladesh. But interestingly, since last couple of years, mortality pattern due to WSSV infection found following a different pattern and showing differences in virulence as well. Apart from WSSV, several symptoms of shrimp mortality indicating the probability of presence of some non-reported disease to Bangladesh, viz., Necrotizing Hepatopancreatitis (NHP), Bacterial White Tail Disease, Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) and Enterocytozoon hepatopenaei (EHP). Among this disease, EMS found positive to a number of shrimp farms (Personal Communication). GSMC 2016 blog reported that EHP disease now 'everywhere in Asia'. Enterocytozoon hepatopenaei (EHP), the newest disease challenging shrimp farming operations, is now present in nearly all Asian countries. Shrimp production increased in 2014, dropped off in 2015, and it may not bounce back in the upcoming year.

Disease is always a problem which harasses the health development of shrimp aquaculture. Both virus and bacteria can be dangerous pathogens of shrimp in aquaculture. Application of traditional antibiotics can alleviate bacteria disease, but traditional strategy used to prevent virus disease in vertebrate is not effective to cure virus disease of shrimp since no adaptive immunity exists in them. WSSV is one of the most dangerous pathogens that is highly virulent in penaeid shrimp. WSSV infection of penaeid shrimp can result in mortality of up to 90–100%.

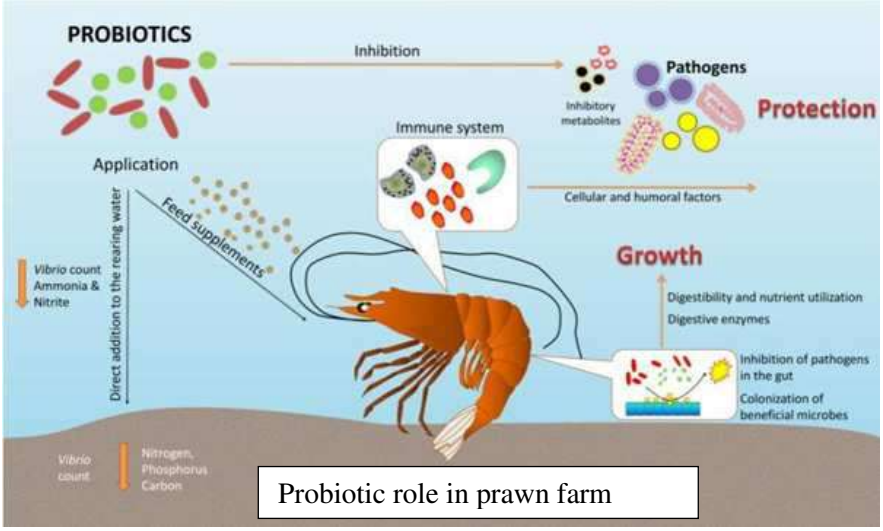
Giant freshwater prawn *Macrobrachium rosenbergii*is a widespread crustacean genus and is distributed globally across the tropical and sub-tropical areas, throughout Indo-Pacific region, and South and Central America (Jayachandran, 2001; Murphy and Austin, 2005). The probable cause of their global distribution might be due to their adaptation in a wide variety of environments from freshwater to brackish water, throughout the mountain streams, lowland rivers, estuaries and coastal lagoons, even at acidic rainforest streams and troglodytic habitats (Murphy and Austin, 2005; Ng. and Lim. 1992; Short and Marquet, 1998). Bangladesh has a great potentiality to culture freshwater prawn due to its suitable geographical and climatic condition, well-established culture techniques, technical and non-technical manpower's, and availability of wild and hatchery produced seeds (Ahmed et al. 2008; Wahab et al. 2012). The average production of 336 $kgha^{-1}$ is reported (Muir, 2003) as increased from the typical yield of 200-250 $kgha^{-1}$ in late 1990s (Rahman et.al. 1999).At the beginning of the study baseline surveyed was done south-west coastal region of Bangladesh(Khulna, Bagerhat, Satkhira and Jessore), and found that mostly traditional prawn-carp poly-culture and rice-prawn rotation were practiced where stocking density

ranged 10000 to 20000 post larvae/hand production ranged from 350-625 kg/ha (2020). Prawn culture is contributing to eliminate poverty, employing rural women and men in small businesses, earning currencies, and uplifting the social and economic conditions of stakeholders (Ahmed et al., 2008). However, with the increased population, life expenses, and local and global demands, people are realizing to boost the production and quality of prawns. Recently, probiotics are used in Bangladesh as a growth promoter and immune enhancing agent for disease protection in *M. rosenbergii* culture (Azad et al. 2019; Ghosh et al. 2016).

Recently, aquaculture industry is moving to eco-friendly and sustainable culture system to maintain the superiority and health of cultured food along with plummeting environmental impacts ( Shingare et al. 2020). Although shrimp industries having a great contribution to the economy of Bangladesh as well many developing countries (Alam and Ahammad 2017). But the industries have faced a lot of challenges in recent decades, hence production and the quality of shrimp commodities are also declining gradually (Ghosh 2018; Azad et al. 2019). Different types of environment-friendly culture methods have been developed. Application of Probiotics and prebiotics is one of the most popular eco-friendly culture systems around the world (Kumar et al. 2016; Shefat 2018; Shingare et al. 2020).

Probiotics are considered as a bio-friendly agent introduced into the aquaculture environment to improve water quality, to manage the pathogenic bacteria in bio-control measures for replacing indiscriminate use of antibiotics in the aquaculture systems as well as to enhance the growth of the cultured animals (Azad et al., 2019; Dash et al., 2017; Ghosh et al., 2016; Mohapatra et al. 2013; Padmavathi et al. 2012). The farming by probiotic technology would open a new horizon to increase the production and sustainably manage the aquaculture ponds. Several authors reported the beneficial role of using probiotics in aquaculture, including shrimp and prawn (Balcazar et al. 2006; Ibrahem, 2015; Kumar et al. 2016; Lara-Flores, 2011; Wang 2008).

The principal mode of action of probiotic bacteria is through competitive exclusion mechanisms in which pathogens are replaced or excluded through the development of a beneficial microbial population on the intestinal surface which leads to a reduction in disease, better health, and thus better growth of the host (Jha 2014; Azad et al. 2019). Thus, probiotics act as the alternatives of antibiotics and chemicals by reducing diseases through maintaining a pollution free environment (Kumar et al. 2016; Fernandes and Kerkar 2019). On the other hand, prebiotics acts as a free carbon source that helps heterotrophic bacteria to digest ammonia. It also ensures enough oxygen, stabilizes the pH and balance the density of algae-bacteria ratio.




Probiotic role in prawn farm

Stocking density plays an important role in survival, growth, production performance and population structure in the culture system (Chowdhury et al. 2017; Sugathan et al. 2014). The growth rate and the stocking density have an intricate relation, as the optimized stocking density facilitates proper use of feed, expedites highest production, keeps healthy environment for fish and leads to sustainable aquaculture (Reza et al. 2015). If the optimal stocking densities are not practiced in the prawn culture system, the production performance significantly decreases due to poor growth regardless to better survival of the stock (Langer 2009; López-Uriostegui et al. 2014). The higher stocking densities in prawn culture may inhibit the growth due to social dominancy of male prawn in the population and increase the cannibalism (Newsholme *et al.* 1987). High stocking density may also negatively impact on the production by food and shelter competition, risk of disease outbreak and mortality, undersized of marketed prawn. On the other hand, stocking density with lower than optimum reduces the net production (de Souza Costa and de Fátima Arruda, 2016; Ellis et al. 2002; Reza et al. 2015). The stocking density for prawn cultivation ranged from 1 to 10/m2 in many different parts of the world (Nagarathinam et al. 2000). In India, the stocking density of *M. rosenbergii* varies from 2 to 3/m2 in mixed culture and 1-2/m2 in all male monoculture (Nair and Salin, 2012). In Bangladesh, it varies from 2 to 3/m2, and 2/m2 has been recommended as the most profitable density (Asaduzzaman et al. 2009).

The main challenge of the prawn fishery is the diseases, considered as the major responsible factor for high mortality and significant economic loss. Diseases could be happened due to the prevalence of pathogenic bacteria, virus, fungi and protozoan parasites. Among them, bacterial diseases are responsible for massive economic losses in the prawn aquaculture (Miao et al. 2019). Using antibiotics to bacterial disease treatment has been prohibited due to its high possibility of residual bioaccumulation in fish tissues and biotransformation to human (Ringø, 2020). The antibiotic also develops antibiotic-resistant bacteria, which has a severe impact on human health and the environment (Zhong et al. 2018, Binh et al. 2018). Researchers are interested in investigating environment friendly green solutions to control fish diseases. Therefore, probiotics have received particular attention as a potential alternative to antibiotics of the aquaculture systems (Binh et al. 2018, Kavitha et al. 2018). Probiotic fight against pathogenic bacteria and enhance innate immunity by enhancing total

hemocyte count (THC), clearance efficiency, phagocytic activity and differential hemocyte count (DHC) (Ahmmed et al. 2020). Today, enhancing the immunity of aquatic animals has received higher priority to control disease and for higher survival. As crustaceans do not contain immunoglobulins (Ig), they use a non-specific immunity approach to resist disease (Rattanachai and Cheng, 2015). Non-specific immunity factors such as superoxide dismutase (SOD) activity, phenoloxidase activity (PO), lysozyme activity, respiratory burst ($O_2^-$), and phagocytic activity also play essential roles in disease resistance and immune response of prawn (Wang and Jaio 2000).

In addition, freshwater prawn aquaculture is threatened due to variety of factors such as misuse of antibiotics and drugs, pollution of environment, and spreading of severe diseases caused by bacterial and viral agents. Some of those bacterial and viral agents are already known; however, many of them have not been reported yet in Bangladesh. Thus, it is now important to identify the new and unknown pathogens, and determination of virulence factors with mitigation measures. There is a national and international concern for preventing and controlling the diseases through new scientific approaches to make health-safe fish/shellfish product. Probiotics can be one of the alternatives to improve culture friendly water and soil quality, prevention of disease producing pathogen, increasing digestibility and immune competence of prawn. Thus, it is also important to isolate potential probiotics bacteria from the culture environment and go for their mass production in laboratory and pond.

In freshwater prawn the innate defense system – also known as natural or non-specific defense system includes both cellular and humoral components which work in jointly coordination for the elimination of all foreign organisms potentially hazardous for the host (Jiravanichpaisal *et al.* 2006). Amylase activity was found to be localized within the midgut gland of *M. rosenbergii*. Amylase is an enzyme that hydrolysis of carbohydrate. While carbohydrates play important functions in several metabolic processes including the Krebs cycle, gluconeogenesis, chitin synthesis, and the formation of steroids and fatty acids (Wigglesworth and Griffith 1994). In Krebs cycle, high rates of partial oxidation of both fuels in lymphocytes and macrophages, and in other cells such as enterocytes, colonocytes,and in neoplastic cells. High rate of glutamine utilization and its importance in such cells has raised the question as to the source of this glutamine in the body (Newsholme *et al.* 1987).

Most of the digestive enzyme plays very important role for metabolism and respiration as well as their immune system. Protease is an important digestive enzyme for animal. Proteolytic enzymes are divided into four groups: serine, cysteine, aspartic and metallo proteases. Serine or alkaline proteases are so named because of their having a “super-reactive” serine in the active site (Simpson et al. 2000). Serine proteases function as important regulatory proteins in activating the prophenol oxidase (pro-PO) and clotting systems of the giant tiger prawn (Supungul et al. 2002; Dong et al. 2018; Xue et al. 2013). Addressing all the issues this research project focused on enhancement of prawn production by using eco-friendly culture techniques with the application of probiotics as well ensuring the health safe prawn production.

## 7. Sub-project general objective(s)

**General objective:** To boast up Shrimp/Prawn production using sophisticated breeding technique and grow out management with reference to disease diagnosis and preventive measures.

## 8. Sub-project specific objectives (component wise)

### Coordination component

Under the principal objective of the NATP, the Coordination component of the BFRI sub project shall have to ensure smooth and efficient implementation of component sub-project activities to achieve desired project output/goal within the stipulated timeframe under strengthened capable research management and coordination system.

### BARC component

i. To coordinate project implementation efforts and integration of activities to generate desired information /technology as per methodology of the sub-projects.
ii. Identify operational deviations and addressing constraints/problems (if any) under a process of strong and regular monitoring of the project activities.
iii. To upgrading the level of output of the sub-project through reviewing of yearly technical progress.
iv. Collect and collate project data, finding and observation and production of compiled Project Completion Report (PCR).
v. Finally, to ensure increased safe fish food production technologies under an environment friendly atmosphere with concomitant increase in rural employment, earning and involvement of rural women through effective coordination among the sub projects.

### BFRI (SRS) component

i. To develop cluster based shrimp (*Penaeus monodon*) farming (CBSF) with special emphasis on disease prevention and traceability
ii. To promote Shrimp/Prawn health management through intensive monitoring and surveillance of bacterial (NHP, EMS/AHPND & EHP) and viral (WSSV, YHV, GAV, TSV, IMNV, MBV & BP) diseases, identification of risk factors, evaluation of immune response and preventive measures
iii. To develop all male PL production technique of Giant Freshwater Prawn (*M. rosenbergii*).
iv. To have an insight of innate immune response over immunogenic agents in shrimp (*P. monodon*).
v. To infer the genetic diversity of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) in the major coastal rivers.

### KU component

i. Production intensification of freshwater prawn *M. rosenbergii* through selected probiotic based eco-friendly culture system
ii. Growth and Quality enhancement of prawn administrating probiotics for augmenting digestibility and immunity, minimizing disease.

iii. Identification of potential probiotics in aqua farm and their mass proliferation in lab and in situ condition as well as in the gut of prawn by using locally available ingredients and their effect on prawn production.
iv. Investigation of storage approaches of identified probiotics from the farm through heat shock and chemical treatment.

9. **Implementing location(s):** Bagerhat and Khulna region.

## 10. Methodology in brief

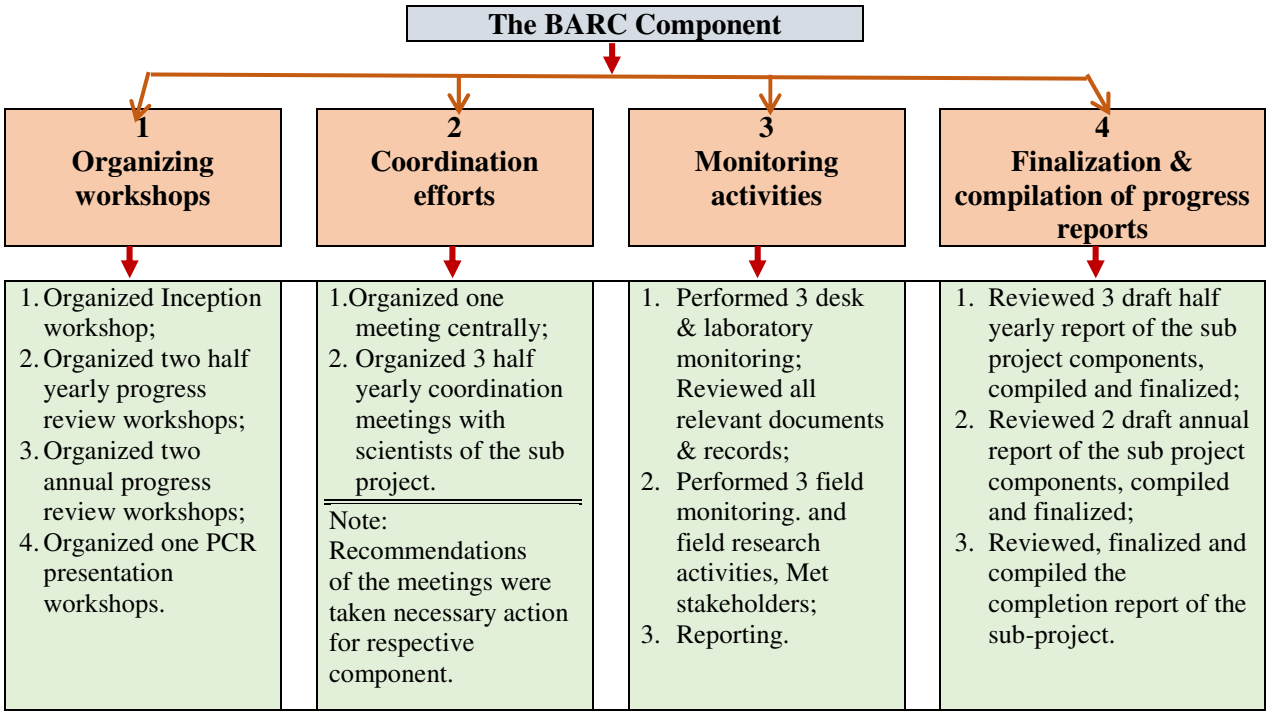
### 10.1. Activities implementation approach of the BARC component

The BARC component as the responsible unit of the sub-project to initiate all potential efforts in the process of implementation of each component projects under the sub-project so that the general objectives and goal of the sub-project can be achieved through smooth and successful completion of each of the specific objectives as per activity time plan of the sub-project document. To ensure that, the Coordination component, taken into consideration its own activity and objectives and duration of the sub-project, thus accordingly designed its own plan of activity (approach) for the proposed period.

Following are the major activities carried out by the BARC component under the plan:

a. Organizing seminars/workshops.
b. Monitoring sub-project activities.
c. Assist Coordination component to establish working linkage within the sub-project components.
d. Review research progress activities and compilation half yearly and annual research progress reports.

The implementation approach and activities thereunder for the BARC component of the sub project shown in the following diagram:




Recommendations of the inception, half yearly and annual research progress review workshops and different coordination meetings are furnished hereunder in **Appendices- BARC: A - D.**

Following table presenting the summary statement of achievements performed by the Coordination component of the sub project

| **Summary statement of achievements** | | |
|---|---|---|
| **Name of activities** | **Performance against each activity** | **Remark** |
| Inception workshop | Organized centrally at BARC in October' 2018 | Attended all PI, Co-PI & expert members. |
| Revision of PP | Done as per recommendations of Inception workshop | |
| Half yearly Prog. Review Workshop (Date) | Organized centrally at BARC in March' 2019, January'2020. | Attended all PI, Co-PI & expert members |
| Ann. Prog. review Workshop (Date) | Organized centrally at BARC in June' 2019 & in September' 2020 | Attended all PI, Co-PI & expert members. |
| Coordination meeting (No) | 03 ***(24.10.19, 19.02.20 & 18.06.20)*** | One Coordination meeting held centrally. |
| Monitoring of field and Lab activities | 04 ***(BARC, BFRI & KU)*** | Covered all components under sub-project. |
| Financial achievement | Approx. 98.00% of total released money & 77.6% of the total approved budget | - |
| Reporting performance | Provided project inception reports, SoE, Half yearly and Annual compiled progress reports of all sub project components as per planned time frame. | Major reports are:<br>• Inception report (1 no) Compiled half yearly progress report (3 no)<br>• Compiled annual progress report (2 no);<br>• Monitoring reports (3 no) |

***Pictorial views of***
***Different workshops, coordination meetings and project monitoring activities***





## 10.2. Research methodology

### 10.2.1. BFRI (SRS) component

#### 10.2.1.1. Expt. 1. Development of Cluster Based Shrimp Farming (CBSF) to cope with disease outbreak in shrimp aquaculture

**Forming cluster**

At the first year of the project, the conceptual model of CBSF was subjected to field trial. For implementing the model, traditional ghers has been converted into clusters having facilities to implement scientific methods of shrimp culture like installation of surrounding net, different water outlet and inlet, water purification chamber, nursing point and grow-out pond. Stocking density was followed in 1st year is 30000/ha & 60000/ha and 60000/ha & 90000/ha in 2nd year.

**Table 1**: Experimental design for 1st year

| SL | Treatment Name | Stocking Density | Replications |
|---|---|---|---|
| 1 | Impact of stocking density on shrimp production | 30000/ha | 2 |
| | | 60000/ha | 2 |

**Table 2**: Experimental design for 2nd year

| SL | Treatment Name | Stocking Density | Replications |
|---|---|---|---|
| 1 | Impact of stocking density on shrimp production | 60000/ha | 2 |
| | | 90000/ha | 2 |

**Pond preparation**

Traditional shrimp farm of 1.316 ha was experimentally converted into cluster. Each pond of the cluster was prepared following dry method of pond preparation. Briefly, pond bottom was dried and CaO were applied at the rate of 1kg/decimal. Then the pond bottom was ploughed and kept under direct sunshine for a week. Before entering water, fine mesh net fencing throughout the dike was done to restrict any kinds of living organisms entering into the culture system. After proper netting, water was allowed through a 0.5 mm mesh net to restrict macro-organisms into the ponds. Then the pond was filled-up with tidal water and treated with 75-100kg/ha Bleaching to kill all living organisms. To regenerate the planktonic community into the pond, molasses custard was implemented at the required dose (mention the dose). The ponds were treated with lime @ 25-50kg/ha for increasing the buffering capacity of the water. Based on the primary productivity, the ponds were fertilized with inorganic fertilizers followed by Urea: 50-60kg/ha., TSP: 25-30kg/a., and $KMnO_4$:15-20kg/ha (considering 3 feet water depth/dec).

**Feeding**

Feed was supplied twice daily @ 8% of body weight for the first month, 5% for the second month and 2-3% for the rest of the period. A total of 30 shrimp from each treatment were sampled using cast net. Weight of the shrimp taken using portable balance for growth monitoring, feed adjustment and disease checking. Water quality was also monitored and recorded at weekly intervals.

## Data collection

Water samples were collected and tested on the spot using a water test kit (HACH FF-3 with digital titrator) between 8.00 to 10.00 A.M.

## 10.2.1.1A. Study-1 Development of PCR protocol for identification and characterization of OIE listed pathogens in Prawn/Shrimp aquaculture

Under this study, for the identification of OIE Listed pathogen for Shrimp and Prawn, PCR protocol were optimized. Pathogen specific primers were synthesized from the gene bank. Then PCR protocol was optimized for the pathogens in the laboratory of SRS, Bagerhat.

**Table 3**. List of primers

| | |
|---|---|
| **Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)** | |
| 389 bp | IHHN389F 5'-CGG-AAC-ACA-ACC-CGA-CTT-TA-3' |
| | IHHN389R 5'-GGC-CAA-GAC-CAA-AAT-ACG-AA-3' |
| 356 bp | IHHN77012F 5'-ATC-GGT-GCA-CTA-CTC-GGA-3' |
| | IHHN77353R 5'-TCG-TAC-TGG-CTG-TTC-ATC-3' |
| 392 bp | IHHN392F 5'-GGG-CGA-ACC-AGA-ATC-ACT-TA-3' |
| | IHHN392R 5'-ATC-CGG-AGG-AAT-CTG-ATG-TG-3' |
| 309 bp | IHHN309F 5'-TCC-AAC-ACT-TAG-TCA-AAA-CCA-A-3' |
| | IHHN309R 5'-TGT-CTG-CTA-CGA-TGA-TTA-TCC-A-3' |
| 831 bp | IHHNG831F 5'-TTG-GGG-ATG-CAG-CAA-TAT-CT-3' |
| | IHHNG831R 5'-GTC-CAT-CCA-CTG-ATC-GGA-CT-3' |
| **Monodon Baculovirus (MBV)** | |
| 261 bp | MBV261F 5'-AAT-CCT-AGG-CGA-TCT-TAC-CA-3' |
| | MBV261R 5'-CGT-TCG-TTG-ATG-AAC-ATC-TC-3' |
| 533 bp | MBV 533 F 5'-CGA-TTC-CAT-ATC-GGC-CGA-ATA-3' |
| | MBV 533 R 5'-TTG-GCA-TGC-ACT-CCC-TGA-GAT-3' |
| 361 bp | MBV 361 F 5'-TCC-AAT-CGC-GTC-TGC-GAT-ACT-3' |
| | MBV 361 R 5'-CGC-TAA-TGG-GGC-ACA-AGT-CTC-3' |
| **Infectious Myonecrosis Virus (IMNV)** | |
| 993 bp | IMNV 993F 5'-AACACAAAATCTGCCAGCAA-3' |
| | IMNV 993R 5'-CCCAACCACCCAAATTCATA-3' |
| 530 bp and 410 bp | IMNV 530 F5'- GCT GCC ACT GTA CCG TAT GT -3' |
| | IMNV 530 R5'- CAA AAT CTG CCG GCA ACA CA -3' |
| | IMNV 410 R5'- GGA TTA GCC GCG CCA GTC -3' |
| **Yellow Head Virus genotype 1 (YHV1)** | |
| 943 bp | YHV943F 5'- CAG CTG GTA GTG TTG TTC GAT GCA -3' |
| | YHV943R 5'- CCA AAC AGC ATA GCT ACT GCC TGG -3' |
| 564 bp | YHV564R 5'- CCG GTG TGC CAA GGT CAG -3' |
| ***Enterocytozoon hepatopenaei* (EHP)** | |
| 510 bp | EHP-510F 5´- GCCTGAGAGATGGCTCCCACGT-3' |
| | EHP-510R 5´- GCGTA CTATCCCCAGAGCCCGA-3' |
| 270 bp | EHP-270F 5´- CCA CGT CCA AGG ATG GCA GC-3' |
| | EHP-270R 5´- CCA TGC TCC CTA TTC GTT CCG C-3' |
| ***Necrotizing Hepatopancreatitis* (NHP)** | |
| 720 bp | NHP720F- 5´- GAG TGG CAG ACG GGT GAG-3' |
| | NHP720R- 5´- GCG ACA CTG AAG GAC GAA CCT C-3' |

## Sampling area

**Table 4.** Sampling area and number of samples

| District | Number of Shrimp Ghers/farms | Number of Shrimp Samples/gher | Sampling Duration | Total sample |
|---|---|---|---|---|
| Khulna | 20/district | 3* | March, April, May (Once in a month) | 720 |
| Bagerhat | | | | |
| Satkhira | | | | |
| Cox's bazar | | | | |

*10 shrimp samples from 3 random site of each gher were collected for clinical observation and finely minced to make 3sample/gher for PCR detection

## Sample collection and identification

Samples were collected from the study area in fresh condition and transported to Shrimp Health Management Laboratory, SRS, Bagerhat in 95% ethanol and peptone water. *All the 10 shrimp from each sampling point/gher were finely chopped and then 20~30 mg were used for the PCR diagnosis followed by DNA/RNA extraction.

## DNA/RNA extraction

Genomic DNA were extracted from tissue samples collected from pleopods. Approximately 25-mg tissues were cut into small pieces, homogenized by micro-tissue-grinder in a 1.5 ml microfuge tube, and total genomic DNA were extracted following the instruction provided with Thermo scientific's Purelink DNA extraction kit.

## DNA/RNA amplification

Both first step PCR and nested PCR were done to confirm the presence or absence of the pathogen. Primer was designed from the sequenced gene from the NCBI gene bank using Serial Cloner 2.6.1. Software. Thermal cycler for polymerase chain reaction (both first and nested PCR) was programmed as follows:

**Table 5**. Thermal cycler condition

| | |
|---|---|
| 95 °C x 3 minutes followed by | |
| 95 °C x 30 seconds | 15-35 cycles |
| 58~62 °C x 30seconds | |
| 72 °C x 30 seconds | |
| 72 °C x 5 minutes | Final Extension |

## Analysis of PCR products by gel electrophoresis and documentation

Both first & nested PCR products taken for gel electrophoresis. To run the process, 1.0% agarose gel was prepared (thickness not more than 0.8cm.) prior to electrophoresis apparatus be assembled. For electrophoresis, 1 X TAE buffer were used as the medium at 100-120 volts.

### 10.2.1.2. Expt.-2. Development of all-male prawn PL production using Neo-female technology

**Experimental design**

**Collection of wild breeds :** Matured wild brood of *M. rosenbergii* were collected and keep in brood rearing tank of SRS prawn hatchery until hatching.

**Hatchery :** After hatching, the larvae were transferred to Larvae Rearing Tanks (LRT). The tanks were stocked at a density of 60–80 larvae/l. Hatchery operation carried out flowing the previously modified hatchery operation protocol by SRS scientists. Larvae metamorphosed into post larvae (PL) after 30 days of rearing.

**Sex reversal microsurgery (phase I) :** Microsurgical Ag ablation was performed by removing the fifth pair of walking legs together with the androgenic gland.

### 10.2.1.3. Expt.-3. An insight into innate immune response of shrimp resisting viral and bacterial disease

**Methodology**

Healthy shrimp PL were collected. PL were reared in the SRS pond complex until it attained average 2 g body weight. Desired sized shrimp were then clustered in groups and kept in 35-L aquaria at 26 $^{O}$C and fed with commercial diet at 5% of body weight before and during experiments. Pleopods from the shrimp were collected and extracted DNA were subjected to PCR detection. Then the pathogen free shrimp selected for the experiment.

**Immune stimulant agents**
As immune stimulant agents, first priority was given to WSSV protein cloned into PcDNA/PET32 Vector plasmid followed by bacterial flagellum/attenuated bacteria/LPS.

Attenuated pathogenic bacteria was used as immune stimulant agents due to unavailability of necessary reagents for cloning part because of corona pandemic. For this experiment, pathogenic vibrio bacteria cultured in nutrient broth and pelleted by centrifuging at 3500rpm. These bacterial pellets were then diluted in PBS and given heat shock at $95^{0}$C for 5 minutes. Then 100 ml PBS solution containing ($1\times10^{8}$ cfu per ml) pathogenic dead bacteria were mixed with per Kg of commercial feed to for the experimental trial. This feed was administrated for three weeks as treatment and haemocytes were counted to see changes in immunogenicity.

**Haemocyte collection**
From each shrimp 0.1 ml haemolymph were collected into a 26-gauge 1 ml sterile syringe containing 0.2 ml of anticoagulant (Trisodium Citrate 30mM, NaCl 338mM, Glucose 115mM, EDTA 10mM).

**Total haemocyte count (THC)**

THC= average of 4 blocks × dilution correction factor $\times 10^4$

Dilution correction factor =

$$\frac{volume\ of\ hemolymph\ extracted + volume\ of\ anticoagulant\ used}{volume\ of\ hemolymph\ extracted}$$

### 10.2.1.1B. Study-2. Identification of genetic variability of *M. rosenbergii* in the major brackish water rivers (Kocha, Payra, Baleswar, Rupsha and Poshur) of Bangladesh

**Sample collection**

Wild populations from the rivers viz., Kocha, Payra, Baleswar, Rupsha, Poshur, Karnafuli, Meghna and Kumar Nod were collected for the study of genetic variability and diversity within the population.

**DNA Extraction**

Genomic DNA were extracted from tissue samples collected from pleopods. Approximately 25-mg tissues were cut into small pieces, homogenized by micro-tissue-grinder in a 1.5 ml microfuge tube, and total genomic DNA were extracted following the instruction provided with Thermo Scientific's Pure link DNA extraction kit.

**Primer specification and PCR optimization**

For the identification of genetic variability, the extracted DNA was amplified with the given primers in table 6.

**Table 6**. Primer sequences of 10 *Macrobrachium rosenbergii* microsatellite loci, including GenBank Accession no. and annealing temperature (Ta)

| Locus | GenBank Accession no. | Primer sequences5'~3' | *T*a ($^oC$) |
|---|---|---|---|
| Mbr-1 | DQ019863 | F: CCCACCATCAATTCTCACTTACC<br>R: TCCTTTTCACATCGTTTCCAGTC | 60 |
| Mbr-2 | DQ019864 | F: TTCCCGACCAATTTCTCTTTCTC<br>R: GGCAAAAATGATCTTGGATTCAC | 60 |
| Mbr-3 | DQ019865 | F: CAACTCTATGTTTCGGCATTTGG<br>R: GGGGAATTTTACCGATGTTTCTG | 62 |
| Mbr-4 | DQ019866 | F: CCACCTACCGTACATTCCCAAAC<br>R: CGGGGCGACTTTTAGTATCGAC | 62 |
| Mbr-5 | DQ019867 | F: CAAGGCTCGTGTCTCTTGTTTC<br>R: GCTTGTACTTGTTCAGCTTTTGC | 62 |
| Mbr-7 | DQ019869 | F: ATAAAAGAGTCGCCAAATGAGCA<br>R: ATTGGGAATTGTTGACCTCCAAG | 62 |
| Mbr-8 | DQ019870 | F: AACCAGCCGACTTAGACTGTGC<br>R: CGCCATTTGCGTCTATCTCTTAC | 62 |
| Mbr-9 | DQ019873 | F: TTGTTTGCTTGTTTAGTGTCAAGG<br>R: CTCCAAAACCGAAAAATCCTCAC | 60 |
| Mbr-10 | DQ019871 | F: ATGACGATGATGAGGAATGAAGC<br>R: TTTCAGGCTATATCAAGCAACAG | 60 |
| Mbr-11 | DQ019872 | F: GTATTGAGAACAAAGGCGCACAG<br>R: ATCTCTTTCCAAAACAGGGCACA | 60 |

## 10.2.2. Khulna university (KU) component

The field experiment was conducted at the pond complex of Fisheries and Marine Resource Technology (FMRT) Discipline of Khulna University. All the experimental pond size was 240 $m^2$. Laboratory work was conducted in the water quality Lab, Disease lab, Post-Harvest Technology Lab of FMRT Discipline.

### 10.2.2A. Activity 1. *Baseline survey to know current culture scenario and available probiotics in Khulna region*

**Method**

At the beginning of the study a baseline survey was conducted to identify available probiotics applied in southwest coastal prawn farms and traders (Khulna, Bagerhat, Shatkhira and Jessore). Data were collected from 60 respondents including farmers, traders, and other beneficiaries. Bacterial quality of each probiotic was determined through examining the growth of bacterial colony on selective agar media by comparing with the company values. *Bacillus* Differentiation Agar (Himedia-M1394), *Lactobacillus* MRS Agar (Himedia-M641), Reinforced Clostridial Agar (Himedia-M154) and KF Streptococcal Agar Base (Himedia-M248) were used for counting the desired bacteria, i.e., *Bacillus* spp. *Lactobacillus* spp. *Clostridium* spp., and *Enterococcus* spp. respectively from the collected probiotics. For other bacterial colony determination selective agar media was used and culture accordingly. The list of selected probiotics is shown in Table 7.

**Table 7.** List of available probiotics found in local market and prawn farms of Khulna region

| SL | Probiotics commercial Name | Used (%) | Composition | Position as per used (%) | Probiotic code name as per production performance in 1st year |
|---|---|---|---|---|---|
| **1** | Aqua Clear S | 47 | *Bacillus mensentericus* $9 \times 10^9$ *CFU*<br>*Bacillus subtillis* $10 \times 10^9$ *CFU*<br>*Bacillus licheniformis* $9.8 \times 10^9$ *CFU*<br>*Lactobacillus acidophilus* $7.5 \times 10^9$ *CFU*<br>*Nitrobactersp* $8 \times 10^9$ *CFU*<br>*Nitrosomonassp* $3 \times 10^9$ *CFU*<br>*Aspergillusoryzae* $9 \times 10^9$ *CFU*<br>*Saccharomyces cerevisiae* $9 \times 10^8$ *CFU* | 1st | (Probiotic-1, P1) |
| **2** | Zymetin | 45 | *Streptococcus faecalis, Clostridium butyricum, Bacillus mesentericus*, (over all bacterial load $1.10 \times 10^6$ CFU/g | 2nd | (Probiotic-2, P2) |
| **3** | Aquaback P | 40 | *Bacillus amyloliquefaciens*, *Bacillus pumilus* strain D1728 | 4th | (Probiotic-6, P6) |
| **4** | Super Biotics | 39 | *Bacillus subtilis* (1 x $10^6$cfu), | 3rd | (Probiotic-3, |

| SL | Probiotics commercial Name | Used (%) | Composition | Position as per used (%) | Probiotic code name as per production performance in 1st year |
|---|---|---|---|---|---|
| | | | Bacteriophage q.s. to 1 gm | | P3) |
| **5** | Biotics | 37 | *Saccharomyces cerevisiae, Lactobacillus acidophilus, Bacillus suntilis* | 5th | (Probiotic-5, P5) |
| **6** | Super PS | 34 | *Rhodococcus; Rhodobacter; Nitrosomonus* | 6th | (Probiotic-4, P4) |
| **7** | Pro W | 26 | *B. licheniformis, B. polymyxa* | | |
| **8** | pH Fixer | 18 | | | |
| **9** | Ammonil | 15 | *B. subtilis, Candida utilis* | | |
| **10** | Greenmax Aqua | 14 | *Bacillus megaterium; Bacillus subtilis; Bacillus licheniformis; Bacillus polimixa, Lactobacillus acidophilus; Pseudomonus sp. Saccharomyces cerevisiae; Nitrobacter sp.; Nitrosomonus* | | |
| **11** | Power PS | 12 | *Bacillus, Nitrobactersp* | | |
| **12** | Probac WS | 11 | *B. subtilis* | | |
| **13** | Viva Pond | 10 | *B. subtilis* | | |
| **14** | Pond D Tox | 9 | *Pracoccuspantotrophus* | | |
| **15** | Biozime | 9 | *S. cerevisiae* | | |
| **16** | Megazeo Pro | 7 | *B. subtilis* | | |
| **17** | Eco toxil | 5 | *Saccharomyces cerevisiae* | | |

### 10.2.2B. Activity 2. *Growth and production performance of prawn at different probiotics for the selection of better competent probiotics*

**Method**

Among seventeen probiotics, six mostly usable probiotics were applied to know growth and production performance of prawn in pond condition. Commercial feed containing 28% protein was applied in control (without probiotics) and all 6 probiotics treated treatments where stocking density maintained 2 juvenile /$m^2$ and probiotics was applied as per instruction of manufacturer (written on packet) (Table 8).

**Table 8.** Growth and production performance of prawn applying 6 top uses commercial probiotics in pond condition with stocking density 2 juvenile/ $m^2$

| Treatment | Feed | Aqua Clear S | Zymatin | Super Biotics | Aquaback P | Biotics | Super PS | Replication |
|---|---|---|---|---|---|---|---|---|
| Control | + | | | | | | | 3 |
| T1 | + | + | | | | | | |
| T2 | + | | + | | | | | |
| T3 | + | | | + | | | | |
| T4 | + | | | | + | | | |
| T5 | + | | | | | + | | |
| T6 | + | | | | | | + | |

## 10.2.2C. Activity 3. *Intensification of prawn in non-aerated and aerated culture system by applying best performed three probiotic from first year experiment*

**Table 9**. Growth and production (kg/ha) performance of *M. rosenbergii* under three probiotics (P1, P2 and P3) without aeration where stocking density maintain 2, 4 and 6/$m^2$

| Treatment | Probiotics Applied | Stocking density (/$m^2$) | Replication |
|---|---|---|---|
| C1 | nil | 2 | 3 |
| T1 | P1 | | |
| T2 | P2 | | |
| T3 | P3 | | |
| C2 | nil | 4 | |
| T4 | P1 | | |
| T5 | P2 | | |
| T6 | P3 | | |
| C3 | nil | 6 | |
| T7 | P1 | | |
| T8 | P2 | | |
| T9 | P3 | | |

**Table 10**. Growth and production performance of *M. rosenbergii* under three probiotics (P1, P2 and P3) with aeration where stocking density maintain 4, 8 and 12/$m^2$.

| Treatment | Probiotics Applied | Stocking density (/$m^2$) | Replication |
|---|---|---|---|
| C1 | nil | 4 | 3 |
| T1 | P1 | | |
| T2 | P2 | | |
| T3 | P3 | | |
| C2 | nil | 8 | |
| T4 | P1 | | |
| T5 | P2 | | |
| T6 | P3 | | |
| C3 | nil | 12 | |
| T7 | P1 | | |
| T8 | P2 | | |
| T9 | P3 | | |

Hatchery produced post larvae (PL-25) of *M .rosenbergii* were acclimatized and reared in 120 $m^2$ outdoor pond for 60 days and juvenile was used in culture ponds. All ponds used in this experiment were rectangular in shape with a maximum depth of 1.5 m and all of them were fully exposed to prevailing sunlight. The experiment was conducted in two different schemes, i) culturing prawn without aeration under three stocking densities (e.g. 2, 4 and 6 $m^{-2}$), and ii) culturing prawn with aeration under three stocking densities (e.g. 4, 8 and 12 $m^{-2}$). Three different commercial probiotics (1, P2, P3) were used in both without aeration and aeration system at different stocking densities to know growth and production of freshwater prawn in pond condition. In both the experiment determined and recorded the growth (g) and production (kg/ha). Therefore, we had nine experimental treatments in each of the experimental schemes. Prawns of all treatments were reared using 28% protein containing feed. Probiotics were applied according to the manufacturer's protocol. In this experiment, probiotics with three different application approaches were used. For example, $P_1$ contained both feed and environmental probiotics, $P_2$feed probiotics and $P_3$environmental probiotic.

### 10.2.2D. Activity 4. *Water and soil parameters in without probiotic and with probiotic*

Water temperature was measured by using a Digital Thermometer and dissolved oxygen (DO) by portable dissolved oxygen (DO) meter,model no HANNA HI9146. Salinity was measured by Refractometer (ATAGO CO. LTD). pH test kit, alkalinity kit and ammonia test kit respectively used to measure the parameters. Nitrite-nitrogen ($NO_2^-$-N) and Nitrate-nitrogen ($NO_3$-N) were measured by Ultraviolet Spectrophotometric Screening Method and ammonia-nitrogen ($NH_3$-N) by Nessler Method.

Most of the soil parameter measurements were done at the Laboratory of Soil Resource Development Institute (SRDI), Khulna. pH and Electric conductivity (EC) were measured by pH meter and EC meter, Organic Matter measured by Standard method. Nitrogen and Phosphorus measured by Micro-Kjeldahl and Olsen's method. Potassium and Sulphur were measured by Flame emission spectrophotometer and Turbidimetric method.

### 10.2.2E. Activity 5*. Proximate composition of cultured prawn in without and with probiotics*

Body basic proximate composition such as content of protein, lipid, ash and moisture were measured in fish nutrition laboratory of Fisheries and Marin Resource Technology Discipline, Khulna University. Prawn proximate composition was determined for control (without probiotic) and three probiotics (P1, P2, P3) at 2, 4 and 6 prawn/$m^2$.

Protein was determination by using micro Kjeldahl method by measuring the percentage of nitrogen (%N) into the sample multiplies with conversion factor (6.25). The percentage of gross portentous nitrogen (%N) was calculated by the following formula:

Percentage of nitrogen (%N) =[((Volume of HCl x Normality of HCl x 0.014)/(Sample weight (g) ))x 100], where % Protein = , where %N = Percentage of nitrogen, 6.25=conversion factor

The percentage of the lipid content was calculated by the following formula:

% Lipid = ((Weight of lipid x 100))/(Sample weight)

The percentage of the moisture content was calculated by the following equation

% Moisture= (Weight of wet sample-weight of dried sample)x100/(Weight of wet sample )

The percentage of ash was determined by the following formula:

% Ash = (weight of Ash x 100)/(Weight of sample)

### 10.2.2F. Activity 6. *Digestive enzyme activity was analyzed in without and with probiotics treated prawn*

Amylase activity was assayed by starch hydrolysis method of Bernfeld (1955) in which the increase in reducing power of buffered starch solutions was measured. The specific activity of amylase was calculated as milligrams of maltose liberated per gram of protein per hour (mg/g/h). The reaction mixture consisted of 0.125 ml of 2% (w/v) starch solution, 0.125 ml of 0.1 M citrate phosphate buffer (pH 7.5) and 0.5 ml enzyme source. The reaction was incubated at 37 °C for 1 hour, and the absorbance was measured at 600 nm against a blank. For the blank, the enzyme source was added just after the incubation period. Maltose solution was used as standard (Bhavan *et al*. 2014).

The protease activity was estimated by using the casein-hydrolysis method by the method of Furne *et al.* (2005). Casein was used as substrate. The reaction mixture contained casein at 1% (w/v) (0.25 ml), 0.25ml of 0.1M glycine – NaOH buffer (pH 10) and 0.1ml supernatant (enzyme source). The mixture was incubated for 1 h at 37ºC. The reaction was stopped by addition of 0.6 ml 8% (w/v) trichloroacetic acid solution; kept for 1 h at 2ºC; centrifuged at 1800 g for 10 min and the absorbance of supernatant was measured at 280 nm against blank.

The lipase activity was determined by the evaluation of the degradation of triacylglycerols, diacylglycerols, and monoacylglycerols to free fatty acids, following the method of Bier (1955). 1 Litre of Polyvinyl alcohol was prepared. A solution of 1% polyvinyl alcohol and 5ml of 0.1 N HCl was heated to 75°C- 85°C . Then they were cooled and filtered. The solution was adjusted to 8.0 with 0.1 N NaOH. Virgin olive oil was added to an aliquot of this solution for obtaining 0.1 M substrate concentration. This mixture was emulsified for 5 min. A mixture of 1 mL of emulsified solution, 0.5 mL of enzyme source and 0.5 mL of phosphate-citrate buffer was incubated for 4 hours at 37°C. To stop the reaction and break the emulsion, 3 mL of 1:1 ethanol-acetone was added. To the reaction mixture, phenolphthalein in ethanol 1% (w/v) was added titrated against 0.01 N NaOH. For the blanks, the same procedure was followed and boiled enzyme was used. Porcine type 2 Lipase was used as standard.

### 10.2.2G. Activity 7. *Assessing of immune enzyme activity (Pro-Phenoloxydase, Pro-PO and Superoxide dismutase, SOD) at without and with probiotic treated prawn*

***Measurement of Prophenoloxidase*** **(proPO)**

Phenoloxidase activity in haemocytes was measured spectrophotometrically by recording the formation of dopachrome from L-dihydrophenylalanine (L-DOPA)(Moullac et al. (1997). Haemocyte suspension was incubated with zymosan and transferred to microtitration plate wells. L-DOPA (L-dihydroxyphenylalanine, 4 $mgml^{-1}$ of cacodylate buffer) was added to haemocyte suspension. After 10 min, absorbance was measured at 490 nm using ELISA reader.

***Determination of* Superoxide dismutase (*SOD)***

Superoxide dismutase enzyme activity was determined according to Marklund and Marklund with some modifications of Jing and Zhao (Marklund and Marklund, 1974). The principle of this method was based on the competition between the pyrogallol autoxidation by $O^{2-}$ and the dismutation of this radical by SOD. In order to calculate the enzyme activity, SOD assay was performed with crude enzyme. Tris-EDTA buffer (2.35 mL) and distilled water (2.00mL) was taken into a test tube. Pyrogallol solution (0.15mL) was added into the tube and immediately vortexed. Then two aliquots 1% and 10% were made with distilled water. Thus, the blank solution was prepared. In case of sample solution, about 20µl of sample was added to the solution after vortexed. After that optical density was measured at 325 nm using ELISA reader. Temperature of the room was maintained at 30±2°C throughout the entire study.

### 10.2.2H. Activity 8. *Antagonistic effect of probiotics against pathogenic bacteria*

Antagonism of isolated probiotics against *Vibrio* spp. by the bacterial isolates was assessed by disk and diffusion method. For this purpose, at first the media was dried to 60°C for 15 minutes and was poured into petri dishes and allowed to solidify. To test the antagonistic effects of probiotics against *Vibrio* spp. on solid media, the disk diffusion test was performed. Agar plates containing 20 ml of agar. After that taking 1ml of probiotic solution and spreading properly on the solid media. After few minutes later soaked a blank disk into *Vibrio* spp. solution. Then, treated disk was aseptically placed using forceps onto the agar surface of the plates inoculated with the probiotic. The plates were incubated at 37°C for 24 hours.

### 10.2.2I. Activity 9. *In-vivo Challenge test using pathogenic bacteria on survival of prawn PL in without and with probiotics*

After completing *in vitro* test, it is very essential to conduct *in vivo* test for determine that is it better suited for observing the overall effects of the experiment on a living subject. *In vivo* is crucial because *in vitro* assays can sometimes yield misleading results. For conducting this study, wild shrimp post larvae (PL) were collected. Then the experiment was conducted in the Laboratories of Fisheries and Marine Resource Technology Discipline, Khulna University. This study was conducted to determine the antagonistic effect of probiotics by counting survival (of prawn against pathogenic *Vibrio* spp. through a challenge test. The *Vibrio* spp. concentrations at this study considered as $10^6$ CFU/mL in an *in vivo* challenge test with shrimp larvae. For the challenge test, 20 PL were stocked in 20 liters plastic tanks containing 15 L water. The PLs were acclimatized for five days before inoculating the bacteria. At that time just feed and probiotics were given. Environmental parameters like dissolve oxygen, temperature and pH were maintained at a constant level. After acclimatization, the experimental tanks were inoculated with desired bacterial density through spectrophotometric observation. Only 5-10% water was adjusted daily to compensate the loss through evaporation and syphoning. Survival was checked daily.

**Table 11.** Experimental design for antagonistic challenge test of probiotics against pathogenic *Vibrio* spp.

| Control | | Treatment | | |
|---|---|---|---|---|
| Negative Control (PL+No Probiotic+ No *Vibrio*) | Positive Control (PL+ No Probiotic+ *Vibrio*) | PL+ *Vibrio*+ Probiotics P1 | PL+ *Vibrio*+ Probiotics P2 | PL+ *Vibrio*+ Probiotics P3 |
| $NC_1$ | $PC_1$ | $Tp1R_1$ | $T_{p2}R_1$ | $T_{P3}R_1$ |
| $NC_2$ | $PC_2$ | $T_{p1}R_2$ | $T_{p2}R_2$ | $T_{P3}R_2$ |
| $NC_3$ | $PC_3$ | $T_{P1}R_3$ | $T_{p2}R_3$ | $T_{P3}R_3$ |

NC= Negative control: Only PL No probiotic and No *Vibrio*; PC= Positive control: PL and*Vibrio* presence but No Probiotic

### 10.2.2J. Activity 10. *Effect of probiotic bacteria on the growth of other beneficial microflora in prawn gut was identified*

This experiment was conducted to evaluate the effect of different probiotics on the gut microbiota of *M. rosenbergii*. The experiment also aimed to see whether the inoculation of probiotic does flourish the amount of other beneficial bacteria in the gut or not? The post-larvae of *M. rosenbergii* were cultured for 120 days in a nursery pond supplemented with proper food and artificial aeration. Ten juveniles of uniform size (mean weight 15±2.3g) were placed in a 25 L plastic tank containing 20 L water and were acclimatized for 1 week before the trials began. The research was designed with 8 treatments and each were triplicated (Table 12).

**Table 12.** Experimental design for the challenge test of probiotic treated prawn with *Vibrio* spp.

| | Prawn and feed | Pathogen (*Vibrio* spp.) | Probiotic (*Bacillus* spp.) | Probiotic (*Lactobacillus* spp.) | Probiotic (*Chlostridium*spp.) |
|---|---|---|---|---|---|
| Cn | + | - | - | - | - |
| Cv | + | + | - | - | - |
| T1 | + | - | + | - | - |
| T2 | + | + | + | - | - |
| T3 | + | - | - | + | - |
| T4 | + | + | - | + | - |
| T5 | + | - | - | - | + |
| T6 | + | + | - | - | + |

*Note*. Indication: "+" denotes applied whereas "–"stands for not applied in the treatment.

### 10.2.2K. Activity 11. *Observation of prawn growth and production at different carbohydrate enriched ingredients*

This study was conducted to evaluate the effect of locally available carbohydrate enriched ingredients on the growth performance of *M. rosenbergii.* Three locally available ingredients like molasses, rice polish, and maize powder were used as carbon source and prawn was fed on commercial feed containing 28% protein. Prawn post larvae (PL) was collected from hatchery and nursed for 45 days. Stocking density was same in all the 7 treatments 3 juvenile/$m^2$, same amount probiotic (P1) 250g/month/ha was applied in all the treatments as seed.

**Table 13.** Experimental design on application of molasses, rice polish, maize powder in prawn culture and growth observation of native beneficial bacteria

| Treatment | Prawn and feed | Carbohydrate enriched ingredients | | |
|---|---|---|---|---|
| | | Molasses | Rice polish | Maize powder |
| **C** | + | _ | _ | _ |
| **T1** | + | + | _ | _ |
| **T2** | + | _ | + | _ |
| **T3** | + | _ | _ | + |
| **T4** | + | + (1/2) | +(1/2) | _ |
| **T5** | + | +(1/2 | _ | +(1/2) |
| **T6** | + | _ | +(1/2) | +(1/2) |
| **T7** | + | + (1/3) | +(1/3) | +(1/3) |

Molasses (25 kg/ha), rice polish (25 kg/ha), maize powder (25 kg/ha),molasses (12.5kg) + rice polish (12.5kg), molasses (12.5kg)+ maize (12.5kg), rice polish (12.5kg) +maize (12.5kg),molasses(8.33kg) + rice polish (8.33kg) + maize (8.33) were applied weekly in T1, T2, T3, T4, T5, T6 and T7 respectively; no carbohydrate enriched ingredient used in control pond. Each treatment was replicated in three equal ponds (Table 13) and prawn was culture for 180 days.

### 10.2.2L. Activity 12. *Locally available carbohydrate enriched ingredients (Molasses, Rich polish and Maize powder) were applied in pond to know the growth of beneficial bacteria*

The experiment was aimed to identify three beneficial bacteria (probiotics) species *Bacillus* spp.*, Chlostridium* spp.*, Lactobacillus* spp. and their load in the ponds where carbon enriched ingredients were used (Table 13).In the control pond no additional carbon source was applied except feed containing 28% protein. To enumerate enrichment of native bacterial colony in different carbohydrates treated pond, water sample was collected from each treated pond and native bacterial load was counted. Stock solution was serially diluted to target fold adding peptone water. Selective agar was weighted and autoclaved and water solution was inoculated through pour plating method. The Petri dish were kept at 37° C for 24 hours and then colony were counted. Counting the colony, the suitable dilution factors were defined for each treatment. Then each defined diluted sample were inoculated, incubated and counted maintaining replications.

### 10.2.2M. Activity 13. *Culture and storage possibility of three main native probiotics (Bacillus, Lactobacillus and Clostridium spp.) through heat shock treatment*

To culture the native three probiotics bacteria (*Bacillus, Lactobacillus and Clostridium* spp) water sample was collected from control pond where no carbohydrate was used. The bacterial growth was observed in collected pond water (control) and applying locally available carbohydrate enriched ingredients like molasses, rice polish and maize powder as per Table 14.

**Table 14.** Experimental design for culture of probiotic bacteria using carbohydrate enriched ingredients (molasses, rice polish, maize powder)

| Treatment | Pond water 1ml | Molasses 1% | Rice Polish 1% | Maize Powder 1% | Replications |
|---|---|---|---|---|---|
| Control | + | – | – | – | 3 |
| T1 | + | + | – | – | |
| T2 | + | – | + | – | |
| T3 | + | – | – | + | |
| T4 | + | + | + | – | |
| T5 | + | + | – | + | |
| T6 | + | – | + | + | |
| ***Note*.** Indication: "+" denotes applied whereas and "–"stands for not applied in the treatment. | | | | | |

***Preparation of Stock solution***

The collected water samples were taken at 15 ml test tubes and centrifuged at 4000 rpm for 4 minutes. After the centrifugation, the stock solution was prepared. To dilute this stock solution, 0.1 ml of the stock was transferred to each 15 ml test tube containing 0.9 ml peptone water solution and this provided a dilution of $10^{-1}$. Again 0.1 ml sample from the dilution-1 tube was transferred to another test tube of 0.9 ml to give a dilution of $10^{-2}$. The process was repeated for the preparation of $10^{-3}$, $10^{-4}$ and $10^{-5}$dilutions respectively. The entire process was conducted with micropipettes and all the activities were conducted inside the bio safety cabinet.

***Media Preparation and Autoclave***

Definite amount of selective plate counting agar was weighed using electric balance and mixed properly through magnetic stirrer as per company's instruction. These were taken in separate volumetric flasks (500 ml) containing distilled water. Then the final volume was adjusted by pouring additional distilled water. After that, the volumetric flasks were kept in autoclave for sterilization. Autoclave was done at a temperature of 121°C and 15 lbs pressure for 2 hours and 30 min. After autoclaving, the agar media were kept in the bio-safety cabinet for 10-20 min for cooling.

***Bacteria Culture***

The prepared media was poured to different petri dishes by 20 ml volume per petri dish for preparing the plates. 100 µL sample of each test tube subjected to 10-fold dilution were transferred to different plates by using micropipettes. The plates were then kept at the incubator at 37°C for 24 hours to let them grow. After the incubation, a single colony from each plate was added to 15 ml test tubes containing 5 ml peptone water. The test tubes containing single colony bacterial culture solution of different species were kept at the incubator at 37°C for 24 hours in order to grow them properly. After incubation, the turbidity of the test tubes were observed using Spectrophotometer to know the growth of bacterial cells.

***Heat Treatment***

The test tube of cultured bacteria was kept in incubator at 45, 50, 55 and 60°Ctemperature for 30 minutes to create encapsulation. 100 µL of each test tube subjected to 10-fold dilution were transferred to different petri dishes of different agar media and incubated overnight (24 hours) for colony counting to obtain initial colony forming unit (CFU). Single colony culture was performed in several test tubes among which the half were used for counting initial colony forming unit (CFU) and the rest were used for storage purpose. Same way heat treatment was done at 50, 55 and 60°C. Heat treatment was done to observe the of storage possibility of indigenous beneficial bacteria.

***Storage***

Definite amount of single colony bacterial culture solution from each test tube was taken into several eppendorf tubes. Then 20% glycerol was added into each eppendorf tube at required ratio (1:1) and mixed well through Vortex machine. The glycerol culture in each eppendorf tube was stored at −80 °C. After storage of 15 days, the stored eppendorf tubes were given a water bath and it turned into liquid from solid. Then the re-culture was done following the above protocol with spread plating method to obtain the final CFU/ml. The survival rate was calculated using the following formula:

**Survival Rate (%) = (Final CFU/ml ÷ Initial CFU/ml) × 100**

The same procedure was repeated for 50, 55 and 60°C respectively.

# 11. Results and discussion

## 11.1. BFRI (SRS) Component

### 11.1.1. Expt. 1. Development of Cluster Based Shrimp Farming (CBSF) to Cope with Disease Outbreak in Shrimp Aquaculture

Under these studies, in the first year, shrimp were cultured for 4 months in two different stocking density. Growth, water quality parameters and disease screening information during the culture period were found satisfactory. No disease outbreak was recorded throughout the culture period, which was one of the great achievements of this study. Besides, from the traditional gross production of around 250-300 kg/ha, with this management intervention, production maximized more than two times (780 kg/ha at the stocking density of 3 PL/$m^{-2}$ in T1R1 and 856 kg/ha at the stocking density of 6 PL/$m^{-2}$ in T2R1) compared to the traditional farming.
Once getting significant production of 856 kg/ha at the stocking density of 6 PL/$m^{-2}$ with aeration, in the second year, emphasize given on to optimize maximum stocking density to increase per ha production. Therefore, instead 3 and 6 PL/$m^{-2}$, treatment designed to 6 and 9 PL/$m^{-2}$ to observe in this higher stocking can be sustained without aeration.

Unfortunately, due to corona pandemic and lockdown situation, stocking of PL became delay. Due to heavy rainfall, salinity fluctuation was higher and average salinity throughout the culture period was around 2 ppt, which is considered as low salinity for the shrimp growth. Without aeration, higher stocking density of 9 PL/$m^{-2}$ found not suitable and the production dropped to 566 kg/ha at T2R2. Due to lower salinity overall survival found 15% less than the first-year survival. For the same reason, production dropped to 734 kg/ha in T1R1 and 653 kg/ha in T1R2 with the stocking density of 6 PL/$m^{-2}$.

Even though the average production dropped to 693.6 kg/ha with the stocking density of 6 PL/$m^{-2}$, is still more than double than the average traditional production of shrimp i.e., 300 kg/ha.

**Table 15.** Shrimp production of different ghers of cluster of 1st year

| | Stocking Density (Per $m^2$) | Avg. Final Weight (x±sd)) | Survival Rate (%) | Production (Per Ha) |
|---|---|---|---|---|
| $T_1R_1$ | 3 | 52±4.36 | 50 | 780 |
| $T_1R_2$ | 3 | 48±3.21 | 48 | 691.2 |
| $T_2R_1$ | 6 | 34±10.58 | 42 | 856.8 |
| $T_2R_2$ | 6 | 35.5±5.15 | 50 | 852 |

**Table 16.** Water quality data (Mean±SD) of different ghers of cluster of 1st year

| | Temperature ($^0$C) | $P^H$ | Salinity (ppt) | Alkalinity (mg/L) | Ammonia (mg/L) | Oxygen (mg/L) | Iron |
|---|---|---|---|---|---|---|---|
| T1R1 | 29.17±1.57 | 8.33±0.24 | 4.83±0.90 | 120±11.55 | 0.43±0.21 | 6.33±1.60 | 0.37±0.20 |
| T1R2 | 28.67±0.94 | 8.17±0.24 | 3.11±1.45 | 102.22±14.74 | 0.31±0.28 | 6.56±0.83 | 0.2±0.16 |
| T2R1 | 29±1.26 | 8.32±0.50 | 3.55±1.67 | 112.73±21.36 | 0.32±0.24 | 6.91±1.50 | 0.57±0.31 |

| | Temperature ($^0$C) | P$^H$ | Salinity (ppt) | Alkalinity (mg/L) | Ammonia (mg/L) | Oxygen (mg/L) | Iron |
|---|---|---|---|---|---|---|---|
| T2R2 | 29.25±1.09 | 8.3±0.33 | 3.6±1.74 | 110±16.12 | 0.3±0.27 | 7.1±1.51 | 0.4±0.19 |

**Table 17.** Shrimp production of different ghers of cluster of 2nd year

| | Stocking Density (Per m$^2$) | Survival Rate (%) | Avg. Final Weight (x±sd)) | Production (Per Ha) |
|---|---|---|---|---|
| T1R1 | 6 (64 Dec) | 35 | 35±2.62 | 734 |
| T1R2 | 6 (65 Dec) | 33 | 33±3.27 | 653.144 |
| T2R1 | 9 (67 Dec) | 22 | 32±3.58 | 633 |
| T2R2 | 9 (72 Dec) | 21 | 30±4.51 | 566 |

**Table 18.** Water quality data (Mean±SD) of different ghers of cluster of 2nd Year

| | Temperature ($^0$C) | P$^H$ | Salinity (ppt) | Alkalinity (mg/L) | Ammonia (mg/L) | Oxygen (mg/L) | Iron |
|---|---|---|---|---|---|---|---|
| T1R1 | 28.26±1.37 | 8.12±0.30 | 2.75±0.96 | 117±13.52 | 0.36±0.12 | 6.54±1.57 | 0.37±0.12 |
| T1R2 | 28.17±0.57 | 8.16±0.21 | 2.11±1.04 | 112.52±12.53 | 0.28±0.17 | 6.78±0.62 | 0.28±0.15 |
| T2R1 | 28.4±1.11 | 8.23±0.42 | 2.55±1.32 | 109.98±24.33 | 0.35±0.21 | 6.83±1.48 | 0.53±0.23 |
| T2R2 | 28.22±0.95 | 8.13±0.13 | 2.68±1.11 | 115±18.54 | 0.29±0.18 | 6.9±1.44 | 0.39±0.16 |

### 11.1.1A. Study-1. Development of PCR protocol for identification and characterization of OiE listed pathogens in Prawn/Shrimp aquaculture

Laboratory based activities are mainly dependent on the collected samples from the farmer's pond and different rivers. Due to the Covid-19 situation of Corona Pandemic, field sampling was hampered for the first couple of months. Due to the lack of available PL, farmers also failed to stock PL in their farms, resulted almost no disease outbreak report in this year. However, PCR Identification protocol for all the OIE Listed Pathogens for shrimp and prawn has been standardized. PCR were optimized based on the given temperature at Table 19.

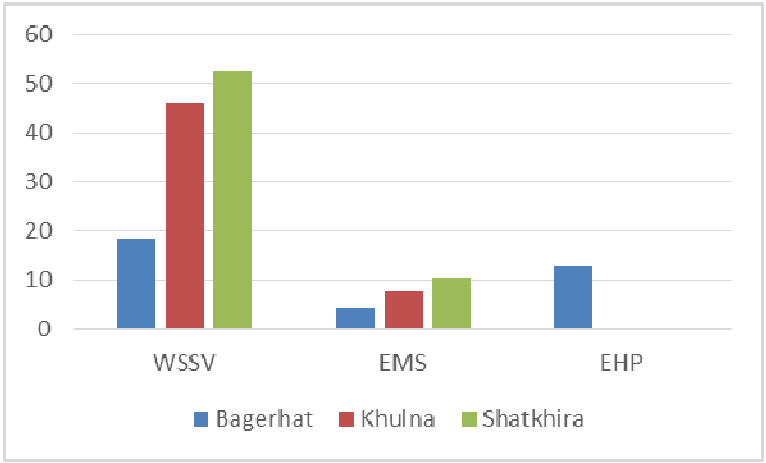



**Fig 1.** District wise distribution of identified pathogens.

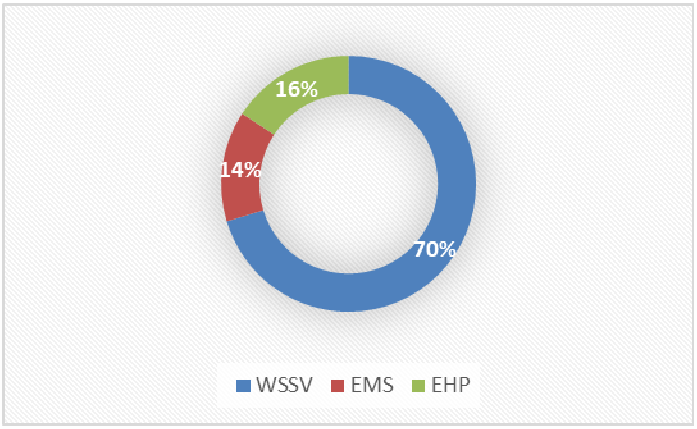



**Fig 2.** Prevalence of identified pathogens.

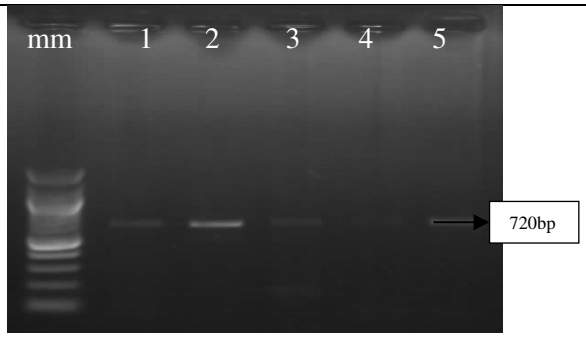



**Fig 3.** NHP Gel UV Illumination Picture for gradient PCR, mm: molecular marker, 1—5: Positive control

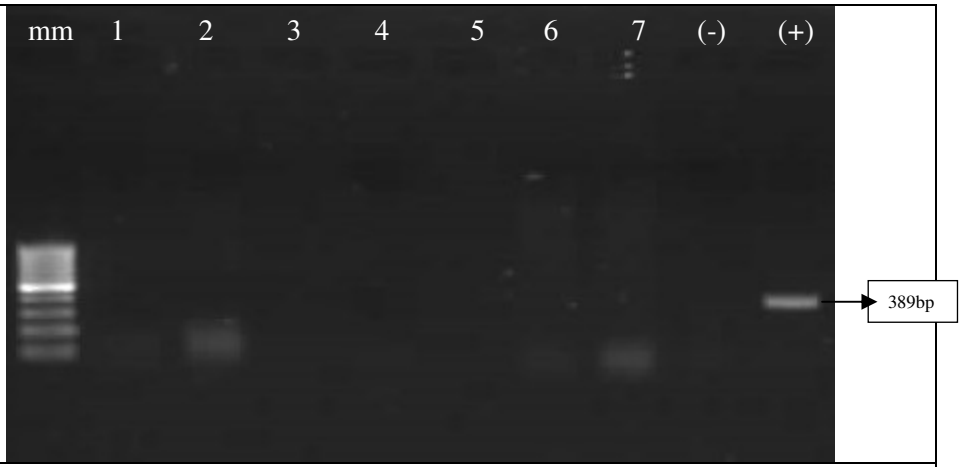



**Fig 4.** IHNNV Gel UV Illumination Picture, mm: molecular marker, 1—7: (Sample-1-7), (-): Negative Control, (+): Positive control

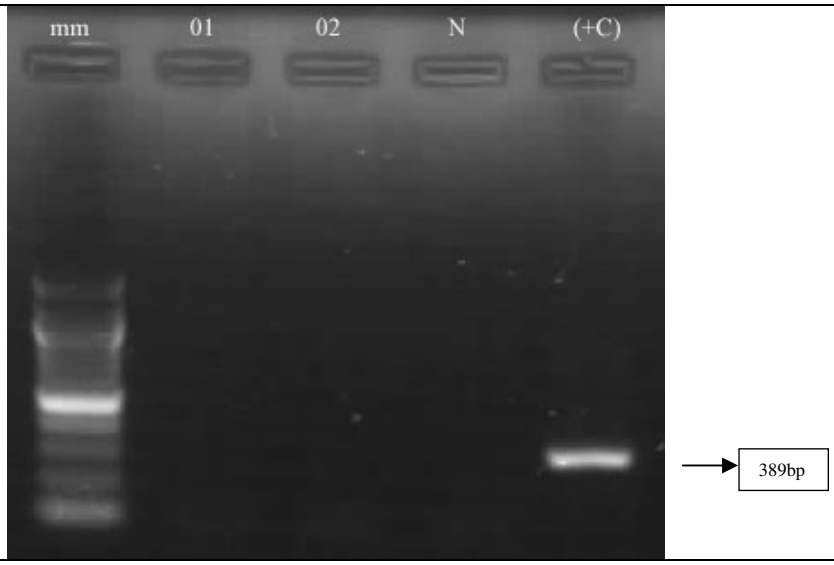



**Fig 5.** YHV Gel UV Illumination Picture, mm: molecular marker, 1—2: (Sample-1-2), (N): Negative Control, (+C): Positive control

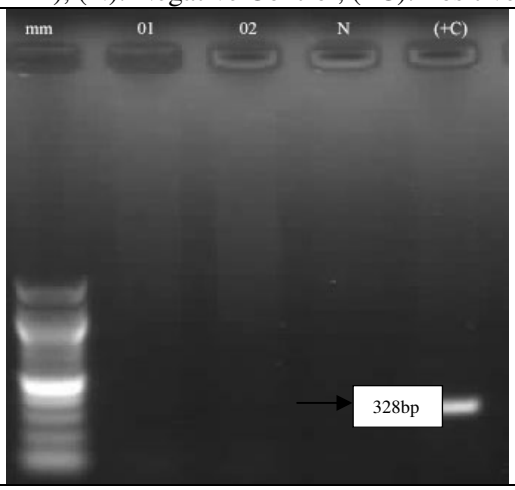



**Fig 6.** IMNV Gel UV Illumination Picture, mm: molecular marker, 1—2: (Sample-1-2), (N): Negative Control, (+C): Positive control

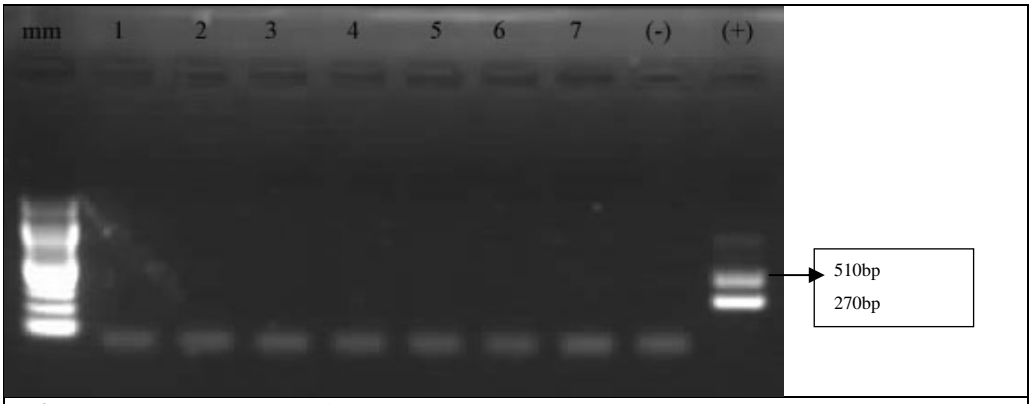



**Fig 7.** EHP Gel UV Illumination Picture, mm: molecular marker, 1—7: (Sample-1-7), (-): Negative Control, (+): Positive control

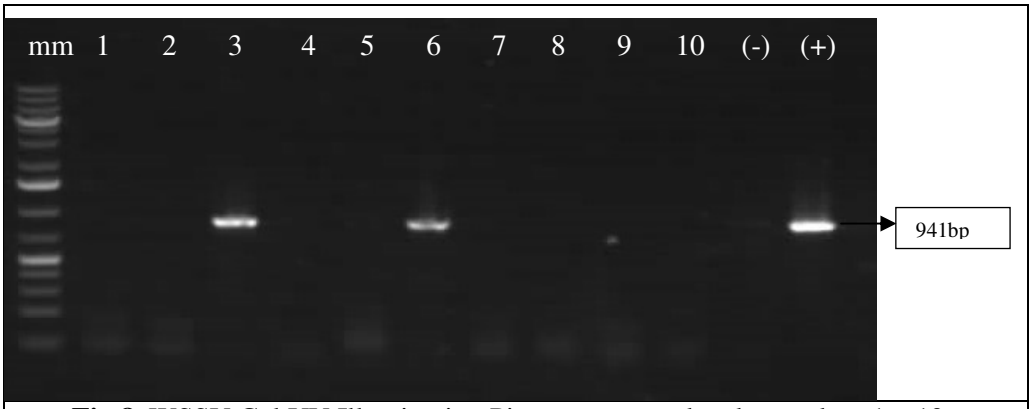



**Fig 8.** WSSV Gel UV Illumination Picture, mm: molecular marker, 1—10: (Sample-1-10), (-): Negative Control, (+): Positive control

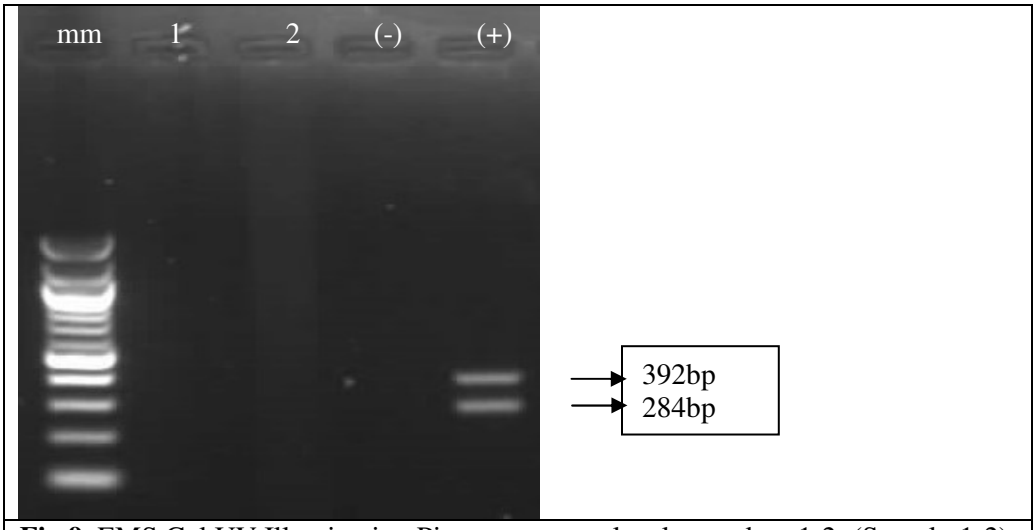



**Fig 9.** EMS Gel UV Illumination Picture, mm: molecular marker, 1-2: (Sample-1-2), (-): Negative Control, (+): Positive control

**Table 19.** PCR optimization protocol for disease identification.

| Pathogen | Temperature ($^0$C) | Time | Number of Cycles |
|---|---|---|---|
| EHP (1st) | 95 | 3 min | 1 |
| | 95, 58, 72 | 25 sec, 30 sec, 35 sec | 34 |
| | 72 | 3 min | 1 |
| EHP (2nd) | 95 | 3 min | 1 |
| | 95, 57, 72 | 25 sec, 30 sec, 35 sec | 34 |
| | 72 | 3 min | 1 |
| IMNV (1st) | 60 | 30 min | 1 |
| | 95 | 2 min | 1 |
| | 95, 60 | 45 sec,450 sec | 38 |
| | 60 | 7 min | 1 |
| IMNV (2nd) | 95 | 2 min | 1 |
| | 95, 65, 72 | 30 sec, 30 sec, 30 sec | 38 |

| Pathogen | Temperature ($^0$C) | Time | Number of Cycles |
|---|---|---|---|
| | 72 | 2 min | 1 |
| IHHN | 95 | 5 min | 1 |
| | 95, 55.7, 72 | 30 sec, 30 sec, 30 sec | 35 |
| | 72 | 5 min | 1 |
| YHV-1 (1st) | 50 | 30 min | 1 |
| | 94, 95, 66 | 2 min, 30 sec, 30 sec | 34 |
| | 68 | 7 min | 1 |
| YHV-1 (2nd) | 95 | 2 min | 1 |
| | 95, 66, 72 | 30 sec, 30 sec, 45 sec | 34 |
| | 72 | 7 min | 1 |
| NHP (1st) | 95 | 3 min | 1 |
| | 95, 56, 72 | 30 sec, 30 sec, 45 sec | 34 |
| | 72 | 5 min | 1 |
| NHP (2nd) | 95 | 3 min | 1 |
| | 95, 56, 72 | 20 sec, 25 sec, 30 sec | 34 |
| | 72 | 5 min | 1 |

### 11.1.2. Expt.-2. Development of all-male Prawn PL production using Neo-female technology

For the experiment production of prawn, known aged PL is essential. Therefore, PL must be produced in hatchery condition. To meet the criteria, Prawn PL was successfully produced in SRS Prawn Hatchery. As PL 45 to PL 60 is required for the microsurgery of androgenic gland ablation, PL were nursed in the hatchery cistern for the required Days.

After getting required aged PL, several batches of PL (500 PLs) operated for micro surgery. Only 2% PL survived but unfortunately all found male when checked. The poor survival rate was due to the stress under the microscope and as male prawn are generally sturdy, so the survived prawn was all-natural male. It was a good sign to become all-individual as male but it would have been better to get some female which were basically male at its birth. Only at that point those individuals could be act as neo-female and can successfully produce all male population of prawn after mating with natural male.

In short, microsurgical ablation of androgenic gland is a complicated task and required subsequent trials. One-year trial is not sufficient for such kind of sophisticated research where timing and perfection is mandatory and it comes by practice. This was a start and with all the established facilities, trial will be continued in the coming days to make all these efforts successful.

### 11.1.3. Expt.-3. An insight into innate immune response of shrimp resisting viral and bacterial disease

Under this experiment, first priority was given to WSSV protein cloned into PcDNA/PET32 Vector plasmid followed by bacterial flagellum/attenuated bacteria/LPS. But supply of few necessary reagents was not possible due to corona pandemic. As the project did not get extension, effort was given to see the immune booster capacity of the killed/attenuated

bacteria administrated with feed.

Feed containing attenuated bacteria administrated for three weeks and as a mean of evaluation of immune response, total haemocytes were counted and compared with the control group. The enriched feed gave almost double haemocytes count compared to the control group (Fig. 10). This is a very good indication of increased immunity as in crustaceans' innate immunity act as the main force of disease defence mechanism where haemocytes play the main role inhibiting pathogenic agents into the biological system of the shrimp. Due to delayed start of the experiment for covid pandemic and the project did not get even no cost extension, conducting challenge experiments were not possible.

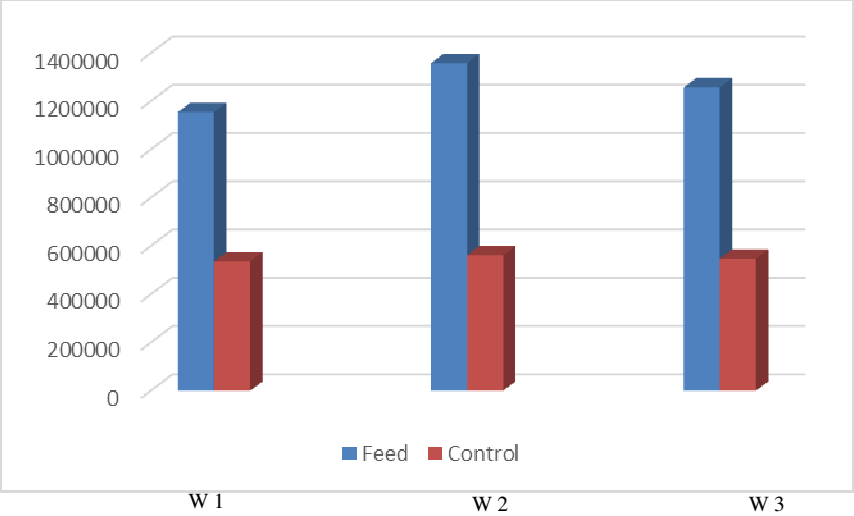



**Fig 10.** Hemocyte count on different weeks.

### 11.1.3A. Study-2. Identification of genetic variability of *M. rosenbergii* in the major brackish water rivers (Kocha, Payra, Baleswar, Rupsha and Poshur) of Bangladesh

Under this study four wild populations from the coastal rivers viz. Kocha, Payra, Baleswar, Rupsha, Poshur, Kornofuli, Meghna and Kumar Nod were collected for the study of genetic variability & diversity within the population. Genomic DNA extracted from the tissue samples collected from pleopods. Approximately 25-mg tissues cut into small pieces, homogenized by micro-tissue-grinder in a 1.5 ml microfuge tube, and total genomic DNA extracted following the instruction provided with Thermo Scientific's Pure link DNA extraction kit.

For the microsatellite study of genetic variation, the entire extracted DNA multiplied by polymerase chain reaction following the given PCR optimization. Getting the PCR products refers to 80% completion of the activity.

**Table 20.** PCR Optimization for microsatellite DNA multiplication

| Microsatellite Location | Temperature ($^0$C) | Time | Number of Cycles |
|---|---|---|---|
| Mbr (1, 2, 9, 10) | 94 | 3 min | 1 |
| | 94, 60, 72 | 30 sec, 45 sec, 1min | 34 |
| | 72 | 7 min | 1 |
| Mbr (3-8) | 94 | 3 min | 1 |
| | 94, 62, 72 | 30 sec, 45 sec, 1min | 34 |
| | 72 | 7 min | 1 |

Getting the PCR products refers to 80% completion of the activity. To evaluate the variability, those primer products should be analyzed by SDS Page gel electrophoresis, but due to corona pandemic procurement of few essential reagents was not possible.

These PCR products stored duly and can be analyzed to study genetic variability if the required reagents get available.

**Plate 1.** Sampling of shrimp

**Plate 2.** Harvesting of shrimp

**Plate 3.** Sampling from different shrimp farm

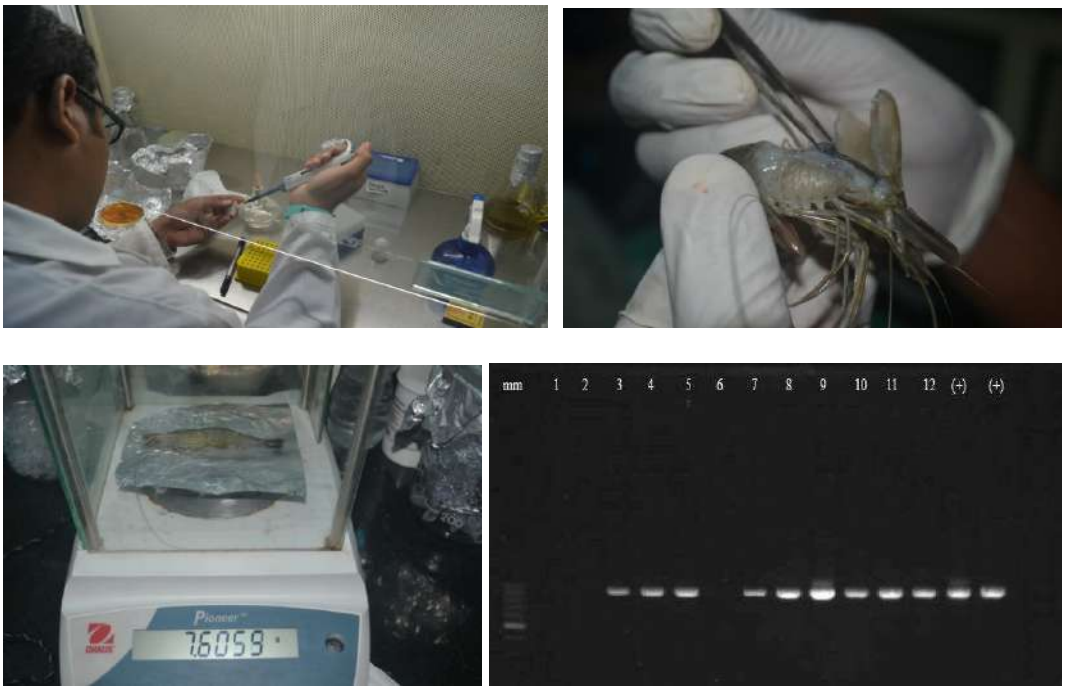



**Plate-4.** Preparation of shrimp sample for disease diagnosis & PCR Band

**Plate-5.** Microsurgery Operation for All male prawn production

**Plate-6.** Hatchery operation after microsurgery operation for All male prawn production

**Plate-7.** Sampling of prawn brood prawn from different river

## 11.2. Khulna University Component

### 11.2.1. Activity-1. Baseline survey to know current culture scenario and available probiotics in Khulna region

From the baseline survey it was observed that mostly traditional prawn polyculture and rice-prawn rotation were practiced where production ranged from 350-625 kg/ha. Seventeen probiotics were found surveying form the local respondents. The mostly useable probiotics and their compositions are shown in table 7.

### 11.2.2. Activity-2. Growth and production performance of prawn at different probiotics for the selection of better competent commercial probiotics.

**Table 21.** Growth (g), survival (%) and production kg/ha performance of freshwater prawn applying 6 commercial probiotics at stocking density 2 juvenile/$m^2$ where replication was 3 in each treatment

| Treatment | Probiotic Name | Initial Wt (g) | Final Wt (g) | Survival (%) | Production (Kg/Ha) | Probiotic Code Name as per Production kg/ha |
|---|---|---|---|---|---|---|
| C | Nil | 6.82 | 40.17±11.8 | 82.38 | 661.64 | Nil |
| T1 | Aqua Clear S | | 48.77±14.3 | 87.94 | 857.70 | Probiotic 1 (P1) |
| T2 | Zymatin | | 48.04±12.6 | 87.55 | 841.20 | Probiotic 2 (P2) |
| T3 | Super Biotic | | 47.43±13.5 | 85.23 | 808.56 | Probiotic 3 (P3) |
| T4 | Aquaback P | | 45.97±11.4 | 85.90 | 789.84 | Probiotic 6 (P6) |
| T5 | Biotics | | 45.90±10.7 | 86.78 | 796.63 | Probiotic 5 (P5) |
| T6 | Super PS | | 46.20±12.9 | 87.16 | 805.44 | Probiotic 4 (P4) |

Considering availability and used (%), 6 commercial probiotics were selected for trial to justify their effect on prawn growth and production. Depending on prawn production/ha the commercial probiotics are denoted as P1, P2, P3, P4, P5, P6 and production were found 857.70, 841.20, 808.56, 805.44, 796.63 and 789.84 kg/ha respectively (Table 21). All the probiotic treated pond showed higher production than the without probiotic 661.64 kg/ha. According to production performance the top three probiotics P1, P2 and P3 were selected for next successive culture.

### 11.2.3. Activity-3. Intensification of prawn in non-aerated and aerated culture system by applying best performed probiotic from first year experiment

**Table 22.** Growth and production (kg/ha) performance of *M. rosenbergii* under different probiotic treatments without aeration

| Treatment | Probiotics Applied | Stocking density (/$m^2$) | Initial weight (g) | Final Weight (g) | Weight Gain (g) | Survival (%) | Production kg/ha |
|---|---|---|---|---|---|---|---|
| C1 | nil | 2 | 2.06±0.35 | 36.24±15.7 | 34.18±12.5 | 85.01 | 616.08 |
| T1 | P1* | | | 49.77±12.5 | 47.71±11.7 | 89.98 | 895.68 |
| T2 | P2* | | | 45.56±11.4 | 43.50±12.3 | 85.02 | 774.69 |
| T3 | P3* | | | 47.92±13.6 | 45.86±11.4 | 86.89 | 832.76 |
| C2 | nil | 4 | 2.55±1.03 | 30.74±14.6 | 28.19±9.7 | 70.27 | 864.08 |
| T4 | P1 | | | 43.04±13.8 | 40.49±8.8 | 74.02 | 1274.28 |
| T5 | P2 | | | 40.34±11.8 | 37.79±8.4 | 72.84 | 1175.30 |
| T6 | P3 | | | 41.61±14.2 | 39.06±9.7 | 75.01 | 1248.60 |
| C3 | nil | 6 | 2.17±0.75 | 25.36±9.5 | 23.19±6.7 | 57.79 | 879.40 |
| T7 | P1 | | | 35.25±11.2 | 33.08±7.3 | 61.12 | 1292.71 |
| T8 | P2 | | | 33.14±9.5 | 30.97±7.6 | 60.97 | 1212.19 |
| T9 | P3 | | | 33.70±7.9 | 31.53±8.2 | 59.95 | 1256.4 |

**Outcome:** In without aeration all three probiotics (P1, P2 and P3) treated prawn showed higher prawn production than the without probiotic (nil), and within the three probiotics P1 showed comparatively higher prawn production in all stocking densities. Stocking density 4/$m^2$ could be suggested for probiotic based without aeration culture system (Production: 1274.28 kg/ha). Net benefit 4,00,818TK/ha could be earned stocking 4 juvenile/$m^2$ (Table 22)

**Table 23.** Cost Benefit analysis probiotic based prawn culture without aeration where stocking density was 20000, 40000 and 60000juvenile/Ha and culture period 180 days

| | Subject | Amount | Unit | Unit Price (Taka) | Total Tk (20000/ha) | Total Tk (40000/ha) | Total Tk (60000/ha) |
|---|---|---|---|---|---|---|---|
| 01 | Mud excavation and dyke repair | 50 | Person | 500 | 25,000/- | 25,000/- | 25,000/- |
| 02 | Labor cost during culture | 60 | Person | 500 | 30,000/- | 30,000/- | 30,000/- |
| 03 | Nylon Net | 500 | Meter | 25 | 12,500/- | 12,500/- | 12,500/- |
| 04 | Post Larvae | 20,000 | Ha | 2 | 40,000/- | | |
| | | 40,000 | Ha | 2 | | 80,000/- | |
| | | 60,000 | Ha | 2 | | | 1,20,000/- |
| 05 | Feed | 1550 | Kg | 55 | 85,250/- | | |
| | | 2200 | Kg | 55 | | 1,21,000/- | |
| | | 2250 | Kg | 55 | | | 1,42,000/- |
| 08 | Probiotics | | | | 35,000/- | 35,000/- | 35,000/- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 09 | Lime | 610 | Kg | 25 | 15,250/- | 15,250/- | 15,250/- |
| 10 | Miscellaneous | | | | 20,000/- | 20,000/- | 20,000/- |
| 11 | Harvesting | | | | 15,000/- | 15,000/- | 15,000/- |
| 12 | Marketing | | | | 10,000/- | 10,000/- | 10,000/- |
| Total Expenditure (Taka) = | | | | | 2,88,000/- | 3,63,750/- | 4,24,750/ |
| Income (Considering 600Tk/kg) | | | | | 5,37,408/- | 7,64,568/- | 7,75,626/- |
| Net Benefit (B-A) | | | | | 2,49,408/- | 4,00,818/- | 3,50,876/- |
| Note: Prawn production was 895.68, 1274.28 and 1292.71kg in stocking density 20000, 40000 and 60000 juvenile/ha. | | | | | | | |

**Table 24.** Growth and production performance of *M. rosenbergii* under different probiotic treatments with aeration

| Treatment | Probiotics Applied | Stocking density (/$m^2$) | Initial weight (g) | Final Weight (g) | Weight Gain (g) | Survival (%) | Production kg/ha |
|---|---|---|---|---|---|---|---|
| C1 | nil | 4 | 1.58±0.47 | 31.25±10.9 | 29.67±6.7 | 79.68 | 996.14 |
| T1 | P1* | | | 44.99±11.3 | 43.41±7.3 | 83.06 | 1494.66 |
| T2 | P2* | | | 40.88±10.6 | 39.30±9.2 | 81.74 | 1336.50 |
| T3 | P3* | | | 43.49±11.7 | 41.91±7.5 | 82.11 | 1428.44 |
| C2 | nil | 8 | 2.05±0.75 | 25.98±8.7 | 22.79±7.5 | 65.01 | 1351.30 |
| T4 | P1 | | | 35.03±7.2 | 32.93±5.9 | 67.99 | 1905.09 |
| T5 | P2 | | | 31.37±6.7 | 29.32±5.6 | 67.25 | 1687.92 |
| T6 | P3 | | | 33.68±7.8 | 31.63±6.3 | 65.14 | 1755.00 |
| C3 | nil | 12 | 2.15±0.5 | 22.15±6.4 | 20.00±8.1 | 51.88 | 1379.04 |
| T7 | P1 | | | 29.12±8.2 | 26.97±7.7 | 59.01 | 2061.70 |
| T8 | P2 | | | 27.88±7.1 | 25.73±5.9 | 57.89 | 1936.97 |
| T9 | P3 | | | 28.43±6.9 | 26.28±5.8 | 59.23 | 2020.63 |

**Outcome**: In probiotic based aerated system stocking density 8/$m^2$(1905.09kg/ha, could be suggested for field application considering the production and cost benefit (Table 24 & 25).

**Table 25.** Cost Benefit analysis probiotic-based prawn culture without aeration where stocking density was 40000, 80000 and 120000 juvenile/Ha and culture period 180 days

| Sl. | Subject | Amount | Unit | Unit Price (Taka) | Total Tk (40,000/ha) | Total Tk (80,000/ha) | Total Tk (1,20,000/ha) |
|---|---|---|---|---|---|---|---|
| 01 | Mud excavation and dyke repair | 50 | Person | 500 | 25,000/- | 25,000/- | 25,000/- |
| 02 | Labor cost during culture | 60 | Person | 500 | 30,000/- | 30,000/- | 30,000/- |
| 03 | Nylon Net | 500 | Meter | 25 | 12,500/- | 12,500/- | 12,500/- |
| 04 | Post Larvae | 40,000 | Ha | 2 | 80,000/- | | |
| | | 80,000 | Ha | 2 | | 1,60,000/- | |
| | | 1,20,000 | Ha | 2 | | | 2,40,000/- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 05 | Feed | 2600 | Kg | 55 | 1,43,000/- | | |
| | | 3300 | Kg | 55 | | 1,81,500/- | |
| | | 4000 | Kg | 55 | | | 2,20,000/- |
| 08 | Probiotics | | | | 35,000/- | 45,000/- | 50,000/- |
| 09 | Lime | 610 | Kg | 25 | 15,250/- | 15,250/- | 15,250/- |
| 10 | Electric Cost/ha | 4000 | unit | 10 | 40,000/ | 40,000 | 40,000 |
| 11 | Miscellaneous | | | | 20,000/- | 20,000/- | 20,000/- |
| 12 | Harvesting | | | | 15,000/- | 15,000/- | 15,000/- |
| 13 | Marketing | | | | 10,000/- | 10,000/- | 10,000/- |
| Total Expenditure (Taka) = | | | | | 4,25,750/- | 5,54,250/- | 6,77,750/- |
| Income (considering 600 Tk/kg) | | | | | 8,96,796/- | 11,43,054/- | 12,37,020/- |
| Net Benefit (B-A) | | | | | 4,71,046/- | 5,88,804/- | 5,59,270/- |
| Note: Prawn production was 1494.66, 1905.09 and 2061.70kg in stocking density 40000, 80000 and 120000 juvenile/ha | | | | | | | |

### 11.2.4. Activity-4. Water and soil parameters in without probiotic and with probiotic

- No significant different was observed in basic water parameters at without probiotic and with probiotic culture system.
- Lower $NH_3$ (0.0898 mg/l), $NO_2^-N$ (0.036 mg/l)were observed in probiotic treated pond than non-probiotic treated water ($NH_3$=0.1052, $NO_2^-N$=0.069 mg/l).

**Table 26.** Water quality parameter between aerated and non-aerated pond

| Parameters | Control | Non-Aerated | | | Aerated | | |
|---|---|---|---|---|---|---|---|
| | | P1 | P2 | P3 | P1 | P2 | P3 |
| pH | 7.56 | 7.72 | 7.64 | 7.74 | 7.74 | 7.73 | 7.84 |
| DO | 4.5 | 5.8 | 5.8 | 5.2 | 6.5 | 6.6 | 5.7 |
| BOD | 2.5 | 2.4 | 2.6 | 2.7 | 2.2 | 2.3 | 2.4 |
| $NO_2$ | 0.069 | 0.0528 | 0.0549 | 0.0575 | 0.0399 | 0.036 | 0.0421 |
| $NO_3$ | 0.1398 | 0.1394 | 0.1373 | 0.143 | 0.1371 | 0.1336 | 0.1372 |
| $NH_3$ | 0.1052 | 0.0971 | 0.0964 | 0.1018 | 0.0949 | 0.0898 | 0.0966 |

**Table 27.** Soil Quality Parameter between aerated and Non aerated pond

| Parameters | Control | Non-Aerated | | | Aerated | | |
|---|---|---|---|---|---|---|---|
| | | P1 | P2 | P3 | P1 | P2 | P3 |
| pH | 7.34 | 7.55 | 7.52 | 7.49 | 7.58 | 7.65 | 7.63 |
| EC | 5.92 | 5.58 | 5.38 | 5.45 | 5.13 | 5.24 | 5.17 |
| OM | 5.953 | 3.762 | 4.65 | 4.187 | 3.674 | 4.09 | 3.73 |
| N | 0.258 | 0.188 | 0.215 | 0.204 | 0.1833 | 0.216 | 0.196 |
| P | 13.438 | 9.856 | 11.123 | 10.05 | 8.728 | 10.86 | 9.922 |
| K | 0.481 | 0.448 | 0.458 | 0.455 | 0.442 | 0.468 | 0.451 |
| S | 0.011 | 0.0099 | 0.0185 | 0.012 | 0.007 | 0.007 | 0.0086 |

### 11.2.5. Activity-5. Proximate composition of cultured prawn in without and with probiotics.

**Table 28.**. Effect different commercial probiotics and stocking densities on proximate composition (mean ± STD) of cultured prawn

| Treatment | Stock densities | Probiotics | Protein% | Lipid% | Moisture% |
|---|---|---|---|---|---|
| Control | 2 | nill | 17.24± 0.354 | 2.60±1.311 | 79.14±2.458 |
| T1 | 2 | P1 | 18.37±0.700 | 2.22±0.275 | 76.56±2.607 |
| T2 | 4 | | 20.21±0.165 | 3.54±0.758 | 74.61±2.784 |
| T3 | 6 | | 19.62±0.463 | 2.92±0.569 | 73.70±1.438 |
| T 4 | 2 | P2 | 16.17± 2.687 | 4.42±1.432 | 74.3±1.654 |
| T5 | 4 | | 17.66± 0.347 | 2.24±0.887 | 78.84±0.393 |
| T6 | 6 | | 17.18± 0.147 | 1.38±0.760 | 79.86±0.337 |
| T 7 | 2 | P3 | 17.44± 0.384 | 1.91±0.852 | 78.78±5.157 |
| T 8 | 4 | | 19.10± 1.595 | 3.25±0.452 | 76.53±2.057 |
| T 9 | 6 | | 17.54± 0.717 | 2.60±0.261 | 75.80±0.526 |

**Outcome**

1. There were no distinct differences of protein and lipid values among cultured prawn in three probiotics and without probiotic.
2. Stocking density 4/$m^2$ showed a bit higher protein values then other stocking.

### 11.2.6. Activity- 6. Digestive enzymes (Protease, lipase, Amylase) activity was analyzed in without and with probiotics treated prawn.

***Digestive enzyme activity assessment***

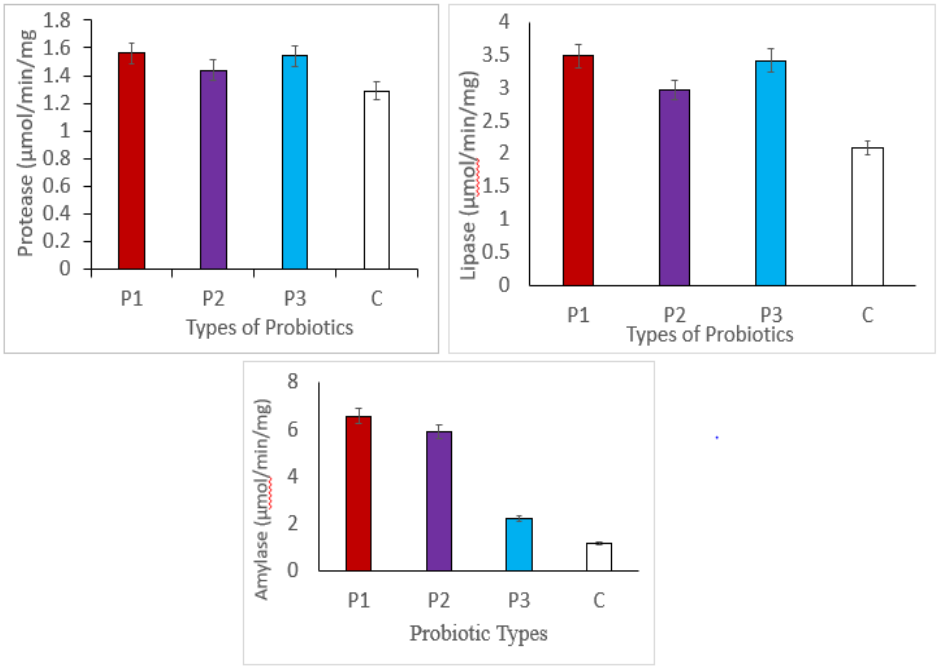



**Fig 11**. Enzyme (Protease, Lipase and Amylase) activities in and probiotic 1 (P1), Probiotic 2 (P2), Probiotic 3 (P3) and without probiotic Control (C).

**Outcome:** All three probiotics treated prawn showed higher protease, amylase and lipase enzyme than the without probiotic treated prawn. Probiotics could increase protease, amylase and lipase enzyme activity in prawn. P1 showed little bit higher enzyme activity than P2 and P3 (Fig. 11}.

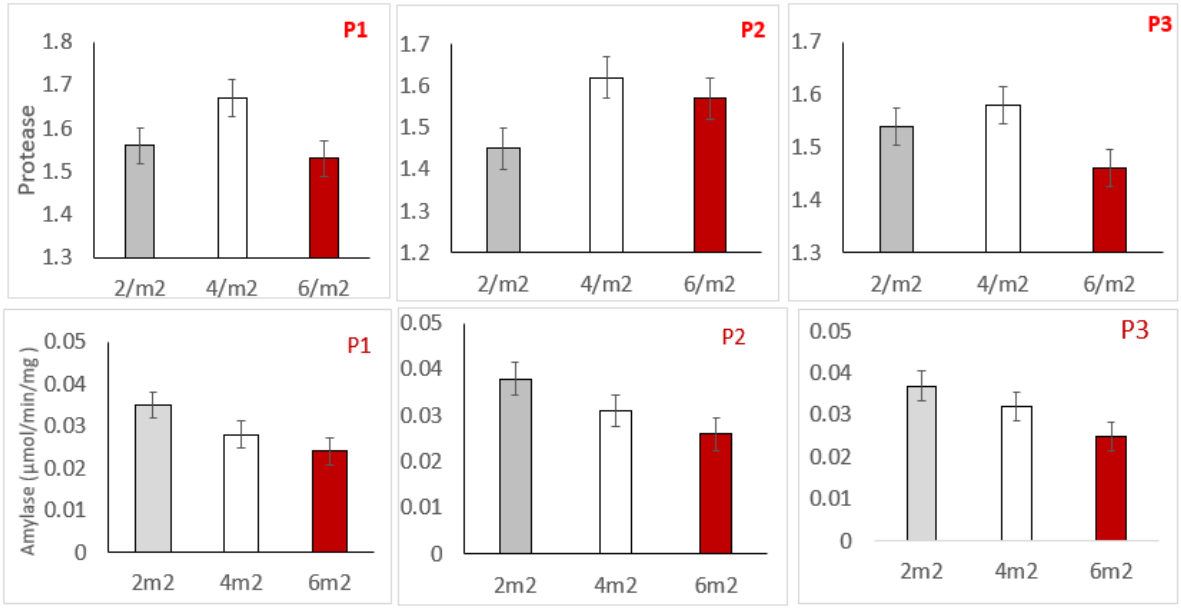



**Fig 12.** Comparison of the of protease and amylase activity at different stocking densities treated by probiotics (P1, P2, P3).

**Outcome :** Stocking density has no distinct effect on enzyme activity among the three probiotics. Protease was found higher in 4/$m^2$ and amylase in 2/$m^2$.

### 11.2.7. Activity-7. Assessing of immune enzyme activity (Pro-Phenoloxydase, Pro-PO and Superoxide dismutase, SOD) at without and with probiotic treated prawn.

***Immune enzyme assessment***

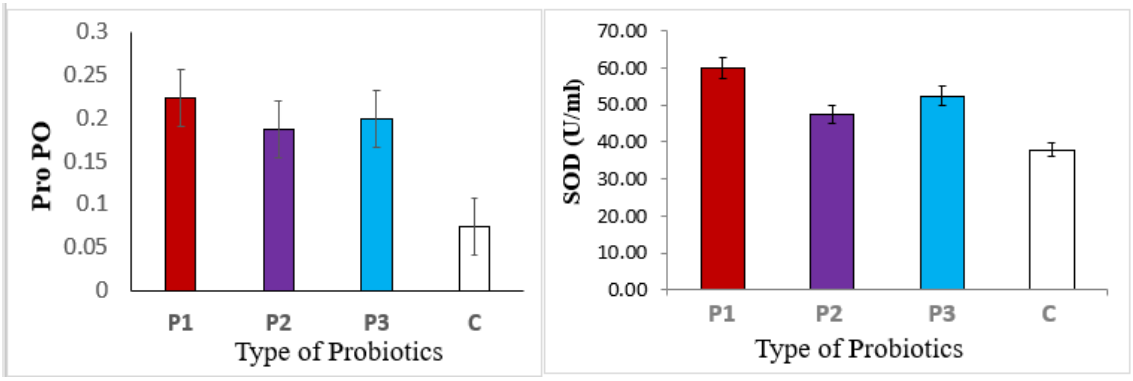



**Fig 13.** A) Pro-Phenoloxidase (Pro-PO) enzyme activity, and B) Superoxide dismutase (SOD) enzyme activity (U/mg) of *M. rosenbergii* rearing with three probiotics (P1, P2 & P3) and without probiotic ( C).

**Outcome**

- Probiotics enhance the activity of immunity enzymes of prawn compare to without probiotic.
- Probiotic-1 (P1) showed little bit higher Pro-PO and SOD than other two probiotics P2 and P3.

### 11.2.8. Activity-8. Antagonistic effect of probiotics against pathogenic bacteria.

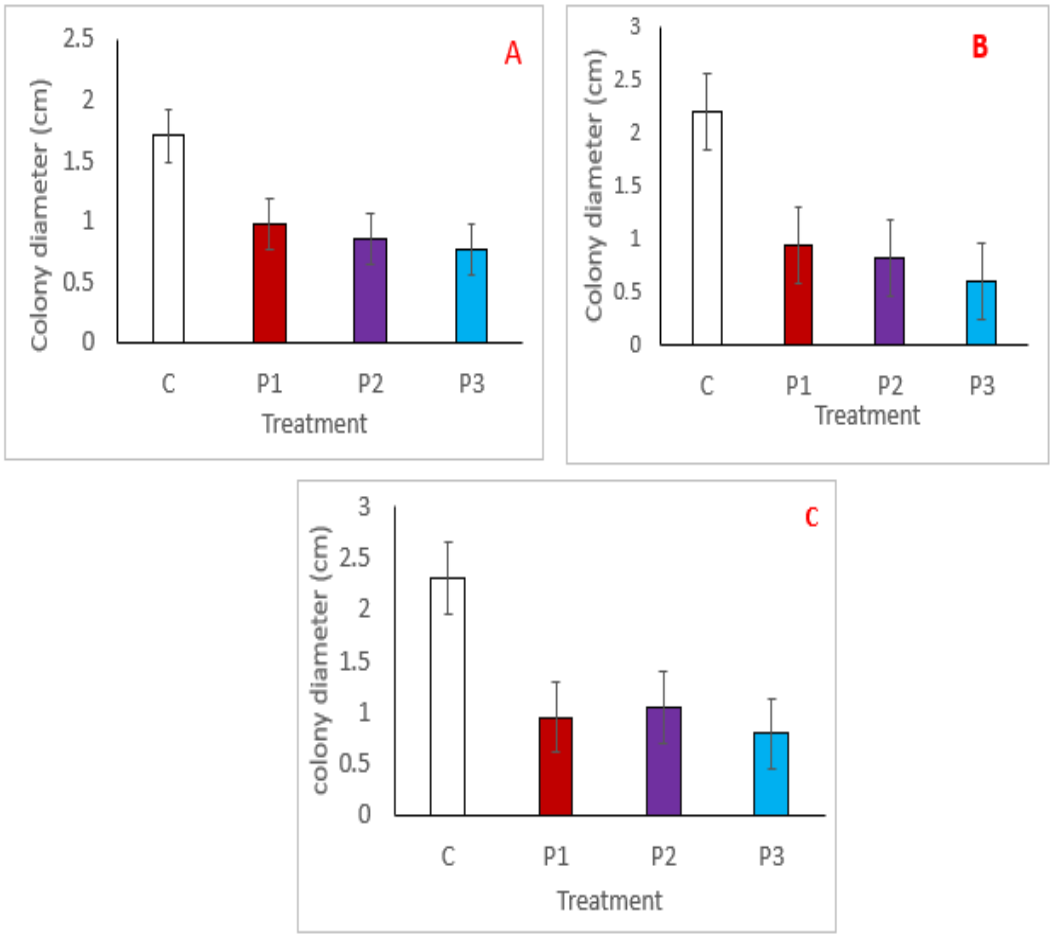



**Fig 14.** Antagonistic effect of probiotics against pathogenic bacteria. *Vibrio* spp. colony diameter (cm) at **A**. Nutrient ager, **B**. Starch Agar, **C**. Tryptone agar media. Values are the mean ± SD.

**Outcome :** Disease challenge test was done applying pathogenic bacteria *Vibrio* spp. in both with and without probiotics in three medias. In all the three medias the colony of harmful pathogenic *Vibrio* spp. was found less where probiotics (P1, P2 and P3) were applied compared to control (without probiotic). Probiotic could inhibit the growth of pathogenic bacteria *Vibrio* spp.(Fig. 14).

### 11.2.9. Activity-9. In-vivo Challenge test using pathogenic bacteria on survival of prawn post larvae (PL) in without and with probiotics

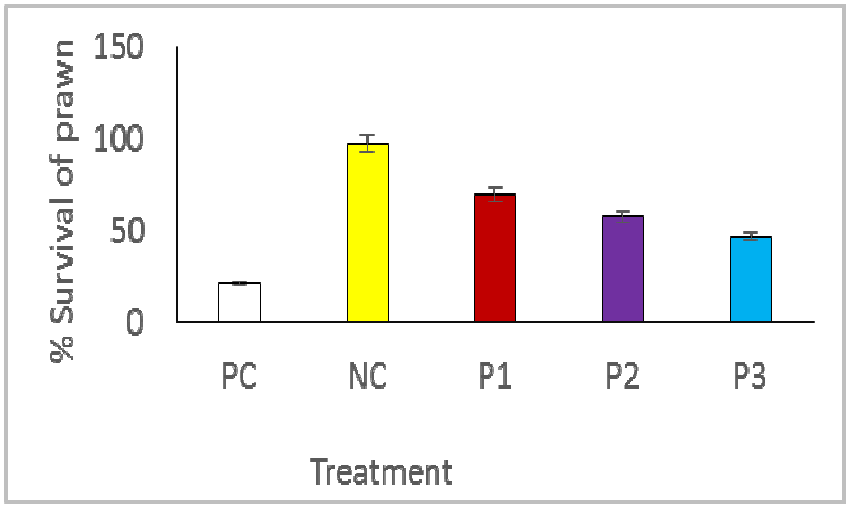



**Fig 15.** Survival (%) of shrimp larvae challenge with *Vibrio* spp. Applying three probiotics (P1, P2 and P3) and without probiotic.[PC= Positive control where *Vibrio* spp. Present but probiotic absent, NC= Negative control where no probiotic and no *Vibrio* spp. And P1-P3=Probiotic with *Vibrio* spp.]

The survival (%) of shrimp larvae with the application of *Vibrio* spp. Was also tested in three probiotics (P1, P2 and P3) and without probiotic (Fig. 15). All three probiotics treated prawn showed comparatively higher survival rate against pathogenic bacteria *Vibrio* spp. Compare to positive control (no probiotic but *Vibrio* spp. Present).

**Outcome :** Probiotics treated prawn showed comparatively higher survival rate against pathogenic bacteria *Vibrio* spp..

### 11.2.10. Activity-10. Effect of probiotic bacteria on the growth of other beneficial microorganisms in prawn gut was identified.

The total inoculation period was 15 days in the laboratory tank-rearing condition for this experiment. Table 29 showed that when beneficial bacteria *Bacillus* spp. was added then it assist to increase itself (T1, 10.20±0.08 Log CFU/g) as well as *Lactobacillus* spp. (T1,7.18±0.07) and *Clostridium* bacterial load (T1, 6.23±0.09 Log (CFU/g)compare to the control (Cn) of the respective bacteria (8.11±0.07. 5.20±0.02 and 4.36±0.13 respectively (Table 24). Same way *Lactobacillus* spp. (T3) and *Clostridium* spp. (T5) showed growth increment on other two beneficial bacteria (Table 29) compared to control.

**Table 29.** Effect of probiotic (beneficial bacteria) on growth of other beneficial bacteria.

| | Cn | Cv | T1 (B) | T2 (B+v) | T3 (L) | T4 (L+v) | T5 (C) | T6 (C+v) |
|---|---|---|---|---|---|---|---|---|
| *Bacillus* spp. | 8.11± 0.07 $^{g}$ | 7.32± 0.06 $^{f}$ | 10.20± 0.08 $^{a}$ | 9.883± 0.05 $^{b}$ | 8.471± 0.13 $^{e}$ | 7.98± 0.08 $^{g}$ | 9.21± 0.06 $^{c}$ | 8.91± 0.04 $^{d}$ |
| *Lactobacillus* Spp. | 5.20± 0.02 $^{d}$ | 5.08± 0.10 $^{d}$ | 7.18± 0.07 $^{b}$ | 7.054± 0.03 $^{b}$ | 5.686± 0.22 $^{c}$ | 5.86± 0.02 $^{c}$ | 7.88± 0.18 $^{a}$ | 7.273± 0.17$^{b}$ |
| *Clostridium* Spp. | 4.36± 0.13$^{ce}$ | 4.11± 0.05$^{cde}$ | 6.23± 0.09 $^{b}$ | 6.12± 0.18 $^{b}$ | 4.53± 0.20 $^{e}$ | 3.72± 0.27 $^{d}$ | 7.06± 0.13 $^{a}$ | 7.35± 0.05 $^{a}$ |

Note. Mean values with different superscript letters in the same row indicate the significant difference among the treatments (one□way ANOVA, $p < 0.05$)., Cn=No probiotics no *Vibrio*, Cv=No probiotics but *Vibrio* present, B= *Bacillus*, L=*Lactobacillus*, C=*Clostridium*

## 11.2.11. Activity-11. Observation of prawn growth and production at different carbon enrich ingredients.

This study was conducted to evaluate the effect of locally available carbon sources on the growth and production performance of *M. rosenbergii* in pond condition where stocking density was 3/$m^{2.}$ After 180 days culture period, prawn production was found higher in all carbohydrate treated pond than the control (Table 30) and highest production was observed (1108.57 kg/ha) in T4 where mixture of molasses and rice polish was used; and 2$^{nd}$highest in T1 (1080.81kg/ha) where only molasses was used. Prawn production was found lowest in control (751.80kg/ha) where no carbohydrate used. The cost-benefit analysis showed that net benefit 3,61,142Taka/ha could be earn applying carbohydrate in prawn culture (Table 30).

**Table 30.** Effect of carbohydrate enriched ingredients on growth and production of prawn

| Treatment | Initial Wt (g) | Final Wt (g) | Survival (%) | Production (Kg/Ha) |
|---|---|---|---|---|
| C | 2.43 ± 0.82 | 32.66±8.24 | 76.73 | 751.80 |
| T1 | | 42.93±13.45 | 83.92 | 1080.81 |
| T2 | | 37.42±11.81 | 81.68 | 916.94 |
| T3 | | 36.79±10.24 | 79.96 | 882.52 |
| T4 | | 43.54±14.60 | 84.87 | 1108.57 |
| T5 | | 41.54±9.32 | 81.98 | 1021.63 |
| T6 | | 36.92±11.53 | 78.63 | 870.91 |
| T7 | | 38.37±11.04 | 81.88 | 942.52 |

**Output**

1. All the carbon enriches ingredients added treatments showed higher production than the control.
2. Within the ingredient added treatments T4 showed highest production where Molasses and rice polish was applied; 2$^{nd}$ highest was found in T1 where molasses was applied.
3. T4 (Molasses+Rice polish), T1 (Molasses) and T5 (Molasses+Mayze powder) could be suggested to apply in Farmers pond for prawn culture as substitute of commercial probiotics.
4. The net benefit 3,61,142 Taka/ha could be earn applying carbohydrate as feed ingredients in prawn culture (Table 31).

**Table 31.** Cost benefit analysis of Carbohydrate (Molasses +Rice polish) based prawn culture where stocking density 30000 juvenile/ha and culture period 180 days

| | Subject | Amount | Unit | Unit Price (Taka) | Total Tk |
|---|---|---|---|---|---|
| 01 | Mud excavation and dyke repair | 50 | Person | 500 | 25,000/- |
| 02 | Labor during culture | 60 | Person | 500 | 30,000/- |
| 03 | Nylon Net | 500 | Meter | 25 | 12,500/- |
| 04 | Post Larvae | 30,000 | Ha | 2 | 60,000 |
| 05 | Supplementary feed | 2000 | Kg | 55 | 1,10,000 |
| 06 | Molasses | 300 | kg | 28 | 8,400 |
| 07 | Rice polish | 300 | kg | 22 | 6,600 |
| 08 | Probiotics | 1.5 | kg | 7500 | 11,250/- |
| 09 | Lime | 610 | Kg | 25 | 15,250/- |
| 11 | Harvesting | | | | 15,000/- |
| 12 | Marketing | | | | 10,000/- |
| Total Expenditure (amount in taka) | | | | | 3,04000/- |
| Income (600 Tk X 1108.57kg) | | | | | 6,65,142/- |
| Net Benefit (B-A) | | | | | 3,61,142/- |

### 11.2.12. Activity-12. Locally available carbon enrich ingredients (Molasses, Rice polish and Maize powder) are applied in pond to know the growth and proliferation of beneficial bacteria

The result from this study found that that highest *Bacillus* (7.236±0.0088 cfu/ml) and *Lactobacillus* (4.11±0.026 cfu/ml) load were observed at T4 (Molasses and Rice polish) whereas highest *Clostridium* at T3 (Maize, 4.27±0.162 Log(cfu/ml) compared to control (*Bacillus*=6.437±0.0449; *Lactobacillus*=3.044±0.122, *Clostridium*=3.12±0.15 Log(cfu/ml). This study proved that carbohydrate ingredients could increase native probiotics in pond system and thus could be used as substitute of commercial probiotics(Table 32)..

**Table 32.** Effect of carbohydrate enriched ingredients on growth of three beneficial bacteria (*Clostridium* spp, *Lactobacillus* spp and *Bacillus* spp) count log cfu/ml in pond water

| | C | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|---|
| *Clostradium*s sp. | 3.12± .15$^{f}$ | 3.82± .038$^{c}$ | 3.852±.059$^{c}$ | 4.27±.0 162$^{a}$ | 4.21± .016$^{a}$ | 3.7± .045$^{c}$ | 4.15±.05$^{ab}$ | 3.65±036$^{de}$ |
| *Lactobacillus* spp. | 3.044± .122$^{f}$ | 4.01± .037$^{a}$ | 3.83±.042$^{b}$ | 3.86±.0 42$^{b}$ | 4.111±.026$^{a}$ | 3.644±.07$^{c}$ | 3.35±.057$^{d}$ | 3.244±.16$^{de}$ |
| *Bacillus* spp. | 6.437± .0449$^{f}$ | 7.10±.027$^{b}$ | 7.202±.023$^{a}$ | 7.1987±.039$^{a}$ | 7.236±.0088$^{a}$ | 6.86±.047$^{d}$ | 6.814±.051$^{de}$ | 6.791±.044$^{e}$ |

Locally available carbohydrate enriched ingredients could increase beneficial bacteria in pond system. The highest *Bacillus* (7.236±0.0088) and *Lactobacillus* (4.11±0.026 log cfu/ml) load were found in pond water where molasses + rice polish was used and lowest in control (*Bacillus*=6.437±0.0449; *Lactobacillus*=3.044±0.12 Log(cfu/ml). *Clostridium* was found highest in maize powder (4.27±.016) and second highest (4.21±.016log cfu/ml) in mixture of molasses and rice polish. Carbohydrate could be used as substitute of commercial probiotics (Table 33).

**Table 33.** Culture and storage possibility of three main native probiotics (*Bacillus, Lactobacillus and Clostridium* spp.) through heat shock treatment

| Temp | Spp. | Control | | | T1 ( Molasses) | | | T2 ( Rice Polish) | | | T3 (Maize Powder) | | | T4 (Molasses+ Rice Polish) | | | T5 (Molasses +Maize Powder) | | | T6 (Rice Polish +Maize Powder) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BS (log10(CFU/ml)) | AS (log10(CFU/ml)) | SR (%) | BS (log10(CFU/ml)) | AS (log10(CFU/ml)) | SR (%) | BS (log10(CFU/ml)) | AS (log10(CFU/ml)) | SR (%) | BS (log10(CFU/ml)) | AS (log10(CFU/ml)) | SR (%) | BS (log10(CFU/ml)) | AS (log10(CFU/ml)) | SR (%) | BS (log10(CFU/ml)) | AS (log10(CFU/ml)) | SR (%) | BS (log10(CFU/ml)) | AS (log10(CFU/ml)) | SR (%) |
| 45°C | *Bacillus* | 4.44 | 4.38 | 86.95 | 5.37 | 5.36 | 97.45 | 5.16 | 5.14 | 95.89 | 5.06 | 5.04 | 94.01 | 5.41 | 5.39 | 96.13 | 6.07 | 6.05 | 94.95 | 5.35 | 5.34 | 97.80 |
| | *Clostridium* | 2.98 | 2.97 | 96.90 | 3.92 | 3.89 | 91.76 | 3.82 | 3.79 | 92.53 | 3.96 | 3.95 | 97.84 | 4.24 | 4.23 | 97.74 | 4.35 | 4.34 | 97.36 | 4.15 | 4.12 | 93.00 |
| | *Lactobacillus* | 2.27 | 2.17 | 78.94 | 3.74 | 3.73 | 96.42 | 2.23 | 2.17 | 88.23 | 3.67 | 3.64 | 93.61 | 2.93 | 2.91 | 95.40 | 2.89 | 2.87 | 94.93 | 3.03 | 2.98 | 88.99 |
| 50°C | *Bacillus* | 4.34 | 4.31 | 93.27 | 5.27 | 5.26 | 97.32 | 5.12 | 5.10 | 95.48 | 5.07 | 5.03 | 91.59 | 5.36 | 5.35 | 96.15 | 6.03 | 6.01 | 95.41 | 5.32 | 5.30 | 97.12 |
| | *Clostridium* | 3.54 | 3.20 | 89.74 | 3.72 | 3.69 | 92.45 | 3.81 | 3.78 | 96.82 | 3.89 | 3.86 | 93.58 | 4.27 | 4.26 | 97.86 | 4.25 | 4.23 | 97.19 | 4.26 | 4.25 | 98.90 |
| | *Lactobacillus* | 2.11 | 2.04 | 84.61 | 3.89 | 3.86 | 92.40 | 3.08 | 3.07 | 96.74 | 3.63 | 3.61 | 96.82 | 2.91 | 2.88 | 93.90 | 2.83 | 2.79 | 92.64 | 2.94 | 2.91 | 93.18 |
| 55°C | *Bacillus* | 4.19 | 4.17 | 94.90 | 5.27 | 5.25 | 94.17 | 5.01 | 4.98 | 94.17 | 4.96 | 4.95 | 97.84 | 5.38 | 5.38 | 98.76 | 5.96 | 5.93 | 93.54 | 5.28 | 5.27 | 96.89 |
| | *Clostridium* | 3.64 | 3.60 | 90.90 | 3.98 | 3.96 | 95.87 | 3.77 | 3.70 | 86.44 | 3.86 | 3.85 | 97.26 | 4.15 | 4.14 | 97.20 | 4.25 | 4.23 | 96.06 | 4.23 | 4.22 | 97.66 |
| | *Lactobacillus* | 1.95 | 1.90 | 88.88 | 3.72 | 3.69 | 92.45 | 3.74 | 3.69 | 87.5 | 3.08 | 3.07 | 96.74 | 2.82 | 2.79 | 94.02 | 2.82 | 2.79 | 94.02 | 3.08 | 3.06 | 95.12 |
| 60°C | *Bacillus* | 4.16 | 4.15 | 97.95 | 5.35 | 5.34 | 97.35 | 5.11 | 5.08 | 95.34 | 4.90 | 4.89 | 97.53 | 5.28 | 5.27 | 97.90 | 5.94 | 5.92 | 96.59 | 5.15 | 5.14 | 96.52 |
| | *Clostridium* | 2.68 | 2.64 | 91.66 | 3.82 | 3.81 | 97.01 | 3.79 | 3.75 | 91.93 | 3.64 | 3.60 | 90.90 | 4.22 | 4.21 | 97.60 | 4.06 | 4.04 | 95.68 | 4.31 | 4.30 | 98.07 |
| | *Lactobacillus* | 1.84 | 1.77 | 85.71 | 3.71 | 3.67 | 90.38 | 3.03 | 3.01 | 94.49 | 2.94 | 2.93 | 97.75 | 2.81 | 2.79 | 95.45 | 2.78 | 2.76 | 95.08 | 2.89 | 2.83 | 88.46 |

Native bacteria (*Bacillus* spp., *Clostridium* spp. and *Lactobacillus* spp.) was culture in pond water sample (1 ml) as control and 1% carbohydrate enriched ingredients (molasses, rice polish, maize powder) at 37°C for 24 hours and then bacterial growth was measured (Log CFU/ml). Before storage bacterial growth found higher in all carbohydrate added treatment (T1-T6). Half of the cultured bacteria was encapsulated with heat shock at 45, 50, 55 and 60°C and stored at -80°C for 15 days. The highest growth of *Bacillus* spp., *Clostridium* spp. and *Lactobacillus* spp.) were found 6.07 (T5), 4.35 (T5) and 3.74 (T1) Log CFU/ml respectively where Molasses + maize powder and molasses were applied. The value of bacterial load was found lower in respective control (pond water). After storage at -80°C for 15 days the survival of all three bacteria was found satisfactory (84.61- 98.90%) (Table 28). Due to Covid-19 pandemic this storage possibility study could not be conduct as per desire level. It needs more detail study.

## 12. Research highlight

### 12.1. BFRI (SRS) Component

**Title of the sub-project**: Development of *in-situ* Breeding Technology of Prawn (*M. rosenbergii*) and Adoption of Sustainable Eco-Friendly Culture of Prawn and Shrimp (*P. monodon*)

**Research highlight: 01**

**Background**

Shrimp industry plays a significant role in foreign exchange earnings, employment generation and poverty reduction in Bangladesh. However, since the last decades shrimp farming has been suffering from various issues associated to culture and production. Farmed shrimp production reduced significantly due to various diseases, WSSV in particular, has become a serious constraint. Major suspected reasons behind the disease outbreak are poor farm management in terms of water quality, poor seed quality, less or no biosecurity, etc. To address these issues, the conceptual model of CBSF subjected to field trial.

**Objective**

To develop cluster-based shrimp (*Penaeus monodon*) farming (CBSF) with special emphasis on disease prevention and traceability

**Methodology**

For implementing the model, traditional ghers were converted into clusters having facilities to implement scientific methods of shrimp culture. The model farm developed with water purification pond, different water outlet and inlet facility and nursing point facility with better quality feed and seed (PL), continuous monitoring of water quality and monitoring the presence of pathogens. Stocking density was $3/m^2$ & $6/m^2$ in the $1^{st}$ year is and $6/m^2$ & $9/m^2$ in the $2^{nd}$ year.

Traditional shrimp farm of 1.316 ha was experimentally converted into cluster. Each pond of the cluster was prepared following dry method of pond preparation. Briefly, pond bottom was dried and CaO were applied at the rate of 1kg/decimal. Then the pond bottom was ploughed

and kept under direct sunshine for a week. Before entering water, fine mesh net fencing throughout the dike was done to restrict any kinds of living organisms entering into the culture system. After proper netting, water was allowed through a 0.5 mm mesh net to restrict macro-organisms into the ponds. Then the pond was filled-up with tidal water and treated with 300-400gm/decimal Bleaching to kill all living organisms. To regenerate the planktonic community into the pond, molasses custard was implemented at the required dose (mention the dose). The ponds were treated with lime @ 100-200gm/decimal for increasing the buffering capacity of the water. Based on the primary productivity, the ponds were fertilized with inorganic fertilizers followed by Urea: 200-250gm/dec., TSP: 100-125gm/dec., and $KMnO_4$:60-80 gm/dec (considering 3 feet water depth/dec). Feed was supplied twice daily @ 8% of body weight for the first month, 5% for the second month and 2-3% for the rest of the period. A total of 30 shrimp from each treatment were sampled using cast net. Weight of the shrimp taken using portable balance for growth monitoring, feed adjustment and disease checking. Water quality was also monitored and recorded at weekly intervals.

**Key findings**

Under this study, in the first year, shrimp were cultured for 4 months in two different stocking densities. Growth rate, water quality parameters and disease screening information during the culture period found satisfactory. No disease outbreak recorded throughout the culture period, which was one of the great achievements of this study. Besides, from the traditional gross production of shrimp is around 250-300 kg/ha, with this management intervention, production maximized more than two times (735.6 kg/ha at the stocking density of 30000 PL/ha and 854.4 kg/ha at the stocking density of 60000 PL/ha) as compared to the traditional farming. Once getting more higher production of 854.4 kg/ha at the stocking density of 6 PL/$m^{-2}$ with no aeration, in the second year, emphasize given on to optimize maximum stocking density to increase per ha production. Therefore, instead of 30000 and 60000 PL/ha, treatment designed to 60000 and 90000 PL/ha to see in this higher stocking can be sustained without aeration. Due to corona pandemic and lockdown situation, stocking of PL became delay. Due to heavy rainfall, average salinity throughout the culture period was around 2 ppt, which is considered as low salinity for the shrimp growth. Higher stocking density of 9 PL/$m^{-2}$ with no aeration found not suitable and the production dropped to 600 kg/ha. Due to lower salinity overall survival found 15% less than the first-year survival. For the same reason, production dropped to 694 kg/ha with the stocking density of 60000 PL/ha. Even though the average production dropped to 694 kg/ha in the second year compared to 854.4 kg/ha with the stocking density of 60000 PL/ha, is still more than double than the average traditional production of shrimp i.e., 300 kg/ha.

**Keywords**: Cluster farming, Shrimp farming, Biosecurity

**Research highlight: 02**

**Background**

Among the known disease, *P. monodon* facing devastating crop loss due to WSD outbreak in Bangladesh. In 2010-2013, an intensive research on the risk factors associating white spot syndrome virus (WSSV) infection into shrimp were carried out and the findings were disseminated to the farmers to reinforce their hold to cope with WSSV infection (Islam et al., 2014). But interestingly, since last couple of years, mortality pattern due to WSSV infection

found following a different pattern and showing differences in virulence as well. Apart from WSSV, several symptoms of shrimp mortality indicating the probability of presence of some non-reported disease to Bangladesh, viz., Necrotizing Hepatopancreatitis (NHP), Bacterial White Tail Disease, Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) and *Enterocytozoon hepatopenaei* (EHP). Among these disease, EMS found positive to a number of shrimp farms (Personal Communication with the PL nurturers and farmers). GSMC 2016 blog reported that EHP disease now 'everywhere in Asia'. *Enterocytozoon hepatopenaei* (EHP), the newest disease challenging shrimp farming operations, is now present in nearly all Asian countries.

## Objective

To promote Shrimp/Prawn health management through intensive monitoring and surveillance of bacterial (NHP, EMS/AHPND & EHP) and viral (WSSV, YHV, GAV, TSV, IMNV, MBV & BP) diseases, identification of risk factors, evaluation of immune response and preventive measures

## Methodology

Under this study, for the identification of OIE Listed pathogen for Shrimp and Prawn, PCR protocol were optimized. Pathogen specific primers were synthesized from the gene bank. Then PCR protocol was optimized for the pathogens at the shrimp health management laboratory of Shrimp Research Station, Bagerhat. Samples were collected from the study area in fresh condition and transported to Shrimp Health Management Laboratory, SRS, Bagerhat in 95% ethanol and peptone water. *All the 10 shrimp from each sampling point/ gher were finely chopped and then 20~30 mg were used for the PCR diagnosis followed by DNA/RNA extraction. Genomic DNA were extracted from tissue samples collected from pleopods. Approximately 25-mg tissues were cut into small pieces, homogenized by micro-tissue-grinder in a 1.5 ml microfuge tube, and total genomic DNA were extracted following the instruction provided with Thermo scientific's Purelink DNA extraction kit. Both first step PCR and Nested PCR were done to confirm the presence or absence of the pathogen. Primer was designed from the sequenced gene from the NCBI Gene bank using Serial Cloner 2.6.1. Software. Thermal cycler for polymerase chain reaction (both first and nested PCR) was programmed as follows.

| | |
|---|---|
| 95 °C x 3 minutes followed by | |
| 95 °C x 30 seconds | 15-35 cycles |
| 58~62 °C x 30seconds | |
| 72 °C x 30 seconds | |
| 72 °C x 5 minutes | Final Extension |

Both first & nested PCR products taken for gel electrophoresis. To run the process, 1.0% agarose gel was prepared (thickness not more than 0.8cm.) prior to electrophoresis apparatus be assembled. For electrophoresis, 1 X TAE buffer were used as the medium at 100-120 volts

## Key findings

From three districts, a total of 109 samples tested for 10 OIE listed pathogens resulted in 2180 PCR test. From the conducted tests, three pathogens viz., WSSV, EMS and EHP found positive.

Among these pathogens EHP is the first reported pathogens in *M. rosenbergii* but also it is the first report in the world and deserve serious attention to improve management practices to help secured production of shrimp and prawn in the future. It also explains sudden mortality issue of marketable size Prawn in the southern region of Bangladesh. Having preventive measures from the beginning of the culture period can help farmer from this unbearable loss.

**Keywords**: OIE, EHP, PCR, Shrimp

**Research highlight: 03**

**Background**

The differential growth between sexes is of great concern in *M. rosenbergii* culture where in males grow much faster than females (Holthuis, 1980). Culture of all-male prawn gave significantly higher yield with shorter culture period than the mixed-sexes and the all-female culture (Sagi et al. 1986; Cohen et al. 1988). Moreover, the manual sexing was not justified by the relatively small increase of income. The androgenic gland of crustaceans is the only source of hormone that controls sex differentiation to maleness and the development of male characters. Therefore, the alternative biotechnological method has been explored for producing an all-male stock of freshwater prawn, among which the neo-female technology is promising (reviewed by Sagi and Aflalo, 2005). The ablation of the androgenic gland at early stage of development caused sex reversal to females (neo-female) (Nagamine et al. 1980) while the implantation of the androgenic gland in female *M. rosenbergii* resulted in sex reversal to males (neo-male) (Malecha et al.,1992). Sagi and Cohen (1990) based on two crosses (a total of 567 offspring) showed that the mating of the neo-female with the normal male resulted in 99.1% and 100% male offspring.

**Objectives**

To develop all male PL production technique of Giant Freshwater Prawn (*M. rosenbergii*);

**Methodology**

Matured wild brood of *M. rosenbergii* were collected and keep in brood rearing tank of SRS prawn hatchery until hatching. After hatching, the larvae were transferred to Larvae Rearing Tanks (LRT). The tanks were stocked at a density of 60–80 larvae/l. Hatchery operation carried out flowing the previously modified hatchery operation protocol by SRS scientists. Larvae metamorphosed into post larvae (PL) after 30 days of rearing. Microsurgical Ag ablation were performed by removing the fifth pair of walking legs together with the androgenic gland.

**Key findings**

For the experiment production of prawn, known aged PL is essential. Therefore, PL must be produced in hatchery condition. To meet the criteria, Prawn PL was successfully produced in SRS Prawn Hatchery. As PL 45 to PL 60 is required for the microsurgery of androgenic gland ablation, PL were nursed in the hatchery cistern for the required days.

After getting required aged PL, several batches of PL (500 PLs) operated for micro surgery. Only 2% PL survived but unfortunately all found male when checked. The poor survival rate was due to the stress under the microscope and as male prawn are generally sturdy, so the survived

prawn was all-natural male. It was a good sign to become all-individual as male, but it would have been better to get some female which were basically male at its birth. Only at that point those individuals could be act as neo-female and can successfully produce all male population of prawn after mating with natural male.

In short, microsurgical ablation of androgenic gland is a complicated task and required subsequent trials. One-year trial is not sufficient for such kind of sophisticated research where timing and perfection is mandatory and it comes by practice. This was a start and with all the established facilities, trial will be continued in the coming days to make all these efforts successful.

**Keywords**: Microsurgery, Androgenic gland, All male PL

**Research highlight: 04**

## Background

Shrimp farming in Bangladesh has expanded rapidly since the early 1980s. The area under shrimp farming expanded from 52,000 ha in 1988 to 213,617 ha in 2013 (DoF, 2014). The second largest source of export earnings in Bangladesh (estimated US $450 million) after readymade garments comes from shrimp and prawn exports (DoF, 2017). Until now, WSD is the only major disease reported and recognized as a key factor for reduction in shrimp production in Bangladesh (Debnath et al. 2016, Islam, et al, 2014). Outside of Bangladesh, the Asian shrimp sector has recently been plagued by several emerging diseases (Thitamadee et al. 2016). Shrimp being export commodity, use of medicines and antibiotics are strictly prohibited. Therefore, to cope with disease outbreak sustainable solutions is mandatory. Along with improve management facility, boost up shrimp immunity is considered to be more effective against therapeutics.

## Objectives

To have an insight of innate immune response over immunogenic agents in shrimp (*P. monodon*)

## Methodology

Attenuated pathogenic bacteria was used as immune stimulant agents due to unavailability of necessary reagents for cloning part because of corona pandemic. For this experiment, pathogenic vibrio bacteria cultured in nutrient broth and pelleted by centrifuging at 3500rpm. These bacterial pellets were then diluted in PBS and given heat shock at $95^0$C for 5 minutes. Then 100 ml PBS solution containing ($1\times10^8$ cfu per ml) pathogenic dead bacteria were mixed with per Kg of commercial feed to for the experimental trial. This feed was administrated for three weeks as treatment and haemocytes were counted to see changes in immunogenicity.

## Haemocyte collection

From each shrimp 0.1 ml haemolymph were collected into a 26 gauge 1 ml sterile syringe containing 0.2 ml of anticoagulant (Trisodium Citrate 30mM, NaCl 338mM, Glucose 115mM, EDTA 10mM).

**Total haemocyte count (THC)**
THC= average of 4 blocks × dilution correction factor $\times 10^4$
Dilution correction factor =

$$\frac{volume\ of\ hemolymph\ extracted + volume\ of\ anticoagulant\ used}{volume\ of\ hemolymph\ extracted}$$

**Key findings**
Under this study attenuated/ killed bacteria was considered as immunogenic agent to boost shrimp immunity and administrated with feed for three weeks. Immunity status was measured with total hemocyte count as in crustaceans, hemocytes act as the major defense mechanism. The feed mixed with attenuated bacteria gave almost double count of hemocytes (1200000 cell/ml) compared to the control group (600000 cell/ml). No doubt this is a tremendous increase to the count of hemocytes and further challenge experiments can give a clear idea for the effectiveness of immunity against disease outbreak and mass mortality.

**Keywords**: Immunity, Shrimp, Hemocyte, Therapeutics

**Research highlight: 05**

**Background**
For sustainable management and improvement of the species, study of its population structure is necessary. To address the issue, Khan et al. (2014) characterized genetic diversity in three river populations of *M. rosenbergii* using microsatellite DNA markers. While they used wild populations, in this study genetic variation between wild and hatchery populations of *M. rosenbergii* had been compared by randomly amplified polymorphic DNA (RAPD) analysis. Comparison of genetic diversity between wild and hatchery populations would enable us to understand population structure of the species. Among different DNA markers, RAPD is easy and simple to work. It functions based on amplification of discrete regions of the genome by polymerase chain reaction with short random primers. This marker has extensive use in estimation of genetic variability and relatedness in many organisms (Hadrys et.al. 1992).

**Objectives 5.** To infer the genetic diversity of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) in the major coastal rivers

**Methodology**
Wild populations from the rivers viz., Kocha, Payra, Baleswar, Rupsha, Poshur, Karnafuli, Meghna and Kumar Nod were collected for the study of genetic variability and diversity within the population. Genomic DNA were extracted from tissue samples collected from pleopods. Approximately 25-mg tissues were cut into small pieces, homogenized by micro-tissue-grinder in a 1.5 ml microfuge tube, and total genomic DNA were extracted following the instruction provided with Thermo scientific's Purelink DNA extraction kit. For the identification of genetic variability, the extracted DNA was amplified with the selected primer sequences of 10 *M. rosenbergii* microsatellite loci.

**Key findings**
For the microsatellite study of genetic variation, the entire extracted DNA multiplied by polymerase chain reaction following the given PCR optimization. Getting the PCR products refers to 80% completion of the activity. To evaluate the variability, those primer products should be analyzed by SDS Page gel electrophoresis, but due to corona pandemic procurement of few essential reagents was not possible.

These PCR products stored duly and can be analyzed to study genetic variability if the required reagents get available.

**Keywords**: *Macrobrachium rosenbergii*, Genetic diversity,

**12.2. KU Component**

**Research highlight: 01**

**Background**
The freshwater prawn (*M. rosenbergii*) has significant role in the economy, employment opportunity and poverty elevation in Bangladesh. But sill farmers are practicing traditional culture system. The baseline surveyed of this study showed that in the southwest Bangladesh ( Khulna, Bagerhat, Jessore and Satkhira), prawn-carp poly-culture and rice-prawn rotation were practiced where stocking density of prawn ranged 10000 to 30000 post larvae/ha and production ranged from 350-625 kg/ha.

**Objective**
Production intensification of freshwater prawn *M. rosenbergii* through selected probiotic based eco-friendly culture system

This study was conducted to investigate the prawn production applying probiotic (beneficial bacteria).

**Methodology**
The study was conducted in the experimental ponds of Fisheries and Marine Resource Technology Discipline of Khulna University. The pond size was 240 $m^2$ and water depth maintained 1.5m. The prawn post larvae (PL) was nursed for 60 days and then stocked in grow out pond with a density of 20000, 40000 and 60000 juvenile/ha in without aeration culture system and 40000, 80000 and 120000/ha in aeration pond. Probiotic was used as per manual given by the manufacturer. Commercial feed containing 32% protein was used throughout the research.
Prawn culture period was 180 days

**Key findings**
In without aeration culture system prawn production was found 895.68, 1274.28 and 1292.71 kg/ha respectively and in aeration culture system production was found 1494.66, 1905.09 and 2061.70 kg/ha respectively. Considering cost-benefit stocking 40000/ha could be suggested for without aeration where net benefit could be 4,00,818 Taka/ha. Again stocking 80000/ha could be

suggested for in aeration practice where net benefit could be 5,88,804 Taka/ha.
This study revealed that probiotics (beneficial bacteria) could increase the activity of digestive enzymes and immune enzymes, thus increase growth, prevent disease and augment survival, ultimately higher prawn production was occurred.

**Key words**: *Macrobrachium rosenbergii*, Probiotics, Aeration, Intensification.

**Research highlight: 02**

**Background**
Freshwater prawn aquaculture is threatened due to variety of factors such as misuse of antibiotics and drugs, pollution of environment, and spreading of severe diseases caused by bacterial and viral agents. There is a national and international concern for preventing and controlling the diseases through new scientific approaches to make health-safe fish/shellfish product. Probiotics can be one of the alternatives to improve culture friendly water and soil quality, prevention of disease, increasing digestibility and immune competence of prawn. Thus, it is also important to isolate potential probiotics bacteria from the culture environment and go for their mass production in laboratory and pond. In freshwater prawn the innate defense system – also known as natural or non-specific defense system includes both cellular and humoral components which work in jointly coordination for the elimination of all foreign organisms potentially hazardous for the host (Jiravanichpaisal *et al.,* 2006). Amylase activity was found to be localized within the midgut gland of *M. rosenbergii*. Amylase is an enzyme that hydrolysis of carbohydrate.

Most of the digestive enzyme plays very important role for metabolism and respiration as well as their immune system. Protease is an important digestive enzyme for animal. Proteolytic enzymes are divided into four groups: serine, cysteine, aspartic and metallo proteases. Serine or alkaline proteases are so named because of their having a "super-reactive" serine in the active site (Simpson, 2000). Serine proteases function as important regulatory proteins in activating the prophenol oxidase (pro-PO) and clotting systems of the giant tiger prawn (Supungul et al. 2002; Dong et al. 2018; Xue *et al.* 2013). Addressing all the issues this research project focused on enhancement of prawn production by using eco-friendly culture techniques with the application of probiotics as well ensuring the health safe prawn production.

**Objective**
Growth and Quality enhancement of prawn administrating probiotics for augmenting digestibility and immunity, minimizing disease.

**Methodology**
Amylase activity was assayed by starch hydrolysis method of Bernfeld, 1955 and (Bhavan *et al*. 2014). The protease activity was estimated by using the casein-hydrolysis method by the method of Furne *et al.* (2005). The lipase activity was determined by the evaluation of the degradation of triacylglycerols, diacylglycerols, and monoacylglycerols to free fatty acids, following the method of Bier (1955).

Prawn immune enzyme *Prophenoloxidase* (proPO) was measured following (Moullac *et al.* (1997) and Superoxide dismutase (*SOD)* enzyme activity was determined according to Marklund

and Marklund (1974) with some modifications of Jha (2014). The survival (%) of shrimp larvae challenge test with the application of *Vibrio* spp. was also tested in three probiotics (P1, P2 and P3) and without probiotic.

**Key findings**

All three probiotics treated prawn showed higher digestive enzyme protease, amylase and lipase than the without probiotic treated prawn. Probiotics could increases protease, amylase and lipase enzyme activity in prawn. P1 showed little bit higher enzyme activity than P2 and P3. Stocking density has no distinct effect on enzyme activity among the three probiotics. Protease was found higher in $4/m^2$ and amylase in $2/m^2$.

The immune enzyme assessment data revealed that Pro-Phenoloxidase (Pro-PO) and Superoxide Dismutase (SOD) concentration were higher in probiotic treated prawn than without probiotic, which reflected the higher immune response in probiotic treated prawn.

Disease challenge test was done applying pathogenic bacteria *Vibrio* spp. in both with and without probiotics. It was found that probiotic could inhibit the growth of pathogenic bacteria like Vibrio sp. Thus, increase survival of prawn against pathogenic bacteria.

Probiotic (beneficial bacteria) could show higher growth of not only in added beneficial bacteria but also other beneficial bacteria. This research revealed that probiotic can increase digestive and immune enzymes and can inhibit growth of harmful bacteria (*Vibrio* sp.).

**Key words**: Probiotics, Digestive and immune enzymes, Minimizing disease.

**Research highlight: 03**

**Background**

While carbohydrates play important functions in several metabolic processes including the Krebs cycle, gluconeogenesis, chitin synthesis, and the formation of steroids and fatty acids (Wigglesworth and Griffith 1994). In Krebs cycle, high rates of partial oxidation of both fuels in lymphocytes and macrophages, and in other cells such as enterocytes, colonocytes,and in neoplastic cells. High rate of glutamine utilization and its importance in such cells has raised the question as to the source of this glutamine in the body (Newsholme *et al.*, 1987). The culture media of bacteria content high percentage of carbohydrate. Therefore, carbohydrate enriched locally available ingredients (molasses, rice polish, maize powder) might increase beneficial bacteria in pond system.

It is already proved that probiotics (beneficial bacteria) could increase fish/shrimp production. A large number of farmers are using commercial probiotics indiscriminately those are imported from foreign countries by high price. The study was aimed to investigate the effect of carbohydrate enrich ingredient on growth of beneficial bacteria as well as production performance of prawn.

**Objective**

Identification of potential probiotics in aqua farm and their mass proliferation in lab and in situ condition as well as in the gut of prawn by using locally available ingredients and their

effect on prawn production.

**Methodology**

To conduct the study the ingredients were used as molasses (25 kg), rice polish (25 kg), maize powder (25 kg), molasses (12.5kg) + rice polish (12.5kg), molasses (12.5kg)+ maize (12.5kg), rice polish (12.5kg) +maize (12.5kg),molasses(8.33kg) + rice polish (8.33kg) + maize (8.33)per hectare per week in T1, T2, T3, T4, T5, T6 and T7 respectively; no carbohydrate enriched ingredient used in control pond. Every month 250g probiotic (P1) was added in pond as seed to accelerate the growth of native beneficial bacteria. Stocking density of prawn was 30000 juvenile/ha and each treatment was triplicated where culture period was 180 days.

The experiment was aimed to identify three beneficial bacteria (probiotics) species *Bacillus* spp.*, Chlostridium* spp.*, Lactobacillus* spp. and their load in the ponds where carbon enriched ingredients were used. In the control pond no additional carbon source was applied except feed containing 28% protein. To enumerate enrichment of native bacterial colony in different carbohydrates treated pond, water sample was collected from each treated pond and native bacterial load was counted. Stock solution was serially diluted to target fold adding peptone water. Selective agar was weighted and autoclaved and water solution was inoculated through pour plating method. The petri dish were kept at 37° C for 24 hours and then colony were counted. Counting the colony, the suitable dilution factors were defined for each treatment. Then each defined diluted sample were inoculated, incubated and counted maintaining replications.

**Key findings**

Beneficial bacteria was found higher in carbohydrate added pond than the control (without carbohydrate). Molasses+Rice polish treated pond showed considerable growth of three main bacteria (*Clostridium* spp, *Lactobacillus* spp. and *Bacillus* spp.). The prawn production was found 1080.81, 916.94, 882.52, 1108.57, 1021.63, 870.91,942.52 and 751.80kg/ha in T1, T2, T3, T4, T5, T6, T7 and control respectively. Prawn production was found higher in all carbohydrate treated pond than the without carbohydrate (Control) and highest in T4 molasses (12.5kg) + rice polish (12.5kg), followed by T1 (molasses) and T5 (molasses+maize). The net benefit 3,61,142Taka/ha could be earn applying carbohydrate in prawn culture. The main three native beneficial bacterial growth (log CFU/ml) was measured in seven treatments and also in control. The highest *Bacillus* (7.236±0.0088) and *Lactobacillus* (4.11±0.026 log cfu/ml) load were found in pond water where molasses + rice polish were used and lowest in control (*Bacillus*=6.437±0.0449; *Lactobacillus*=3.044±0.12 Log(cfu/ml). Clostridium was found highest in maize powder (4.27±.016) and second highest (4.21±.016log cfu/ml ) in mixture of molasses and rice polish. Carbohydrate could be used as substitute of commercial probiotics.

Locally available carbohydrate enrich low-cost ingredients can increase native beneficial bacteria in pond system and thus could be used as substitute of commercial probiotics.

**Key words**: *Macrobrachium rosenbergii*, Carbohydrate, Substitute of probiotics.

## B. Implementation Status

### 1. Procurement (Component wise)

### i. Coordination component: Not applicable

### ii. BARC component

| Description of equipment and capital items | PP Target | | Achievement | | Remarks |
|---|---|---|---|---|---|
| | Physical (No.) | Financial (Tk.) | Physical (No.) | Financial (Tk.) | |
| (a) Office equipment | Procurement of Computer & Accessories (Desktop-2 Computer, Laptop-2, UPS-2, Scanner-1, .Multimedia Projector-1) | 4,56,000.00 | Procurement of Computer & Accessories (Desktop-2 Computer, Laptop-2, UPS-2, Scanner-1, .Multimedia Projector-1) | 4,56,000.00 | |
| (b) Lab &field equipment | | | | | |

### iii. BFRI (SRS) component

| Description of equipment and capital items | PP Target | | Achievement | | Remarks |
|---|---|---|---|---|---|
| | Physical (No.) | Financial (Tk.) | Physical (No.) | Financial (Tk.) | |
| (a) Office equipment | 6 | 266000 | 6 | 263500 | |
| (b) Lab &field equipment | 17 | 3910000 | 17 | 3575800 | |
| (c) Other capital items | 6 | 237500 | 6 | 214300 | |

### iv. KU component

| Description of equipment and capital items | PP Target | | Achievement | | Remarks |
|---|---|---|---|---|---|
| | Physical (No.) | Financial (Tk.) | Physical (No.) | Financial (Tk.) | |
| (a) Computer & Accessories | GD-3/1st Y | 140000 | GD-3/1st Y | 140000 | Achieved |
| (b) Procurement of furniture | GD-1(a)/2st Y | 69626 | GD-1(a)/2st Y | 69626 | Achieved |
| Lab & field equipment | | | | | |
| Microscope | GD-2/1st Y | 138885 | GD-2/1st Y | 138885 | Achieved |
| Microbial Media | GD-5/1st Y | 219097 | GD-5/1st Y | 219097 | Achieved |
| Chemicals & Apparatus | GD-4/1st Y | 314132 | GD-4/1st Y | 314132 | Achieved |
| Chemicals | GD-1/2nd Y | 221900 | GD-1/2nd Y | 221900 | Achieved |
| Apparatus | GD-2/2nd Y | 192500 | GD-2/2nd Y | 192500 | Achieved |
| Microbial media | GD-3/2nd Y | 185600 | GD-3/2nd Y | 185600 | Achieved |
| Chemical and apparatus | GD-1/3rd Y | 212000 | GD-1/3rd Y | 212000 | Achieved |
| Spectrophotometer | GD-4/2nd Y | 564000 | GD-4/2nd Y | 564000 | Achieved |

## 11. Establishment/renovation facilities: Not applicable

## III. Training/study tour/ seminar/*workshop*/conference organized

| **BARC component** | | | | | |
|---|---|---|---|---|---|
| Description | Number of participants | | | Duration (Days) | Remarks |
| | Male | Female | Total | | |
| Inception Workshop (1 no) | 56 | 7 | 63 | 1 day | All workshops held at the Conference room of BARC as per schedule of activity of the Coordination component |
| Half yearly Research Prog. Review Workshop (2 no.) | 65+ 62 | 9+8 | 144 | 1+1 = 2 days | |
| Annual Research Prog. Review Workshop (2 no.) | 60+63 | 7+8 | 138 | 1+2 =3 days | |
| Project Completion Report Review Workshop (1 no) | 45 | 6 | 52 | 1 day | |
| **BFRI (SRS) Component:** | | | | | |
| (a) Training | | | | | |
| (b) Workshop | | | | | |
| (c) Field Day | 40 | 0 | 40 | 02 Days (13/09/2020 & 16/09/2020) | |
| **KU Component:** Not applicable | | | | | |
| (a) Training | | | | | |
| (b) Workshop | | | | | Not applicable |
| (c) Others (if any) | | | | | |

## C. Financial and physical progress (Combined & Component wise)

### i. Combined

| Items of expenditure/activities | Total approved budget | Fund received | Actual expenditure | Balance/ unspent | Physical progress (%) | Reasons for deviation |
|---|---|---|---|---|---|---|
| a. Contractual staff salary | 3255787 | 3235853 | 3088593 | 147260 | 94.86 | Not applicable |
| b. Field research/lab expenses and supplies | 11781664 | 9791876 | 9211949 | 579927 | 78.19 | |
| c. Operating expenses | 1825343 | 1593132 | 1327264 | 265868 | 72.71 | |
| d. Vehicle hire and fuel, oil & maintenance | 1044513 | 866894 | 751114 | 115780 | 71.91 | |
| e. Training/workshop/seminar etc. | 123600 | 123600 | 123600 | 0 | 100.00 | |
| f. Publications and printing | 483000 | 103500 | 83000 | 20500 | 17.18 | |
| g. Miscellaneous | 568167 | 538151 | 521627 | 16524 | 91.81 | |
| h. Capital expenses | 5863126 | 5512511 | 5512511 | 0 | 94.02 | |
| Total | 24945200 | 21765517 | 20619658 | 1145859 | 82.66 | |

## ii. Coordination component

| Items of expenditure/activities | Total approved budget | Fund received | Actual expenditure | Balance/ unspent | Physical progress (%) | Reasons for deviation |
|---|---|---|---|---|---|---|
| a. Contractual staff salary | 313200 | 430000 | 230930 | 199070 | 73.73 | Not applicable |
| b. Field research/lab expenses and supplies | 0 | 0 | 0 | 0 | 0 | |
| c. Operating expenses | 490000 | 339700 | 42225 | 297475 | 8.62 | |
| d. Vehicle hire and fuel, oil & maintenance | 200000 | 140177 | 48000 | 92177 | 24.00 | |
| e. Training/workshop/seminar | 0 | 0 | 0 | 0 | 0 | |
| f. Publications and printing | 0 | 0 | 0 | 0 | 0 | |
| g. Miscellaneous | 60000 | 60000 | 20000 | 40000 | 33.33 | |
| h. Capital expenses | 90000 | 90000 | 90000 | 0 | 100.00 | |
| Total | 1153200 | 1059877 | 431155 | 628722 | 37.39 | |

## iii. BARC component

| Items of expenditure/activities | Total approved budget | Fund received | Actual expenditure | Balance/ unspent | Physical progress (%) | Reasons for deviation |
|---|---|---|---|---|---|---|
| a. Contractual staff salary | 135000 | 135000 | 135000 | 0 | 100.0 | Not applicable |
| b. Field research/lab expenses and supplies | 0 | 0 | 0 | 0 | 0 | |
| c. Operating expenses | 100178 | 100178 | 100103 | 75 | 99.9 | |
| d. Vehicle hire and fuel, oil & maintenance | 154543 | 154543 | 154543 | 0 | 100.0 | |
| e. Training/workshop/seminar | 123600 | 123600 | 123600 | 0 | 100.0 | |
| f. Publications and printing | 300000 | 20500 | 0 | 20500 | 0.0 | |
| g. Miscellaneous | 72679 | 72679 | 72580 | 99 | 99.9 | |
| h. Capital expenses | 456000 | 456000 | 456000 | 0 | 100.0 | |
| Total | 1342000 | 1062500 | 1041826 | 20674 | 77.6 | |

## iv. BFRI (SRS) component

Fig in Tk

| Items of expenditure/activities | Total approved budget | Fund received | Actual expenditure | Balance/ unspent | Physical Progress (%) | Reasons for deviation |
|---|---|---|---|---|---|---|
| a. Contractual staff salary | 1104877.00 | 968143.00 | 1019953.00 | -51810 | 92.31 | Not applicable |
| b. Field research/lab expenses and supplies | 8056500.00 | 6066877.00 | 5486950.0 | 579927 | 68.11 | |
| c. Operating expenses | 690000.00 | 622184.00 | 653866.00 | -31682 | 94.76 | |
| d. Vehicle hire and fuel, oil & maintenance | 405000.00 | 279220.00 | 255617.00 | 23603 | 63.12 | |
| e. Training/workshop/seminar | 0.00 | 0.00 | 0 | 0 | 0 | |
| f. Publications and printing | 100000.00 | 0 | 0 | 0 | 0.00 | |
| g. Miscellaneous | 160123.00 | 130162.00 | 153737.00 | -23575 | 96.01 | |
| h. Capital expenses | 4393500.00 | 4053600.00 | 4053600.00 | 0 | 92.26 | |
| Total | 14910000.00 | 12120186.00 | 11623723.00 | 496463.00 | 77.96 | |

### iv. KU component

Fig in Tk

| Items of expenditure/activities | Total approved budget | Fund received | Actual expenditure | Balance/ unspent | Physical Progress (%) | Reasons for deviation |
|---|---|---|---|---|---|---|
| a. Contractual staff salary | 1702710 | 1702710 | 1702710 | 0.00 | 100.00 | Not applicable |
| b. Field research/lab expenses and supplies | 3725164 | 3724999 | 3724999 | 0.00 | 100.00 | |
| c. Operating expenses | 545165 | 531070 | 531070 | 0.00 | 97.41 | |
| d. Vehicle hire and fuel, oil & maintenance | 284970 | 292954 | 292954 | 0.00 | 102.80 | |
| e. Training/workshop/seminar | 0 | 0 | 0 | 0.00 | 0 | |
| f. Publications and printing | 83000 | 83000 | 83000 | 0.00 | 100.00 | |
| g. Miscellaneous | 275365 | 275310 | 275310 | 0.00 | 99.98 | |
| h. Capital expenses | 923626 | 912911 | 912911 | 0.00 | 98.84 | |
| Total | 7540000 | 7522954 | 7522954 | 0.00 | 99.77 | |

## D. Achievement of Sub-project by objectives (Tangible form): Technology generated / developed

### BFRI (SRS) Component

| General/specific objectives of the sub-project | Major technical activities performed in respect of the set objectives | Output (i.e., product obtained, visible, measurable) | Outcome (short term effect of the research) |
|---|---|---|---|
| To develop cluster-based shrimp (*Penaeus monodon*) farming (CBSF) with special emphasis on disease prevention and traceability | i. Traditional ghers has been converted into clusters having facilities to implement scientific methods of shrimp culture viz., installation of surrounding net, different water outlet and inlet facility, water purification chamber, nursing point and grow-out pond<br>ii. Stocking density was followed in 1st year is 3/$m^2$ & 6/$m^2$ and 6/$m^2$ & 9/$m^2$ in 2nd year. | i. Growth, water quality parameters during the culture period found satisfactory<br>ii. No disease outbreak recorded<br>iii. 735.6 kg/ha at the stocking density of 3 PL/$m^{-2}$ and 854.4 kg/ha at the stocking density of 6 PL/$m^{-2}$ achieved in the 1st year<br>iv. With no aeration and higher stocking density of 9 PL/$m^{-2}$ found not suitable and the production dropped to 600 kg/ha<br>v. Due to lower salinity production dropped to 694 kg/ha with the stocking density of 6 PL/$m^{-2}$ in the 2nd year | Even though the average production dropped to 694 kg/ha in the second year compared to 854.4 kg/ha with the stocking density of 6 PL/$m^{-2}$, is still more than double than the average traditional production of shrimp i.e., 300 kg/ha. It can thereby double the shrimp production of the traditional farming system with minimum modifications. |

| | | | |
|---|---|---|---|
| To promote Shrimp/Prawn health management through intensive monitoring and surveillance of and viral diseases, identification of risk factors, evaluation of immune response and preventive measures | i. Pathogen specific primer designed and synthesized<br>ii. PCR protocol were optimized.for the identification of OIE listed pathogen for Shrimp and Prawn<br>iii. Prevalence of pathogens determined | i. For the very first time, Enterocytozoon hepatopenaei (EHP) reported in Bangladesh.<br>ii. This is the first report in the world affecting EHP in *Macrobrachium rosenbergii* | Unclassified mortality in Prawn (*M. rosenbergii*) been long term unsolved case. On average 80~110 prawn mortality caused tremendous loss to the farmers. Identification of the causative agent would help to take better prevention measures. |
| To develop all male PL production technique of Giant Freshwater Prawn (*M. rosenbergii*) | i. Matured wild brood of *M. rosenbergii* were collected and keep in brood rearing tank<br>ii. Hatchery Operation carried out to get viable known aged post larvae of prawn<br>iii. As PL 45 ~ 60 is required for the microsurgery, PL were nursed in the hatchery cistern for the required days<br>iv. Ablation of androgenic gland attempted by microsurgery | i.A total 500 PLs operated for micro surgery.<br>ii.Only 2% PL survived but unfortunately all found male when checked. The poor survival rate was due to the stress under the microscope. | Microsurgical ablation of androgenic gland is a complicated task and required subsequent trials. It would be better to have an extension of another year to master the microsurgical approaches, if succeed, could be a great opportunity for the prawn industry of the country. |
| To have an insight of innate immune response over immunogenic agents in shrimp (P. monodon) | v. Pure culture of target bacteria<br>vi. Mass culture of bacteria in aseptic condition<br>vii. Confirm bacterial number and proceed to attenuation through heat treatment<br>iii. Mixed with feed and maintain schedule administration. | iii. Increased the total count of hemocyte cell to double in the treatment group than the control group. | Application of such medicated feed can boost immunity and thus ensure better protection against disease outbreak. |
| To infer the genetic diversity of Giant Freshwater Prawn (*M. rosenbergii*) in the major coastal rivers | i. Wild populations from the rivers viz., Kocha, Payra, Baleswar, Rupsha, Poshur, Kornofuli, Meghna and Kumar Nod were collected<br>ii. Genomic DNA were extracted from tissue samples collected from | i. PCR products stored at -20$^0$C refrigerator<br>ii. SDS page vertical gel electrophoresis techniques is required to analyze the genetic variability but unfortunately due to corona pandemic procurement of some | Output of the study could help to choose suitable source of brood stock for further brood domestication programme. |

| | | | |
|---|---|---|---|
| | pleopods<br>iii. The extracted DNA was amplified with 10 pair of microsatellite DNA specific primer | necessary reagents was not possible. | |
| **KU component** | | | |
| Effect of probiotic on Prawn growth and production | Prawn was cultured in without probiotic and probiotic treated pond with the stocking density of 20000 juvenile/ha where culture period was 180 days. | Probiotic treated pond showed higher production (P1, 857.7 kg/ha) compare to non-probiotics (661.64 Kg/ha). | Probiotic will be used in the prawn farm thereby higher production will be found compared to conventional practices. |
| Intensification of freshwater prawn (*M. rosenbergii*) through probiotic based culture system increasing stocking density | Study was conducted applying stocking density 20000, 40000 and 60000 juvenile/ha in without aeration culture system and 60000, 80000 and 120000 juvenile/ha in with aeration system. | Prawn production was found 895.68, 1274.28 and 1292.71 kg/ha in without aeration system and 1494.66, 1905.09 and 2061.70 kg/ha in aeration as per stocking density mentioned. | Intensification of prawn production can be done applying probiotic in prawn farming. Farmer can practice stocking of 40,000 and 80,000 juvenile/ha in without aeration and with aeration respectively considering utmost benefit. |
| Application of locally available low cost carbohydrate enriched ingredients (molasses, rice polish, maize powder) as substitute of commercial probiotics | The study was conducted adding carbohydrate enriched ingredients as molasses, rice polish, maize powder, molasses + rice polish, molasses + maize, rice polish +maize ,molasses+ rice polish + maize per ha per week where stocking density of prawn were maintained 30,000 juvenile/ha. And no carbohydrate was used in control pond. | The highest prawn production was found 1,108.57 kg/ha applying molasses + rice polish. The second highest production was found 1080.81kg/ha in molasses applied pond. Prawn production was 751.80 kg/ha, where no additional carbohydrate was applied (control) | Locally available carbohydrate enrich low-cost ingredients can increase beneficial bacteria in pond system and thus could be used as substitute of commercial probiotics. |
| Effect of probiotic (beneficial bacteria) on digestive (Protease, Lipase, Amylase) and immune response enzymes (Prophenoloxidase, Pro-PO;Superoxide dismutase,SOD) | The enzymatic activities of Protease, Lipase and Amylase were estimated following the Furne et al. (2005), Bier (1955) and Bhavan et al. (2014) respectively.<br>Activity of immune enzyme Pro-PO) and SOD were determined following Moullac et al., (1997)<br>And Marklund and Marklund, (1974) repectively. | Probiotic can increase the function of digestive enzymes (Protease (21%), Lipase (68%), and Amylase (455%)) and immune response enzymes (Pro-Phenoloxidase (198%), Superoxide Dismutase (58%). | Probiotics could increase digestive enzyme and immune enzyme activities, thus increase growth, prevent disease and augment survival, ultimately enhance prawn production. |

## E: Information/knowledge generated/policy generated

| **BFRI (SRS) component** | | | |
|---|---|---|---|
| General/specific objectives of the sub-project | Major technical activities performed in respect of the set objectives | Output (i.e., product obtained, visible, measurable) | Outcome (short term effect of the research) |
| To develop cluster-based shrimp (*Penaeus monodon*) farming (CBSF) with special emphasis on disease prevention and traceability | i. Conversion of traditional ghers into clusters to implement scientific methods of shrimp culture<br>ii. Stocking density was followed in 1st year is 3/$m^2$ & 6/$m^2$ and 6/$m^2$ & 9/$m^2$ in 2nd year. | i. Disease outbreak was prevented.<br>ii. Without aeration, 854.4 kg/ha shrimp produced at the stocking density of 6 PL/$m^{-2}$ achieved | Average traditional production doubled with minimum modifications. |
| To promote Shrimp/Prawn health management through intensive monitoring and surveillance of and viral diseases, identification of risk factors, evaluation of immune response and preventive measures | i. PCR protocol optimized .for the identification of OIE listed pathogen for Shrimp and Prawn<br>ii. Prevalence of pathogens determined | i. New pathogen reported.<br>ii. PCR test facility established | Disease screening and diagnosis capacity increased |
| To develop all male PL production technique of Giant Freshwater Prawn (*M. rosenbergii*) | i. microsurgery attempted for ablation of androgenic gland by | i. Preliminary success of microsurgery achieved, however, sex reversal not observed. | It was a first experimental trial in Bangladesh. Further experiment is required. |
| To have an insight of innate immune response over immunogenic agents in shrimp *(P. monodon*) | i. Attenuated bacterial stock was prepared<br>ii. Administrated with feed | i. Immunity boosted with the increased the number of hemocyte cells. | Immunostimulants can help preventing disease outbreak. . |
| To infer the genetic diversity of Giant Freshwater Prawn (*M. rosenbergii*) in the major coastal rivers | i. Sample collected from different rivers<br>ii. DNA extracted and amplified using the microsatellite marker specific primer. | i. PCR products stored for the SDS page analysis | Can help to develop brood domestication programme in future. |
| **KU component** | | | |
| Effect of probiotic on Prawn growth and production | To see probiotic effect prawn was cultured with the stocking density of 20000 juvenile/ha in without probiotic and with probiotic. | Probiotic treated pond showed higher production (P1, 857.7 kg/ha) compare to non-probiotics(661.64 Kg/ha). | Prawn farmers will get higher production using probiotics in prawn culture system. |
| Intensification of freshwater prawn (*M.* | Three different stocking density 20000, 40000 | In without aeration highest prawn | Considering available input and cost-benefit |

| | | | |
|---|---|---|---|
| *rosenbergii*) through probiotic based culture system increasing stocking density | and 60000 juvenile/ha in without aeration culture system and 60000, 80000 and 120000 juvenile/ha in with aeration system. | production was found 1292.71 and 2nd highest 1274.28kg/ha, on the other hand it was 2061.70 and, 1905.09 kg/ha respectively in aeration system. | stocking density 40,000 and 80,000 juvenile/ha could be suggested for without aeration and with aeration respectively. |
| Application of locally available low cost carbohydrate enriched ingredients (molasses, rice polish, maize powder) as substitute of commercial probiotics | Available carbohydrate enriched ingredients molasses, rice polish, maize powder, where used in pond with 30,000 juvenile/ha. | The highest prawn production was found 1,108.57 kg/ha applying molasses12.5kg + rice polish 12.5kg/ha. | Prawn farmers are suggested to use locally available carbohydrate enrich low-cost ingredients besides feed to increase production. |
| Effect of probiotic (beneficial bacteria) on digestive (Protease, Lipase, Amylase) and immune response enzymes (Prophenoloxidase, Pro-PO;Superoxide dismutase,SOD) | i. The enzymatic activities of Protease, Lipase and Amylase were estimated following the Furne et al. (2005),<br>ii. Bier (1955) and Bhavan et al. (2014) respectively. Activity of immune enzyme Pro-PO) and SOD were determined following Moullac et al., (1997), and Marklund, (1974) respectively. | Probiotic increased the function of digestive enzymes (Protease (21%), Lipase (68%), and Amylase (455%)) and immune response enzymes (Pro-Phenoloxidase (198%), Superoxide Dismutase (58%). | Probiotics can be suggested for disease control and production enhancement of Prawn. |

## F. Materials development/ Publication made under the Sub-project

| Publication | Number of publications | | Remarks (e.g. paper title, name of journal, conference name, etc.) |
|---|---|---|---|
| | Under preparation | Completed and published | |
| **i. BFRI (SRS) Component** | | | |
| Technology bulletin/ booklet/leaflet/flyer etc. | Leaflet 01 | | Development of Cluster Based Shrimp Farming (CBSF) to Cope with Disease Outbreak in Shrimp Aquaculture |
| Journal publication | 2 | | 1. Cluster Based Shrimp Farming (CBSF): an approach to mitigate disease outbreak and enhance production<br>2. First Report on *Enterocytozoon hepatopenaei* (EHP) causing cronic mortality in *Macrobrachium rosenbergii* |
| Video clip/TV Program | | | |

| | | | |
|---|---|---|---|
| News Paper/Popular Article | 1 | | Attenuated/ killed bacteria can act as immunogenic agent to boost shrimp immunity: an intervention. |
| **ii. KU component** | | | |
| Technology bulletin/ booklet//flyer etc. | | Leaflet (1) | Application of probiotics and carbohydrate enriched ingredients for intensification of freshwater prawn |
| Journal publication | 2 One is under review | | Pro-Phenoloxidase and superoxide dismutase based immunemodulatory effect of commercial probiotic on *Macrobrachium rosenbergii* cultured at different stocking densities, Journal: Fish and Shellfish Immunology, Elsevier |
| Electronic/Video clip/TV program | | KU website (2020) | Probiotic based prawn culture |
| News Paper/Popular Article | 2 | | Probiotic based prawn culture technology in (draft prepared) :<br>1.National News Paper<br>2. Krishikotha<br>Draft prepared. |
| Other publications, if any | | Fish Week (Matshya Pokkh) 2021 | Role of probiotic in freshwater prawn (*Macrobrachium rosenbergii*) production and enhancement of native beneficial bacteria applying low cost carbohydrate enriched ingredients |

## G. Description of generated technology/knowledge/policy

### i. Technology fact sheet

**BFRI (SRS) component**

**Title of the technology**

Development of Cluster Based Shrimp Farming (CBSF) to Cope with Disease Outbreak in Shrimp Aquaculture

**Introduction**

In Bangladesh, Shrimp sector, playing a significant role in foreign exchange earnings, employment generation and poverty reduction. Since the last decades the sector has been suffering from various issues and problems related to culture and production. In shrimp (*P. monodon*) culture, various diseases, WSSV in particular, has become a serious constraint, thereby, farmed shrimp production reduced significantly. Major suspected reasons behind the disease outbreak are poor farm management in terms of water quality, poor seed quality, less or no biosecurity, etc. To address these issues, the conceptual model of CBSF subjected to field trial. For implementing the model, traditional ghers were converted into clusters having facilities to implement scientific methods of shrimp culture. The model farm facilitated with water purification chamber, different water outlet and inlet facility and nursing point facility with better

quality feed and seed (PL) along with continuous monitoring of water quality and presence of viral particle (random check by Polymerase Chain Reaction, PCR). Main focus of this study was to form a community among the neighboring farm owners and sharing their ghers to adopt the model where their amount of land will be considered as initial investment along with further inputs and profit will be shared after a complete cycle according to their share (land+money).

**Description**

Under this study, in the first year, shrimp were cultured for 4 months in two different stocking densities. Growth, water quality parameters and disease screening information the culture period was found satisfactory. No disease outbreak recorded throughout the culture period, which was one of the great achievements of this study. Besides, from the traditional gross production of shrimp is around 250-300 kg/ha, with this management intervention, production maximized more than two times (735.6 kg/ha at the stocking density of 30000 PL/ha and 854.4 kg/ha at the stocking density of 60000 PL/ha) compared to the traditional farming. Once getting significant production of 854.4 kg/ha at the stocking density of 60000 PL/ha with no aeration, in the second year, emphasize given on to optimize maximum stocking density to increase per ha production. Therefore, instead 30000 and 60000 PL/ha, treatment designed to 60000 and 90000 PL/ha to see in this higher stocking can be sustained without aeration. Due to corona pandemic and lockdown situation, stocking of PL became delay. Due to heavy rainfall, average salinity throughout the culture period was around 2 ppt, which is considered as low salinity for the shrimp growth. With no aeration and higher stocking density of 90000 PL/ha found not suitable and the production dropped to 600 kg/ha at. Due to lower salinity overall survival found 15% less than the first-year survival. For the same reason, production dropped to 694 kg/ha with the stocking density of 60000 PL/ha.

**Suitable location/ecosystem:** Khulna, Satkhira, Bagerhat, Cox'sbazar

**Benefits**

Even though the average production dropped to 694 kg/ha in the second year compared to 854.4 kg/ha with the stocking density of 60000 PL/ha, is still more than double than the average traditional production of shrimp i.e., 300 kg/ha. Cluster based shrimp farming in Bangladesh will contribute in higher amount of healthy shrimp production by reducing the disease incidence possibilities and contribute in increasing export earnings.

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## KU Component

### Title of the technology
Application of Locally Available Low Cost Carbohydrate Enriched Ingredients as Substitute of Commercial Probiotics

### Introduction
The freshwater prawn (*M. rosenbergii*) has significant role in the economy, employment opportunity and poverty elevation in Bangladesh. But sill farmers are practicing traditional culture system. The baseline surveyed of this study showed that in the southwest Bangladesh (Khulna, Bagerhat, Jessore and Satkhira), prawn-carp poly-culture and rice-prawn rotation were practiced where stocking density of prawn ranged 10000 to 30000 post larvae/ha and production ranged from 350-625 kg/ha. The soil and water properties of Bangladesh is very much suitable for freshwater prawn culture. This technology could be used from coastal low saline water to any freshwater body like pond, lake, paddy filed, canal, bill, ditches etc.

### Description
Carbohydrate enriched locally available ingredients (molasses, rice polish, maize powder) were applied in the prawn culture pond to identify the growth of native beneficial bacteria (probiotics). To conduct the study the ingredients were used as molasses (25 kg), rice polish (25 kg), maize powder (25 kg), molasses (12.5kg) + rice polish (12.5kg), molasses (12.5kg)+ maize (12.5kg), rice polish (12.5kg) + maize (12.5kg), molasses (8.33kg) + rice polish (8.33kg) + maize (8.33) per hectare per week; no carbohydrate enriched ingredient used in control pond. Every month, 250g probiotic (P1) was added in pond as seed to accelerate the growth of native beneficial bacteria. Stocking density of prawn was 30000 juvenile/ha and each treatment was triplicated where culture period 180 days. The main three native beneficial bacterial growths (log CFU/ml) was measured in seven treatments and also in control. Beneficial bacteria (*Clostridium* spp, *Lactobacillus* spp. and *Bacillus* spp.) found higher in carbohydrate added pond than the control (without carbohydrate). Prawn production was found higher (1108. 57 kg/ha) in carbohydrate (Molasses 12.5 Kg + Rice polish 12.5 Kg) treated pond than the without carbohydrate (751.80 kg/ha).The net benefit 3,61,142 Taka/ha could be earn applying carbohydrate in prawn culture.

**Suitable location/ecosystem:** Khulna & Bagerhat coastal region

### Benefits
In Bangladesh, the Prawn culture system is still on a traditional stage. But this culture system will be a promising sector for the betterment of our national economy through the application of effective technology. This study firmly reveals that prawn production could be increased at several fold applying probiotics. Locally available carbohydrate enrich low-cost ingredients can increase native beneficial bacteria which boost up the production of freshwater prawn. Therefore carbohydrate enriched ingredients could be the substitute of commercial probiotics.

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**ii. Effectiveness in policy support (if applicable)**

As per clause 3, the most important objective of NFP-1998 is to achieve economic growth through exporting fish/shrimp. As an important foreign exchange earning source, under the range of NFP-1998 (Claus-5), culture of shrimp/prawn in coastal region has been also considered and therefore, increase of shrimp/prawn production by developing appropriate culture technology (Sub-Claus 8.12 of Claus-8 "Coastal shrimp aquaculture policy")has been re-emphasized. Technologies like environment GAP approach based cluster farming of shrimp, enhancement of prawn seed survival rate through all male seed production technology and use of local ingredient based probiotics in shrimp/prawn farming, all these together will be able to contribute for meeting the policy guidelines through increased amount of healthy exportable shrimp/prawn production in the country.

**H. Technology/Knowledge generation/Policy support (as applied)**

**i. Immediate impact on generated technology (commodity & non-commodity)**

Average production of traditional shrimp farming can increase by the implementation of the generated cluster-based shrimp farming technology. And also revealed that probiotics (beneficial bacteria) could increase the activity of digestive enzymes and immune enzymes, thus increase growth, prevent disease and augment survival, ultimately increase prawn production.

**ii. Generation of new knowledge that help in developing more technology in future**

- Identification of new pathogen, EHP in Prawn can offer a new area of intensive research for the sustainable production of *M. rosenbergii*
- Knowledge on the immunity boost up using attenuated bacteria in shrimp can offer new opportunity onto the development of immune boosters for crustaceans.
- Effect of specific probiotic (beneficial bacteria) on other harmful and beneficial bacteria.
- Suitable probiotic dose could be investigated to for digestive enzyme and immune enzyme.
- Biochemical quality of produced product by probiotics.
- Emphasize should be given to increase indigenous natural probiotics for the replacement of commercial foreign probiotics.

**iii. Technology transferred that help increased agricultural productivity and farmers' income**

Shrimp being an export-oriented commodity, will get more values and acceptance if the culture system shifted to cluster-based shrimp farming because of having better management and traceability options. Farmers will be more benefited as it helps to short the market chain and minimize the risk of total crop loss.

The freshwater prawn (*M. rosenbergii*) has significant role in the economy, employment opportunity, poverty elevation and foreign currency earning in Bangladesh. Baseline survey of this study showed that in the southwest Bangladesh ( Khulna, Bagerhat, Jessore and Satkhira), prawn-carp poly-culture and rice-prawn rotation were practiced where stocking density of prawn ranged 10000 to 30000 post larvae/ha and production ranged from 350-625 kg/ha. This study revealed that applying probiotics with a stocking density of 40000 juvenile/ha prawn production could be increased up to 1274.28 kg/ha from where net benefit could be earn 4,00,818 Taka/ha/year. Prawn farmers will be benefited applying this simple probiotic based prawn culture increasing their household earning.

## iv. Policy support

### BFRI (SRS) Component

In Bangladesh, Shrimp sector, playing a significant role in foreign exchange earnings, employment generation and poverty reduction. Since the last decades the sector has been suffering from various issues and problems related to culture and production. With moderate improvements, adopting community-based shrimp farming can double the national gross production of shrimp from the extensive culture practice. Along with the improvement in culture systems, disease screening should be an integral part to ensure sustainable production. Further research can be carried out to produce all male prawn to ensure maximum profit from a limited farming area. Immune stimulants can play major role to cope disease outbreak in shrimp and prawn sector. Further interventions can be carried out along the line.

### KU component

The giant freshwater prawn (*M. rosenbergii*) is playing a significant role in the economy, employment opportunity and poverty elevation in Bangladesh. There is a great opportunity to earn foreign currency through export health safe prawn commodity. Probiotics (beneficial bacteria) applied eco-friendly prawn culture technology could intensify prawn production with emphasizing on both food safety and health safety. Antibiotic and harmful chemical free quality prawn draws the attention of foreign buyers. This study proves that probiotics and carbohydrate enriched ingredients could increase prawn production at a satisfactory level. Further research needs to be carried out on the application doses of carbohydrate enriched ingredients.

## I. Information regarding desk and field monitoring

### i. Desk monitoring (description & output of consultation meeting, monitoring workshops/seminars etc.) information of the sub-project

| Date of the programs | Program descriptions | Implementation Unit | Remarks/output |
|---|---|---|---|
| 31 May, 2018 | Signing of the Letter of Agreement (LoA) | PIU-BARC, NATP-2 | Duly signed as per the terms and condition of LoA |
| 06 November, 2018 | Inception workshop | Fisheries Division, BARC | The meeting was to inform the PIs of the sub-project how follow the PPR 2008 for the recruitment of manpower and procurement of equipment's, chemicals and reagents as per budget. |
| 21 June, 2019 | Monitoring workshop | Fisheries Division, BARC | Progress of procurements of equipment's, chemicals and reagents and research activities |
| 23 July, 2019 | Financial management workshop | PIU-BARC, NATP-2 | Procurement of equipment's , chemicals and reagents as per the rule of PPR 2008 and the audit rules |
| 24 October, 2019<br>19 Feb, 2020<br>!8 June 2020 | Coordination meeting | Fisheries Division, BARC | Discussion among the coordinator, director, PIs and Co-PIs about the guidelines how to achieve the goal collaborately |
| 20 November, 2019 | Annual progress review workshop | Fisheries Division, BARC | Presentation on annual research progress through multimedia presentation, .Received review comments on progress and future works |
| June,2019<br>September, 2020<br>20 November, 2019 | Annual progress review workshop | PIU-BARC, NATP-2 | Presentation on annual research progress through multimedia presentation, .Received review comments on progress and future works |
| March, 2019<br>June, 2020 | Half yearly progress review workshop | Fisheries Division, BARC | Presentation on half yearly research progress through multimedia presentation and all the suggestions were complied |
| 7 September, 2020 | Virtual Meeting on Progress Monitoring | PIU-BARC, NATP-2 | Presentation of research progress through multimedia presentation. Received review comments. |
| 23 September, 2020 | Annual progress review workshop | PIU-BARC, NATP-2 | Presentation on annual research progress through multimedia presentation and the suggestions from the workshop were complied. |
| 24 October, 2020 | Annual progress review workshop | PIU-BARC, NATP-2 | Presentation on annual research progress through multimedia presentation and the suggestions |

| Date of the programs | Program descriptions | Implementation Unit | Remarks/output |
|---|---|---|---|
| | | | from the workshop were complied. |
| 26 December, 2020 | Preparation process of Project Completion Report (PCR) of PBRG sub-project of Fisheries | Nutrition Unit, BARC | Detail discussion was on the guidelines how to write and submit the draft of PCR of the sub-project in time. Discussion was very effective |
| 16 June, 2021 | Virtual Meeting on Progress Monitoring of PBRG Sub-projects | PIU, NATP-2, BARC | Further discussion on submission of technology fact sheet and submission of PCR in time |

## ii. Field monitoring (date & no. of visit, name and addresses of team visit and output)

| SL | Monitoring team | Date(s) of visit | Total visit (No.) | Output |
|---|---|---|---|---|
| **Component 1 (BFRI)** | | | | |
| 01 | 13.07.2019, 28.08.2020 | Technical division/ Coordination component, BARC | 03 | Improved the methodology & designing of the study relating to nutritional and health status. |
| 02 | 28/07/2019<br>24/08/2020<br>13/09/2020 | PIU-BARC, NATP-2 | 03 | Improved the knowledge of sub-project implement techniques |
| 03 | 20/05/2019 | Internal monitoring (BLRI) | 05 | Speed up the sub-project activities |
| 04 | 2019-01no.<br>2020-02 no. | Others visitors (KGF& PKSF) | 03 | Shared the diversified sub-project activities and knowledge |
| **Component 2 (KU)** | | | | |
| 01 | 20/05/2019 | Team leader:<br>Dr. Md. Monirul Islam, MD (Fish)<br>BARC, Farmgate, Dhaka.<br>Member:<br>Md. Al Mobasher Hussen,<br>Senior Training Officer, BARC<br>Md. Jashim Uddin Chowdhury,<br>DD (Budget), BARC<br>Md. Hasan Mahmud,<br>CDA, PIU-BARC, NATP-2 | 03 | Improved the methodology & designing of the study relating to nutritional and health status. |
| 02 | 13.06.2019<br>25.08.2020 | Technical division/ Coordination component, BARC/BFRI | 02 | Completing the baseline study & comparing the end result with baseline study.<br>Setting control site nutritional and health status |

### iii. Weather data, flood/salinity/drought level (if applicable) and natural calamities

**Average last three years weather information of greater Khulna region**

| Parameters | Seasons | | | | | | Remarks |
|---|---|---|---|---|---|---|---|
| | Pre-Monson (January – April) | | Monson (May – August) | | Post Monson (Sept – December) | | |
| | Max | Min | Max | Min | Max | Min | |
| Av. Rainfall(mm) | 136 | 1 | 300 | 146 | 187 | 5 | |
| Av. Temperature (OC) | 34.43 | 12.63 | 35.04 | 25.63 | 33.67 | 15.1 | |
| Av. Humidity (%) | 77.67 | 70.67 | 86.0 | 78.0 | 84.33 | 78.33 | |
| Flood (year & category) | | | | 01 | | | Mild flood occurred in 1920 |
| Av. Salinity (ppt) | 23.67 | 7.0 | 9.0 | 4.0 | 22 | 4.0 | |
| Natural calamity (Frequency & category) | | | | | Cyclone category 3 In 2019 | | "Titli" |

## J. Sub-project auditing (covers all types of audits performed)

### i. Coordination component

| Types of audit | Major observation/ issues/ objections raised; if any | Amount of Audit (Tk.) | Status at the sub-project end | Remarks |
|---|---|---|---|---|
| Financial & Performance Audit by FAPAD on 20.11.19 for the year 2018-2019 | No objection raised, found all relevant documents updated as per guideline | 2,34,410.00 | Financial management of the component found running smoothly till the end of the project. No query or objection raised at any stage of operation by the audit teams. | Financial management & project performance found satisfactory |
| Financial & Performance Audit by FAPAD on 17.11.20 for the year 2019-2020. | No objection raised, found all relevant documents updated as per guideline | 12,500.00 | | Financial management & project performance found satisfactory |
| Financial & Performance Audit by FAPAD on 28.10.21 for the year 2020-2021. | No objection raised, found all relevant documents updated as per guideline | 1,84,245.00 | | Financial management & project performance found satisfactory |

## ii. BARC component

| Types of audit | Major observation/ issues/ objections raised; if any | Amount of Audit (Tk.) | Status at the sub-project end | Remarks |
|---|---|---|---|---|
| Financial & Performance Audit by FAPAD on 31.10.19 for the year 2018-2019 | No objection raised, found all relevant documents updated as per guideline | 681864.00 | Financial management of the component found running smoothly till the end of the project. No query or objection raised at any stage of operation by the audit teams. | Financial management & project performance found satisfactory |
| Financial & Performance Audit by FAPAD on 09.12.20 for the year 2019-2020. | No objection raised, found all relevant documents updated as per guideline | 101535.00 | | Financial management & project performance found satisfactory |
| Financial & Performance Audit by FAPAD on 11.10.2120 for the year 2020-2021. | No objection raised, found all relevant documents updated as per guideline | 211602.00 | | Financial management & project performance found satisfactory |

## iii. BFRI (SRS) component

| Types of audit | Major observation/ issues/ objections raised; if any | Amount of Audit (Tk.) | Status at the sub-project end | Remarks |
|---|---|---|---|---|
| Financial & Performance Audit 2018-19 (26/11/19-28/11/19) | No objection raised, Found all relevant documents updated as per guideline | 37,48,624.00 | Financial management of the component found satisfactory. No query or objection raised at any stage of operation by the audit teams. | Financial management & project performance found satisfactory |
| Financial & Performance Audit 2019-20 (04/11/20-17/11/20) | No objection raised, Found all relevant documents updated as per guideline | 68,10,675.00 | | |
| Financial & Performance Audit 2020-21 (26/10/21-03/11/21) | No objection raised, Found all relevant documents updated as per guideline | 9,43,404.00 | | |

**iv. KU component**

| Types of audit | Major observation/ issues/ objections raised; if any | Amount of Audit (Tk.) | Status at the sub-project end | Remarks |
|---|---|---|---|---|
| Financial & Performance Audit 2018-19 (28-10-2019) | No objection raised, Found all relevant documents updated as per guideline | 2236956.00 | Financial management of the component found satisfactory. No query or objection raised at any stage of operation by the audit teams. | Financial management & project performance found satisfactory |
| Financial & Performance Audit 2019-20 (10-12-2020) | No objection raised, Found all relevant documents updated as per guideline | 2652205.00 | | Financial management & project performance found satisfactory |
| Financial & Performance Audit 2020-21 (14-10-2021) | No objection raised, Found all relevant documents updated as per guideline | 2633793.00 | | Financial management & project performance found satisfactory |

## K. Lessons Learned

- Prior to release PL of prawn/shrimp in culture ponds/ghers a longer period nursing is required to prevent/reduce initial mortality during culture;
- Shrimp production can be raised by minimal upgradation of the farming system if proper effort is given.
- Antigenic particles can improve innate immunity in shrimp;
- This study revealed that probiotics could increase the activity of digestive enzymes and immune enzymes, which has massive role on disease protection and survival of prawn;
- Intensification of prawn culture could be done with application of probiotics as well as carbon enriched ingredients;
- Aeration has a significant effect on growth and production of prawn;
- Probiotic could be a good alternative of antibiotics and chemicals to prevent diseases in an eco-friendly approach thus produce health-safe prawn. Ultimately, the use of antibiotic and chemicals could be minimized;
- Farmer usually stock PL direct to grow-out pond which is responsible for higher mortality. Post-larvae of prawn should be nursed 45-60 days to let them grow about 3-5g juvenile which supports faster growth, higher survival and thus enhance total production.

## L. Challenges (if any)

**BFRI (SRS) component**

- Availability of required reagents by desired time is quite impossible in Bangladesh for basic and advanced research;
- High cannibalistic behavior of shrimp is a great concern for challenge experiments and

growth performance monitoring.

- The culture system of Bangladesh mainly depends on rain water. Culture hamper due to late rain or sometimes over rain;
- Last some years, scarcity of Hatchery produced PL is a major challenge in prawn culture system.
- Prawn shows a remarkable size variation thus large dominate one the smaller and thereby abduction of growth, increasing mortality happens which can be a big issue for low production.

## M. Suggestions for future planning (if any)

**A**. Shrimp farming in Bangladesh is still dominated by extensive farming system. By moderate upgradation of the farms from extensive to a cluster based modified improve system, production can be improved significantly. Along with the development of the culture system, future initiative must be taken to conduct some advance basic work on the immunological aspects and therapeutics/drug design for the ultimate sustainable development of the sector.

**B**. The existing shrimp culture system will be a promising sector for the betterment of our national economy through the application of effective technology. This study firmly reveals that prawn production could be increased at several fold applying probiotics and bottom up aeration system besides quality feeds.

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| **Signature of the Coordinator** | **Counter signature of the Head of the organization/authorized representative** |
|---|---|
|  |  |
| ***(Dr. Md. Khalilur Rahman)*** | ***(Dr. Yahia Mahmud )*** |
| Date: 30.12.21 | Date: 30.12.21 |
| ***Director (Res. & Plan.)*** | ***Director General*** |
| ***Bangladesh Fisheries Research Institute*** | ***Bangladesh Fisheries Research Institute*** |

## Appendices

### BARC component

**Annexure –1 (BARC): A**

**Recommendation of the inception workshop and status of action taken**

| Recommendations | Action Taken |
|---|---|
| **Component 1 ( SRS-BFRI)** | |
| • Citation of adequate literature and references in the text on success and failure of cluster based fish/shrimp farming in other regional countries suggested; | Literature and references cited properly. |
| • Possibility of all male conversion of prawn should be investigated. Similar attempts taken by other countries and success in this regard (if any) should be reviewed in depth to find out the local adoption mechanism of the technology; | Described and reflected properly. |
| **Component 3 (KU)** | |
| • Process of selection of probiotics need to be explained in the text; | Explained as per suggestion. |
| • Identification of probiotics (under objective 3) should be done before selection of probiotics for application (as under objective 1); | Rearrangement done. |
| • No discussion on baseline findings/information focused by the PI; | Baseline study undertaken as per activity plan and finding presented in the next meeting; |
| • Activities of the project should not exceed 36 months. Necessary corrections in this respect suggested; | Revision of activity schedule done. |
| • Year wise reflection of project output discouraged; | To be taken care of; |

**Annexure -1 (BARC): B**

**Recommendation of the half yearly workshops**

| Recommendations of the First Half Yearly Workshop | Action taken |
|---|---|
| **Component 1 (SRS-BFRI)** | |
| • Majority of works so far done only on cluster based shrimp farming which is still continuing; Maximum works under the objectives (3, 4 & 5) are still to complete within the limited remaining project duration which require re thinking and rescheduling of project work plan through better coordination and effective efforts to achieve considerable and acceptable progress of the project | – |
| **Component 2 (KU)** | |
| • Suggested to use code name/number against all | Followed |

| | |
|---|---|
| commercial probiotics products under study hiding their original trade names | |
| • Advised to incorporate baseline information of the study | Done accordingly |
| • Suggested analysis of BCR in respective part (where necessary) of the study | Followed. |
| **Recommendations of the Second Half yearly Workshop** | **Action taken** |
| **Component 1 (SRS-BFRI)** | |
| • Repetition of culture based shrimp farming require for the acceptance of the result obtained in the first trial | Attempts taken |
| • Major objectives of the component still untouched. Cost incurred so far is not comparable with output of the project | Project time extension required |
| **Component 2(KU)** | |
| • Enough literature review suggested in each respective study areas; | Followed |
| • Clear focus on the results of impact of composition of selected of prebiotics on the culture system, survival and growth of species suggested | Followed accordingly |

**Annexure – 1 (BARC): C**

**Recommendation of the Annual workshop**

| **Recommendations of the First Annual Workshop** | **Action taken** |
|---|---|
| **Component 1 (SRS-BFRI)** | |
| • No baseline information was presented; | - |
| • For analysis of research samples, the PI should take immediate measure to make the unused and new lab equipment (particularly the PCR machine) operational for completing the previous incomplete sample analysis; | Attempts taken |
| • Cluster farming under a research management system should have consistencies among the ponds use under the research (like in size, depth along with management practices etc). Here in this case, as the pond utilizing are hired private owned have different in size variation may mislead the research date; | - |
| **Component 2 (KU)** | |
| • Results of baseline study not presented in the workshop; | - |
| • As per the report, number of locally used probiotics showed as only 7 which is questionable. This finding should be further verified; | Done accordingly |
| • Feasibility study of few locally available probiotics (yogurt, yeast etc) suggested; | - |
| • Growth performance of *M. rosenbergii* in the initial year of research and impact of probiotics application on it should be focused clearly in the research report; | Done |

| | |
|---|---|
| • Treatments under each control trials were not explain in the presentation; | Explained in the draft report |
| **Recommendations of the Second Annual Workshop** | **Action taken** |
| **Component 1 (SRS-BFRI)** | |
| • Except the specific objective No. 3 of the sub project component, lot of research works still to do with rest of the objectives of the component while there is a time limit which is very limited. On the other hand, coming winter will reduce the privilege of most of the field-based research of the project due to temperature lowering. Only laboratory activities can be continued that may support to achieve partial result of the research only; | - |
| **Component 3(KU)** | |
| • Cost benefit analysis for probiotics based every culture efforts should be a must; | Followed |
| • Output and outcome of the sub project component shown is not noted down properly; | Reflected in the report |

**Annexure – 1 (BARC): D**

**Recommendation of the coordination meetings**

| **Central Coordination meeting at BARC** | |
|---|---|
| **Recommendations** | **Action taken** |
| • Base line study under both the components should be completed immediately | Completed |
| • Selection of shrimp ponds under cluster farming research should be prioritized based on size and type of culture and the culture environmental condition (where possible) | Followed where possible |
| **Other Coordination meetings (Two Virtual meetings)** | |
| **Component 1 (SRS-BFRI)** | |
| • Results of first trial on cluster farming is not up to the level of satisfaction. More trials with good management practices and farmers involvement require to reach the target. | Attempts taken |
| • As per newly determined end line date of research activity completion of the project, the PI should pay special attention also on all other objectives of the sub project | Maximum effort given |
| **Component 2 (KU)** | |
| • Composition and active agents of each probiotics should present clearly for each samples under study | Included in the report |
| • Clear picture of impact on growth and survival of fish/shrimp, disease frequency, benefit - cost for each treatment should be focused | Reflected in the report |






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