# ASSESSMENT OF HERBAL EXTRACT FOR GROWTH AND NUTRITIONAL QUALITY OF JUVENILE FRESHWATER PRAWN (*Macrobrachium rosenbergii*) UNDER BIOFLOC SYSTEM

**MD. SYFUL ISLAM**

**DEPARTMENT OF BIOTECHNOLOGY**

**SHER-E-BANGLA AGRICULTURAL UNIVERSITY**

**SHER-E-BANGLA NAGAR, DHAKA -1207**

**DECEMBER, 2020**

# ASSESSMENT OF HERBAL EXTRACT FOR GROWTH AND NUTRITIONAL QUALITY OF JUVENILE FRESHWATER PRAWN (*Macrobrachium rosenbergii*) UNDER BIOFLOC SYSTEM


**BY**

**MD. SYFUL ISLAM**

**Registration No: 18-09299**


A Thesis Submitted to
The Department of Biotechnology, Faculty of Agriculture
Sher-e-Bangla Agricultural University, Dhaka-1207
In partial fulfilment of the requirements
for the degree of

**MASTER OF SCIENCE (MS)**
**IN**
**BIOTECHNOLOGY**

**SEMESTER: JULY- DECEMBER, 2020**

**Approved by**


……………………………………

**Dr. A. M. Shahabuddin**
Professor
Department of Aquaculture
Faculty of Fisheries, Aquaculture
and Marine Science
Sher-e-Bangla Agricultural University

**Supervisor**

……………………………………

**Dr. Md. Ekramul Hoque**
Professor
Department of Biotechnology
Faculty of Agriculture
Sher-e-Bangla Agricultural University

**Co- Supervisor**

………………………………………………

**Dr. Mohammad Nazrul Islam**
**Chairman**
Examination Committee
Department of Biotechnology
Sher-e- Bnagla Agricultural University, Dhaka-1207.

DEPARTMENT OF BIOTECHNOLOGY
Sher-e-Bangla Agricultural University (SAU)
Sher-e-Bangla Nagar, Dhaka-1207

# *CERTIFICATE*

*This is to certify that thesis entitled, **"ASSESSMENT OF HERBAL EXTRACT FOR GROWTH AND NUTRITIONAL QUALITY OF JUVENILE FRESHWATER PRAWN (Macrobrachium rosenbergii) UNDER BIOFLOC SYSTEM"** submitted to the Faculty of Agriculture Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (MS)** in Biotechnology, embodies the result of a piece of bona fide research work carried out by **Md. Syful Islam** Registration No. **18-09299** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

*I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged & style of the thesis have been approved and recommended for submission.*

**Dated: December, 2020**
**Dhaka, Bangladesh**

……………………………….
**Prof. Dr. A. M. Shahabuddin**
**Supervisor**

*Dedicated To*

*My Beloved Parents*

*Who Always*
*Inspired Me To*
*Continue My Study*

# *ACKNOWLEDGEMENT*

*At first, I am expressing my gratefulness and acclamation to Almighty Allah for his kindness who empowered me to complete this thesis properly in time.*

*It is a great pleasure to express my heartfelt respect, deepest sense of gratitude, profound appreciation and ever indebtedness to my reverend supervisor* ***Dr. A. M. Shahabuddin****, Professor and Chairman, Department of aquaculture, Faculty of Fisheries, Aquaculture and Marine Science, Sher-e-Bangla Agricultural University (SAU), Dhaka who has guided me immensely with his sincere guidance, pedagogic supervision, careful planning, constructive criticism, and continuous encouragement and all kinds of supports from the very beginning to complete the research.*

*I wish to disclose my sincere respect and indebtedness to my Co-supervisor,* ***Dr. Md. Ekramul Hoque,*** *Department of Biotechnology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka for his utmost co-operation, constructive suggestion, and creating opportunity to work with another department for completing the research work as well as preparation of the manuscript of the thesis.*

*I am also grateful to Ministry of Science and Technology, People's Republic of Bangladesh for selecting me National Science and Technology (NST) Fellow and funding.*

*I express my immense gratefulness to all of teachers specially Shahriar Himel, Md. Abdul Hannan Sohag sir for their cordial co-operation and supplying herbal extract that helped to complete the research work comfortably.*

*I would like to show my gratefulness and give special thanks to beloved Fatema Chadni Apu, PhD student, Department of Fisheries, University of Dhaka for her immense affection and encouragement from the beginning that pave the way to complete the whole work.*

*I thank all of my close friends, beloved junior Md. Khairul Alam, lab attendants and well-wishers to cooperate and help me during working in the lab.*

*At last but not the least, I am indebted to my beloved parents, my family members for their sacrifice, invaluable affection, inspiration, encouragement for completion this study.*

***December, 2020*** ***The Author***
***SAU, DHAKA***

# ASSESSMENT OF HERBAL EXTRACT FOR GROWTH AND NUTRITIONAL QUALITY OF JUVENILE FRESHWATER PRAWN (*Macrobrachium rosenbergii*) UNDER BIOFLOC SYSTEM


## ABSTRACT

The study was conducted in the Biofloc Lab, Department of Aquaculture, Sher-e-Bangla Agricultural University to assess the potentiality of *Macrobrachium rosenbergii*, growth performance and its nutritional quality in Biofloc Technology (BFT) from July to November, 2020. Total five treatments were applied including T-1 (Control), T-2 (Extract of Ginger), T-3 (Extract of Amla), T-4 (Extract of Garlic) and T-5 (Extract of Garlic and higher stocking) with three replications. The initial mean stock weight of prawn were 3.14±0.19, 3.04±0.27, 3.26±0.16, 3.36±0.06 and 3.02±0.36g in T-1, T-2, T-3, T-4 and T-5 respectively. After completing the research duration, the highest mean weight gain and percentage weight gain were 10.9±0.3g and 297.8±16.2% noticed in the T-3. The survival rate was maximum (99.5±0.8%) in the control treatment. The best FCR and SGR were 1.2±0.1 and 2.1% also noticed in the T-3. During the research 35% protein containing commercial feed was given and floc was produced at balanced level which was found 21.7% protein. From the proximate composition analysis, the highest protein percentage of prawn 74.13±.68% was found in the T-3. The best growth performance and nutritional value was observed in T-3 (Extract of Amla) compared to other treatments and also tells the immense possibility of *M. rosenbergii* culture in biofloc in the era of aquaculture and microbial biotechnology for mitigating the demand of animal protein.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF PLATES

# LIST OF APPENDICES

## LIST OF ABBREVIATIONS AND ACRONYMS

| **Abbreviations** | **Full Meaning** |
|---|---|
| **%** | Percentage |
| **&** | And |
| < / > | Less than / Greater than |
| ± | Two Possible Value/ Range |
| **$^0$C** | Degree Celsius |
| **ADG** | Average Daily Weight Gain |
| **AIA** | Acid Insoluble Ash |
| **BFT** | Biofloc Technology |
| **cm, cm$^{-1}$** | Centimetre, Per centimetre |
| **DM** | Dry Matter |
| **DO** | Dissolve Oxygen |
| **EA** | Extract of Amla |
| **EDTA** | Ethylene Diamine Tetra Acetic acid |
| **EG** | Extract of Ginger |
| **EGa** | Extract of Garlic |
| **et al.** | And others |
| **etc.** | And other similar things |
| **FCR** | Feed Conversion Ratio |
| **FV** | Floc Volume |
| **g** | Gram |
| **ha, ha$^{-1}$** | Hector, Per hector |
| **HP** | Horse Power |
| **HS** | Higher Stocking |
| **kg, mt.** | Kilogram, Metric ton |
| **$KMnO_4$** | Potassium per Manganate |
| **L, L$^{-1}$** | Litre, Per litre |
| **LI** | Lipid Intake |
| **m$^3$, m$^{-3}$** | Cubic metre, Per cubic metre |
| **mg/l** | Milligram per litre |
| **ml, ml/l** | Millilitre, Millilitre per litre |

| | |
|---|---|
| **mm** | Millimetre |
| **$NH_3$** | Ammonia |
| **pH** | Puissance of Hydrogen |
| **PI** | Protein Intake |
| **PL** | Post Larvae |
| **ppm** | Parts per million |
| **PVC** | Poly Vinyl Chloride |
| **$R^2$** | Regression value |
| **RAS** | Recirulatory Aquaculture System |
| **RGR** | Reactive Growth Rate |
| **SD** | Standard Deviation |
| **SGR** | Specific Growth Rate |
| **SR** | Survival Rate |
| **T** | Treatment |
| **TDS** | Total Dissolved Solids |
| **WG** | Weight Gain |
| **C:N** | Carbon : Nitrogen |

# CHAPTER I

# INTRODUCTION

Bangladesh is considered one of the most suitable regions for fish culture in the world, with the world's largest flooded wetland and the third largest aquatic biodiversity in Asia after China and India. Fish supplements about 60% of Bangladeshi people’s daily animal protein intake (DoF, 2016). Bangladesh has ranked 3$^{rd}$ in the world in inland fish production, 5$^{th}$ in aquaculture production and 11$^{th}$ in marine fish production in 2018 (FAO, 2018). Fish is a leading export commodity helping Bangladeshi nations to improve their trade balance and believe that it will offer opportunities for developed countries to promote and adopt good trading practices and noticeably increases national wealth (Abila, 2003).

Fish farming is dominated by Asia, which has produced 89 percent of the global total in volume terms in the last 20 years. In the same time, the shares of Africa and the Americas in fish farming have increased than previous proportion. Outside China, Bangladesh have consolidated its shares in world aquaculture production to varying degrees over the past two decades. In 2018, the contribution of Bangladesh in inland waters capture production in the world was 10% and 2.93% was in aquaculture production among Asian 88.69% contribution (FAO, 2020).

Bangladesh is blessed with huge open water resources with a wide range of aquatic diversity. Biodiversity is also enriched, comprising almost 260 freshwater and 475 marine water fish species (Hussain, 2010). But it’s a matter of regret that aquaculture production and aquatic biodiversity has been decreased and deteriorated due to several natural and anthropological activities like degradation of wetland resources, inadequate knowledge on culture tactics, improper utilization of lands, chemicals and technologies, destruction of environment, etc. Besides the global population continues to grow, food production industries such as aquaculture will need to develop in a sustainable way. Aquacultural practices must be sustainable and minimally destructive to the environment, maintain quality and safety standards, and enable efficient use of space and natural resources and possibilities for expansion (Pérez-Rostro *et al*., 2014). One option is environmentally congenial and cost-effective aquaculture system called ‘Biofloc Technology (BFT)’.

‘Bio’ means life and ‘floc’ means mass of organic particle. Biofloc consists of microorganisms such as heterotrophic bacteria, algae, fungi, ciliates, flagellates, rotifers, nematodes, metazoans and detritus that conglomerate together and perform symbiotic processes to maintain the water quality, maintain bio-security, support high density of aquatic animals (Manan *et al*., 2017).

Biofloc technology (BFT) is defined as “the use of aggregates of bacteria, algae, or protozoa, held together in a matrix along with particulate organic matter for the purpose of improving water quality, waste treatment and disease prevention in intensive aquaculture systems” (El-Sayed, 2020). It is considered as an efficient alternative system since nutrients could be continuously recycled and reused. It is mainly based on the principle of waste nutrients management, in particular nitrogenous compounds into microbial biomass that can be used by the cultured species or be collected and processed into feed ingredients. Such technique is based on *in situ* microorganism production which plays three major roles: (a) maintenance of water quality, by the uptake of nitrogen compounds generating *in situ* microbial protein; (b) nutrition, increasing culture feasibility by reducing feed conversion ratio (FCR) and a decrease of feed costs; and (c) competition with pathogens (Halima *et al*., 2019). Biofloc technology removes toxic metabolites and microbial mass protein production helps to retain more nitrogen in the form of fish or shrimp biomass (Devi & Kurup, 2015). Biofloc technology is one kind of microbial biotechnology which can be utilize for fish growth and development program.

Biofloc technology (BFT) is based on the maintenance of high levels of microbial bacterial floc in suspension using constant aeration and addition of carbohydrates to allow aerobic decomposition of the organic material. Biofloc technology deals with manipulation of C/N ratio to convert toxic nitrogenous wastes into the useful microbial protein and helps in improving water quality under a zero or limited water exchange system. It may act as nutrition source for aquatic organisms along with some bioactive compounds that will boost growth, survival, and defence mechanisms. It can also consider as a novel approach for health management in aquaculture by stimulating innate immune system of animals. Biofloc can be a novel approach for disease management rather than conventional approaches such as antibiotic, antifungal, probiotic and prebiotic application. Beneficial microbial bacterial floc and its derivative compounds such as organic acids, polyhydroxy acetate and polyhydroxy butyrate, could resist the growth of other pathogens, thus serves as a natural probiotic and immunostimulant (Ahmad *et al*., 2017).

Historically BFT was first developed in French Research Institute for exploitation of the Sea, Oceanic centre of Pacific (Ifremer-COP) (Emerenciano *et al*., 2011). It is become a popular technology for the production of fish like Tilapia, *Penaeus monodon*, Pacific white shrimp (*Litopenaeus vannamei*) giant freshwater prawn (*Macrobrachium rosenbergii*) (Prajith, 2011). Ifremer started a research programme “Ecotron” in 1980 to better understand this system. Commercial level application of BFT started in 1988 by Sopomer farm (Rosenberry, 2010) and Belize aquaculture farm of Central America producing approximately 26 tons/ha/cycle using 1.6 ha lived grow out ponds. Nowadays, BFT have being successfully introduced and expanded in small and large-scale shrimp farming in Asia, Latin and Central America, USA, South Korea, Brazil, Italy, China and others. In addition, many research institutions and universities of Bangladesh are intensifying research in BFT such as nursery and grow-out management practices, feed and nutrition, microbial biotechnology and economics.

Freshwater prawn (*Macrobrachium rosenbergii*) also known as the giant river prawn or giant freshwater prawn, is a commercially important species of palaemonid freshwater prawn. It is found throughout the tropical and subtropical areas of the Indo-Pacific region, from India to Southeast Asia and Northern Australia. It is one of the biggest freshwater prawns in the world and is widely cultivated in several countries for food. (Motoh & Kuronuma, 1980). *M. rosenbergii* is the most preferred species for culture, which is commercially called 'Scampi' and popularly known as 'Giant freshwater prawn' or 'Malaysian prawn. Due to its fast-growing nature, hardiness, requirement of low protein diets, better domestic and export market and higher returns, this species is recommended for farming in many tropical and sub-tropical countries (Paul *et al*., 2016).

*M. rosenbergii* farming is currently one of the most important sectors of the national economy and during the last two decades, its development has attracted considerable attention because of its export potential. The prawn sector as a whole is the second largest export industry after readymade garments (Karim *et al*., 2019). In 2017-18 there were about 49 *P. monodon* (Bagda) hatcheries and 46 *M. rosenbergii* (Galda) hatcheries and about 1,41,204 lac bagda post larva (PL) and about 521 lac golda post larvae (PL) were produced in these hatcheries. Total shrimp and prawn production including capture has been increased from 1.60 lakh MT in 2002-03 to 2.54 lakh MT in 2017-18 (DoF, 2018). During the recent past decades, hatchery and nursery developed very rapidly which for enhancing commercialization of aquaculture. But the seed quality of both finfish and shrimp/prawn, scarcity of quality brood stock, hygiene and safe fish and fishery products production are now major threats for aquaculture expansion. According to National Shrimp Policy 2014, Government has emphasized on different aspects of this sector like flourish the shrimp industry, raise employment opportunity, alleviate poverty, export earnings and meet up the nutritional demand of the people (Shamsuzzaman *et al*., 2016).

*M. rosenbergii* is a striking looking prawn in which the second pair of walking legs can really justify the genus name meaning ‘large arms’. The largest males can attain a total length from tip of rostrum to the end of the telson of 320 mm compared to 250 mm for the largest females. *M. rosenbergii* can tolerate a wide range of salinities (0 to 25 ppt) and a wide range of temperatures (14 to 35°C). For growth, the optimal temperature is 29 to 31°C and the optimal pH is 7.0 to 8.5 ppm (New, 1995). *M. rosenbergii* inhabits freshwater but the larval and post larval phases are spent in brackish water (Cheng & Chen, 1998). In general, the body form is typical of a decapod crustacean with the head and thorax fused into a cephalothorax. The rostrum at the front end of the cephalothorax is very prominent with 11-14 dorsal teeth and 8-10 ventral teeth. Males can attain larger size than females and in dominant males, the second walking legs are much longer and thicker. The abdomen of the male is narrower and the female, as well as having a wider abdomen, has longer pleura (the overlapping plates of cuticle extending from the exoskeleton) and these combined forms a chamber for incubating the eggs carried on the pleopods. The colours of *M. rosenbergii* can vary according to where the prawns are found but the body can be greenish-grey. The chelipeds of dominant males are bright blue but more yellowish in non-dominant males and females and the ventral side is pale and translucent (CABI, 2018).

Bangladesh, one of the most important deltas in the world with its numerous and diversified natural resources. It's a country having 17 crore population within the small area, ranked 8th in the world and the number is increasing continuously. In most of the places, basic resources needed for aquaculture water and land is not available for fish culture. At present, farmers are intending to go intensive, super-intensive culture system. But the traditional intensification system in aquaculture results water quality deterioration which are leading stress and disease outbreak of fish. Antibiotics, different chemicals and growth promoters are used by farmers for getting good results which is totally condemned. Also, continual usages of antibiotics lead animals to become resistant to pathogenic bacteria which lower the treatment effect. In this consequence, Biofloc Technology (BFT) can be a technical alternative that may lead to the aquaculture sector at revolutionary stage though it's in infant stage in our country. The use of biofloc technology in commercial aquaculture is insufficient and this technique is not fully standardized. Biofloc technology is an integration of fish and microbial biotechnology which has different aspects on fish culture, growth and development. Present study will deal with most demanding freshwater prawn and the expected result would provide valuable information and new dimension in the era of aquaculture and aquatic biotechnology and that may help to contribute to the national development.

## Objectives

Based on the above mentioned context and prospects, the research work will be carried out for the following objectives;

1) To assess the potentiality of freshwater prawn (*Macrobrachium rosenbergii*) culture in biofloc technology.
2) To analyse the growth performance of *M. rosenbergii* in biofloc based culture system.
3) To evaluate the nutritional quality of cultured fish in biofloc technology.

# CHAPTER II

# REVIEW OF LITERATURE

In aquaculture biofloc technology is considered as blue revolution now-a-days. From a short investigation of Emerenciano *et al*. (2017) it can easily be understood that biofloc is modern *in-situ* microorganism-based culture system. There are three main major roles of biofloc were highlighted, those are; (a) maintenance of water quality, by the uptake of nitrogen compounds generating *in situ* microbial protein (b) nutrition, increasing culture feasibility by reducing feed conversion ratio (FCR) and a decrease of feed costs and (c) competition with pathogens.

Aquaculture is an activity in constant growth that requires maximizing resources and spaces. Castro-Nieto *et al*. (2012) have told about the most recent technology “biofloc systems” which seeks to solve the problems of water pollution and improve the use of water resources, in addition to recycling the nutrients found in the water by a community of heterotrophic bacteria. This technology has permitted in the aquaculture farms to reduce and eliminate water exchange in the time of providing added value through the products resulting from microbial metabolism.

Das & Mandal (2018) discussed about an effective tool in aquaculture sector named biofloc technology. That’s a bioremediation tool for various environmental problems, means of poverty alleviation and meeting the demand of food in the coming years. Biofloc system became more economic as it operated under zero water exchange system thereby reducing cost of water exchange and decreasing the commercial diets of fish in fish ponds. They stated that consumption of macro aggregates can increase nitrogen retention from added feed by 7-13%. BFT is known to prevent the accumulation of toxic nitrogen metabolites by stimulating and manipulating the carbon/nitrogen ratio (C/N) and converting these metabolites to microbial flocs. The presence of diverse group of bacteria especially *Bacillus* sp. and *Lactobacillus* sp. indicate that biofloc can be considered as a source of potential probiotics besides acting as valuable protein inputs.

### Source of Seed, Different Water Quality Parameters

Gain *et al*. (2015) carried out an interviewing survey of 75 prawn farmers in the south-western part (Khulna and Bagerhat) of Bangladesh. From their survey they found average stocking density, survival rate and production in Khulna and Bagerhat region using wild post larvae (PL) were 7000 PL and 6750 PL, 45 to 90% and 40 to 85%, 167 and 159 kg/acre respectively. For hatchery produced PL the average stocking density, survivability and production in Khulna and Bagerhat region were 9000 PL, 53% and 124 kg/acre and 9250 PL, 55% and 120 kg/acre respectively. The study also preferences to the wild source for farming and hatchery source could be better using improved technology and good maintenance for PL production as wild source is getting unavailable for various reasons.

An investigation was done by Denga *et al*. (2018) to identify the effect of using different carbon source in biofloc system. In this evaluation control and three types of Carbon sources like tapioca starch (TS), plant cellulose (PC) and the combination of tapioca starch and plant cellulose were used for getting the best result. Average weight gain ratio and microbial richness found in the TS using tanks. After 42 days observation, they concluded and suggested the organic carbon source for biofloc fish farming.

Serra *et al*. (2015) conducted another experiment of different carbon source's effect in biofloc system was done for *Litopenaeus vannamei*. They considered molasses can sugar, dextrose and rice bran for nursery and last two sources for grow-out phases in tanks with a volume of 800 L at 35 and 70 days respectively. In the nursery and grow-out experiment molasses and dextrose gave the least concentration of ammonia in tanks water though rice bran shows better production performance. The research concluded that dextrose and molasses sources use as a good substrate for heterotrophic bacteria to use in neutralizing ammonia and improving the water quality.

Mannan *et al*. (2012) tried to find out the impact of water quality on fish growth, survival and development in managed aquaculture farms. They carried out the observations with three different treatments in Tangail region of Bangladesh. The results of the observation indicated that the temperature was ranged from 26-32$^0$C; pH 6.5-9.2; transparency 10-36 cm; DO 4-8 mg/l; $CO_2$ 3-8 mg/l; BOD 0.1-2.7 mg/l; alkalinity 20-150 mg/l and ammonia-nitrogen 0.1-2.1 mg/l, which were good to perform better developing process in case of fish farming. They stated that the water quality parameters influenced the growth and development of fish in managed aquaculture system.

Chand (2000) worked on the importance of aeration on growth and development of *Macrobrachium rosenbergii* culture for nine months grow-out period. The study comprised three treatments in different stocking densities which were driven by one electric motor. At the end of the culture period, survival rates of prawn were 54.1%, 40.7% and 43.7% in $T_1$, $T_2$ and $T_3$ respectively, whereas the average size of prawn were 60.1g, 54.7g and 59.2g. The research also stated that the aeration increased the DO level by 0.7-2.0 mg/l during 4 hours operation and helped in reducing DO stratification in the pond.

The process of biofloc formation is mandatory in biofloc technology (BFT) for the rearing of marine shrimp. At the same time, there is an increase in total suspended solids (TDS) levels and a decrease in alkalinity and pH. This decrease in alkalinity and pH is attributed to the absorption of inorganic carbon by the bioflocs and biofilms of autotrophic bacteria. Furtado *et al*. (2015) carried out another experiment on effect of different alkalinity levels in biofloc culture system. In their research contained of four treatments with three replicates each: 75, 150, 225 and 300 mg $CaCO_3$/L. Sodium bicarbonate ($NaHCO_3$) was applied to maintain the alkalinity at the tolerable level. After 49 days of treatments, higher ammonia level was found in the $T_1$ compared to $T_2$, $T_3$ and $T_4$. They stated that higher alkalinity level favours biofloc formation and the development of nitrifying bacteria.

The use of probiotics to the freshwater prawn *Macrobrachium* sp. culture in biofloc systems, pointing out the benefits in growth, survival, developments in the immune system, pathogenic control, and water quality. Martínez *et al*. (2020) showed a brief account on using different probiotics in BFT system. In their review, they stated *Bacillus subtilis, B. cereus*, *Pseudomonas fluorescens*, *Clostridium butyricum* and different commercial probiotic (Prosol, Aquapro, etc.) are using as probiotic source and also suggested those probiotics for shrimp culture in BFT system.

Biofloc system is an alternative to conventional aquaculture systems as it minimizes effluents, improves water quality, ensures greater biosafety due to minimal water exchanges and also serves as an additional food source for prawn. Vitamins and minerals have broad participation in the metabolism of aquatic organisms; are required in small quantities for their healthy growth, reproduction, and health. In biofloc culture system the production of *M. rosenbergii* does not require the addition of vitamin and mineral supplementation in the feed (Ballester *et al*., 2018).

Temperature is considered to be a major factor affecting feed intake, growth and developments for aquatic animals. As consequences Niu *et al*. (2003) conducted a research on temperature effect with three different range like $23^0$, $28^0$ and $33^0$C. They stated that temperature has no adverse effect on growth efficiency but better range ($28^0$C) of temperature has positive impacts on weight gain, oxygen consumption and feed intake efficiency.

Biofloc is a culture system with the combination of high amount of microorganism, ions and minerals. Most of the times these waters are in high in calcium that may lead to mineral deposition on carapace, delayed molting and stunted growth of the shrimp. In Aquaculture, it's a regular practice to use chelators like EDTA to reduce hardness and calcium levels in water. Jaganmohan & Kumari (2018) conducted a study and showed that use of EDTA at 1.5 ppm concentration gave better result for degrading hardness of water and helping for molting of prawn.

**Growth, Survival Rate and Production**

A study was done by Ballester *et al*. (2017) on the finding out the best way to culture freshwater prawn *Macrobrachium rosenbergii* in case of getting highest production for the context of neutralizing the day by day demand of protein and nutrients. In this research two most recognised way of culture was selected like RAS and Biofloc Technology (BFT) with 0.20 $m^2$ experimental units and 50 L volume. Proper maintenance was done during experimental session and they identified the difference in in feed conversion rate with better values on RAS treatment and showed the possibility of rearing *M. rosenbergii* in biofloc system technology.

Biofloc nursery system is a profitable alternative for locations contributing to sustainable use of water and improved nutritional quality of the prawns. Considering this, Pérez-Fuentes *et al*. (2013) set out an experimental design with two nursery rearing systems: biofloc and traditional cultivation for identifying the best way of cultivation. Growth, survival rate and body composition of the *Macrobrachium rosenbergii* were recorded and evaluated from six months culture. Daily water parameters and monthly length -weight were recorded during experiment. At the end of the study the research show >85% survival rate for both cultivating way but the final size was significantly higher in the biofloc system (11.54 ± 1.87 $g^{-1}$, 15.18 ± 8.27 $cm^{-1}$) than in the traditional system (10.67 ± 2.26 $g^{-1}$, 12.57 ± 7.89 $cm^{-1}$). The most important thing is protein (51.19%) and lipid (13.84%) content in harvested prawns was significantly higher in the biofloc system than conventional culture system.

An investigation was set by Paul & Rahman (2016) to assess the growth, survival and production for juveniles of *M. rosenbergii* where five different supplementary feeds from local market were used. The mean survival rates were found at 86.46, 81.33, 75.33, 79.44 and 78.33% in T1, T2, T3, T4 and T5 treatments. The highest result was found for treatment 1 where 31.50% protein containing feed was given and the weight gain was 48.03g and net production was 166.14 kg/ bigha.

Rajkumar *et al*. (2016) carried out an experimental study with three biofloc treatments and one control in triplicate in 500 L capacity indoor tanks to assess the best result of growth and survival rate for *L. vannamei.* In their experiment sugarcane molasses, tapioca flour, wheat flour and clean water as control were used as carbon source for 60 days trial. Shrimp PL with an average body weight of 0.15- 0.02 g were stocked at the rate of 130 PL $m^{-2}$ and the result gave the indication on better growth performance and survival rate for addition of wheat flour as carbon source and also indicate the better water quality during experiment.

To find out the growth performance between male and female freshwater prawn *M. rosenbergii*, an assessment work was set by Fatema *et al*. (2011), a group of researchers of Bangladesh Agricultural University for 120 days with nine earthen ponds. During research work all types of necessary parameters were recorded from three replications. After the time frame, the growth and production performance, the male prawn showed better growth and production performance than female prawn in all treatments.

**Effect on Stocking Densities**

Negrini *et al*. (2017) worked on fresh water giant prawn *M. rosenbergii* for evaluating the effect on different stocking densities on productive performance in biofloc system. In that research work 0.20 m² were used as experimental units where experimental tanks were at different stocking densities (50, 100, 150, 200 and 250 pieces $m^{-2}$) and reared during 60 days. *M. rosenbergii* juveniles, with an initial weight of 0.315 ± 0.06 g and initial length of 33.34 ± 2.26 mm were selected. Prawns stocked at the density of 50 $m^{-2}$ showed significant higher ($P < 0.05$) survival rate (73%) and significantly lower values ($P < 0.05$) for feed conversion rate (1.28). The different stocking densities evaluated did not affect the weight and length of prawns. The recommended density for growing *M. rosenbergii* in the biofloc system is 50 ind $m^{-2}$.

Aftab Uddin *et al*. (2020) performed an experiment on biofloc technology rearing system to determine the various observations during culture time (120 days). They selected shrimp (*Penaeus monodon*) for their experimental species as it is a highly demanded species for Bangladesh context. That experiment was mainly based on different stocking densities (400 PL/$m^3$ in CW, 400 PL/$m^3$ in BFT1, 450 PL/$m^3$ in BFT2 and 500 PL/$m^3$ in BFT3) and its effect on growth performance, microbial abundance and water quality. They showed that the overall final biomass in BFT1 (5.88 ± 0.12 kg/$m^3$) was significantly higher ($P < 0.05$) than that of other BFT groups and the control (3.40 ± 0.09 kg/$m^3$). The concluded that the biofloc technology with lower stocking density was reducing the total ammonia nitrogen, nitrite-N, and nitrate-N in water and significantly increased total heterotrophic bacteria and reduced vibrio-like bacterial populations.

A study was conducted to evaluate the effect of different stocking densities of prawn on three male morphotypes named blue claw (BC), orange claw (OC) and small male (SM). Three trials 20, 30 and 40 juvenile $m^{-2}$ including different mixing percentage of three morphotypes were carried out in replicates. After 4 months of culture, the highest survival rate combined with growth performance was from 20 juvenile $m^{-2}$ stocking density with 21% BC, 62.5% OC and 16.5% SM, respectively (Banu *et al*., 2016).

Paul *et al*. (2016) have made an observation after working on the effect of different stocking densities on the growth, survival and production performance of the freshwater prawn (*Macrobrachium rosenbergii).* The post larvae with mean initial weight of 0.22±0.04g were stocked under gher conditions at 40, 50, and 80 PL/decimal and cultured for 50 days. The culture ponds were treated properly and a supplementary feed of 28.82% crude protein was given to the prawns. Prawns were fed artificial diet with 10%, 7% and 5% of their body weight at 1st, 2nd and 3rd respectively and were continued till the end of the experiment. The results indicated that at low stocking density (40post larvae/decimal) the growth, survival and production were higher. Prawns attained a final mean body weight of 38.71±3.79g and 21.59±2.64g in low and higher stocking densities respectively. The highest survival rate was obtained from low stoking density (90.83%) and the lowest survival rate was obtained from high stoking density (47.92%). Hence, the growth performance was significantly ($P < 0.05$) decreased with increasing stocking density.

A research was performed by Hossain & Islam (2006) to optimize stocking density of freshwater prawn (*Macrobrachium rosenbergii*) in carp polyculture for 3 months in 10 experimental ponds of $80m^2$. Five stocking densities of prawn, 2500, 5000, 7500, 10 000 and 12500 ha $^{-1}$ were assigned to treatments T1, T2, T3, T4 and T5 respectively. The densities of catla, rohu, and silver carp were 2500, 5000, and 2500 $ha^{-1}$ respectively in each treatment where every pond has two replicas. The production of prawn was significantly higher (361.3 kg $ha^{-1}$) in T5 with a highest stocking density of 12500 prawn $ha^{-1}$.

**Length-Weight Relationship and Condition Factor**

A research investigation was held in Northern Province of Sri-Lanka including two large and three small culture based perennial reservoirs by Rajeevan *et al*. (2018) to find out the length-weight relationship of *M. rosenbergii*. In their research they randomly collected 300 prawns from the year round. Non-linear regression and Fulton's condition factor were used for calculation and the mean condition factor (K value) of *M. rosenbergii* ranged from 1.3928 – 1.9505 and overall K value was 1.6130. K values were higher in Puthumurippu (1.9505) and lower in Vavunikulam (1.3928). Smaller reservoirs had higher condition factor values than larger perennial tanks and *M. rosenbergii* were in a good health condition in these five reservoirs.

To determine the length-weight relationship of *M. rosenbergii*, Kunda *et al*.(2008) collected a total of 100 shrimps from the experimental plots of integrated rice shrimp culture. They collected samples from August to December and obtained highly significant relationships between length and weight. Standard length-weight relationship of *Macrobrachium rosenbergii* was obtained: $\text{Log } W = -2.0518341 + 3.07513132 \text{ Log } L$ and the co-efficient of correlation (r) was 0.99. The mean values of condition factor (K) and relative condition factor (Kn) were 1.0893674 and 1.0012671 respectively, which suggest good condition of the prawn in rice fields.

Lalrinsanga *et al*. (2012) investigated length-weight relationship and condition factor of *M. rosenbergii* for different culture phases, developmental stages and sexes. Regression lines differed among the culture phases, developmental stages as well as between sexes. Significant difference in the slope was observed among different culture phases with nursery animals showing significantly lower slope. The most significant variation in the slope was observed among different developmental stages. Sex wise comparison also exhibit highly significant variation between male and female.

**Economic Viability**

Ahmed *et al*. (2008) stated that there is scope to increase production and income from prawn farms in extensive and semi-intensive systems with a reduction in production costs and increased market prices provided by an information from scientific journal. The study was considered production technology, cost structure and profitability of freshwater prawn (*M. rosenbergii*) farming in a new area of Mymensingh district in the north central part of Bangladesh.

The continuous expansion of aquaculture sector has no doubt triggered debate on environmental issues and has accelerated global demand for fishmeal and fish oil in equal measure. Ogello *et al*. (2014) wish to show the potentiality of biofloc technology (BFT) towards improving yield, safety and economic sustainability of cultured tilapia. They observed tilapia growth rates of up to 0.3 g. $day^{-1}$ yielding up to 300 mt. $ha^{-1}$ in managed biofloc ponds. They defined biofloc technology as inexpensive thus making it the forgotten asset for present and future aquaculture.

Sontakke & Haridas (2018) selected milkfish which is one of the most preferred and cultured brackish water finfish species across Southeast Asia to see the economic viability of biofloc system than traditional pond rearing system. The experiments were also focused on the growth and survivability of the species in both systems. In their research, survivality was found 80% and 52% and the highest net income Rs. 4, 86,015 and Rs 3,32,420 were obtained from biofloc based system and traditional pond culture. The study declared that milkfish culture in biofloc is more profitable and viable than traditional culture system.

# CHAPTER III

# MATERIALS AND METHODS

This chapter describes the various tools and techniques about biofloc technology, research location, freshwater prawn culture, analysis of growth parameters, etc. for the fulfilment of the research objectives. The flowchart of the overall research work is given below:

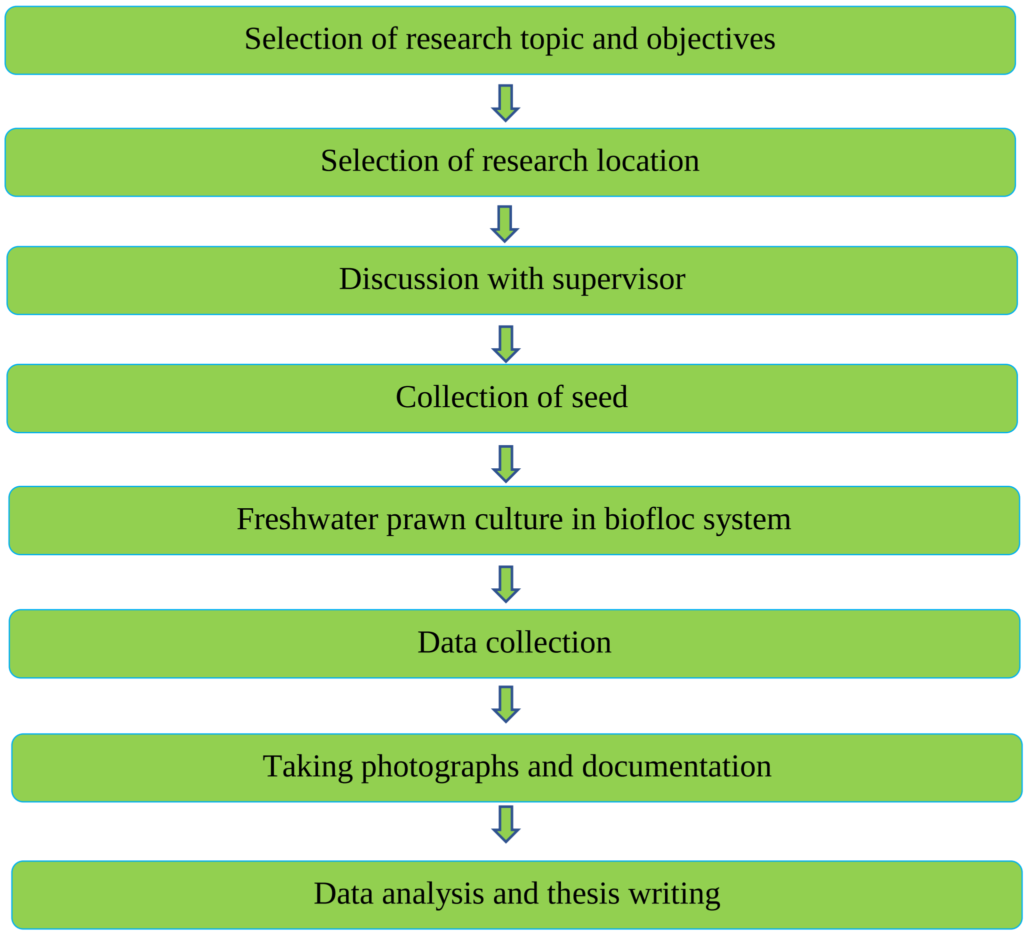



**Figure 01**: Flowchart of the overall research work.

### 3.1 Study Location

The study was performed in the newly established laboratory named "Biofloc Lab" under Department of Aquaculture, Faculty of Fisheries Aquaculture and Marine Science at Sher-e-Bangla Agricultural University which is located in the central part of the capital of Bangladesh. It is one of the most prominent biofloc lab in Bangladesh. Different fish species culture activities are continuing round the year for the development of aquaculture tactics and fulfilling the increasing demand of human protein. At present, various types of indigenous and exotic species are being cultured in this lab as different experimental purposes like tilapia (*Oreochromis mossambicus*), koi (*Anabas testudineus*), thai shol (*Channa striata*), shing (*Heteropneustes fossilis*), magur (*Clarius batrachus*) etc. Freshwater giant prawn *Macrobrachium rosenbergii* was selected as culture species in biofloc technology for the very first time in Bangladesh for assessing its culture suitability and evaluating its survival, growth and other parameters.

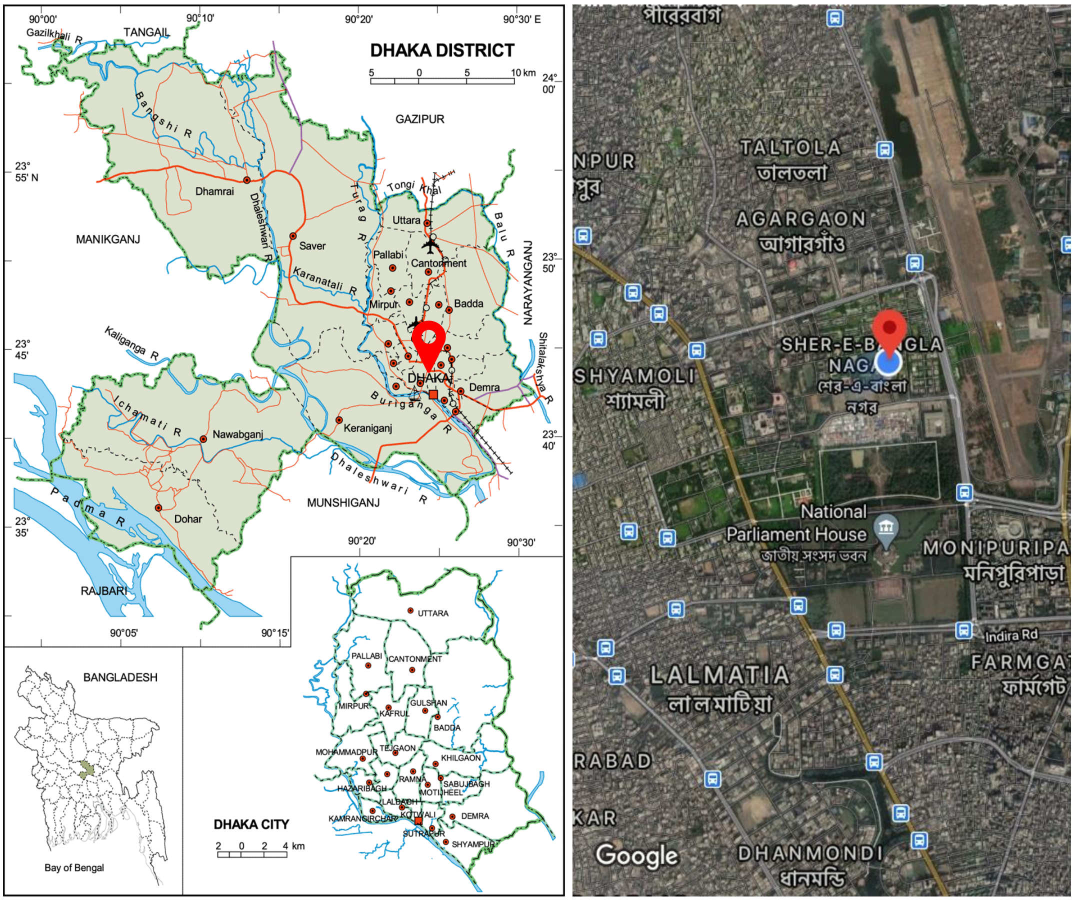



**Figure 02:** Map and Satellite view of the research location.

### 3.2 Duration of the Study

The timeframe was set for the study from mid-July to November, 2020 for freshwater prawn culture in biofloc system according to the researchers and supervisors schedule. Basically, the lab of biofloc culture is engaged in different cultures and research works all the year round. The duration was set considering the prawn seed availability and culture opportunity in that lab.

### 3.3 Experimental Design and Setup

Biofloc research and dissemination of the respective modern aquaculture practice the lab was designed by the Faculty members of Fisheries, Aquaculture and Marine Science of Sher-e-Bangla Agricutlural University, Dhaka. The experiment was started to perform in tarpaulin tanks containing 2000L capacity. Water filtration was set for purifying and 1 HP motor was set for proper aeration system.  The three dimensional view of the biofloc lab is given below;

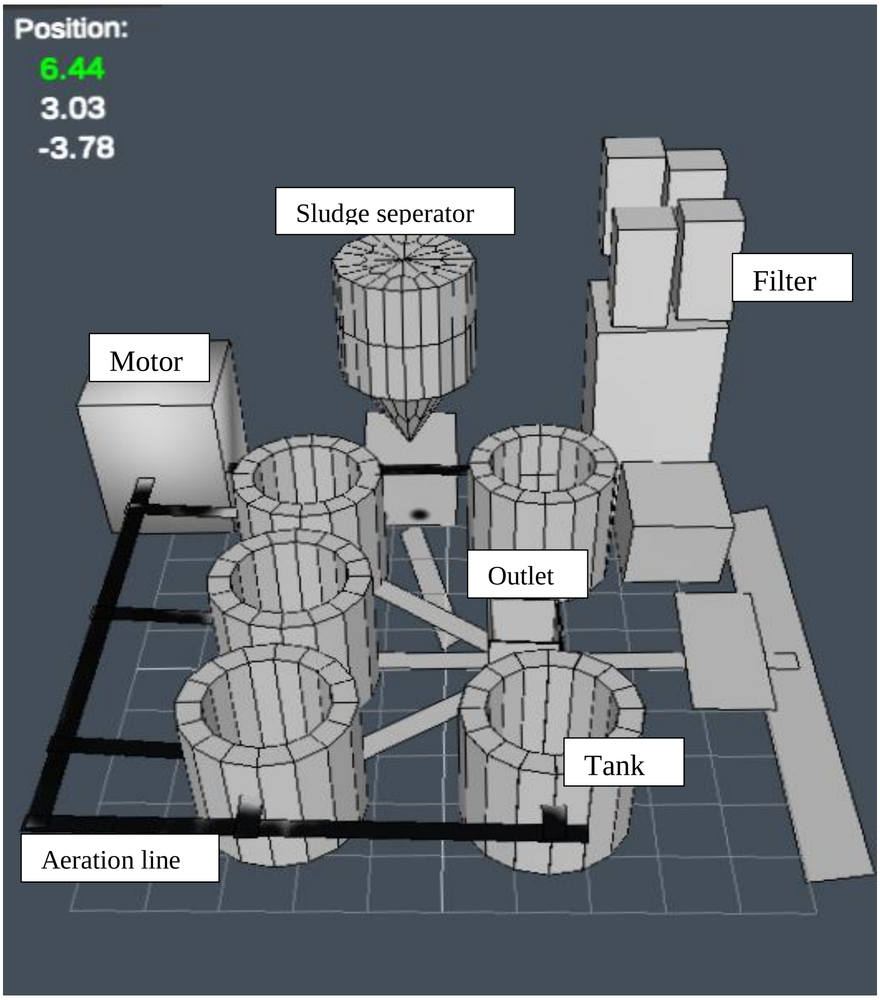



**Figure 03:** Biofloc lab design and set up.

The lay-out of the experiment is given in the following diagram:

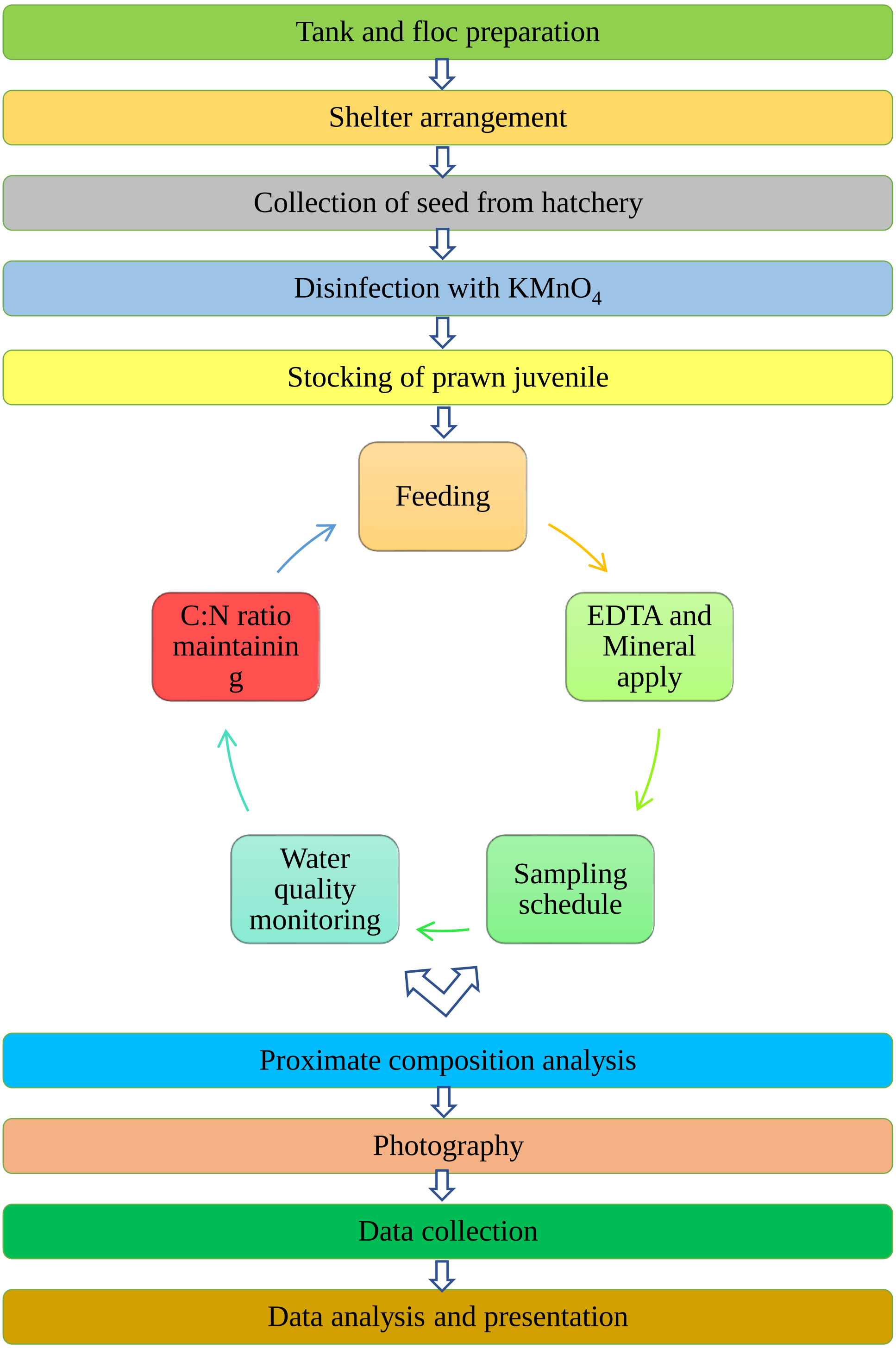



**Figure 04:** Lay-out of the research work in Biofloc lab.

### 3.4 Pre-stocking Management

Pre-stocking management means some sequential managerial activities before stocking of fish species in tanks or ponds which include tank and floc preparation, shelter arrangement, seed collection, disinfection, stocking of seed and so on.

#### 3.4.1 Tank Preparation

The first requirement of biofloc based culture is tank that can be made of any hardy materials. In this study, tanks are made of iron structure that is covered by PVC coated water proof tarpaulin. Tank was treated by $KMnO_4$ (potash) and lime before pouring with water. After filling the tanks with water through purifying aeration was given in all tanks. Salt and lime were given as 500g and 50g per 1000 litre respectively for increasing total dissolve solids and enriching the water quality.

**Figure 05:** External and internal view of experimental tank used in the research work.

#### 3.4.2 Floc Preparation

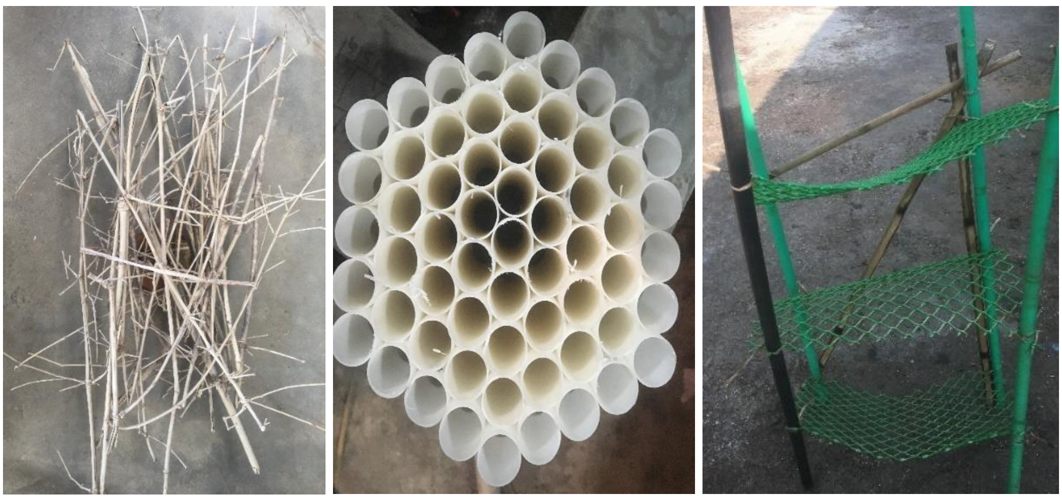
Floc means the aggregation of microbes like bacteria, algae, protozoa, metazoan, etc. In biofloc based culture, the microbial floc is used to convert the ammonia into protein and also used as food substance for aquatic species. After preparing the water in the tank with continuing aeration for two days then 10-20 g of probiotic and 50g raw sugar per 1000L water was applied for microbial growth. Then a slight amount (approx. 2-3 g) of commercial feed was applied for nitrogen formation which will help to start the microbial growth formation. In this study "Everfresh Pro" was used for floc formation. After seven days, it was found that floc has already formed to 10ml per 1.0 litre which was understood by the observation in Imhoff Cone.


**(A)** **(B)**

**Figure 06:** (A) Probiotic used in floc formation; (b) Quantity of floc in Imhoff Cone.

### 3.4.3 Shelter Arrangement

There are different types of shelter were used during freshwater prawn culture period such as dry branches of bamboo, plastic net and pipe structure and hexagonal structure of plastic pipe. As freshwater prawn has carnivorous behaviour, they tried to eat another if any species get weak and after the time of molting. They can protect themselves from others and hide themselves in such suitable place.



**(A)** **(B)** **(C)**

**Figure 07:** Different types of shelters. (A) Bamboo branches; (B) Substrate; (C) Standing structures made of plastic.

### 3.4.4 Collection of Seed

Good seed is the precondition of good outcome for any culture. In this research work seed was collected from a hatchery source of Mymensingh. For getting good results organoleptic tests that means healthy, good looking, disease free seeds were selected for collection purposes. Juvenile freshwater prawn was collected for experimental work. Different sizes of juvenile were collected ranged from 1 g to 5 g.

**Figure 08**: Seed collected in polybag and samples.

### 3.4.5 Disinfection of Seed

After collecting the seed from any sources, it should be disinfected. Juvenile freshwater prawn was disinfected by potassium per manganate ($KMnO_4$) called as potash. Approximately 1g was applied in the polybag of seeds. Then within a minute the seeds were given to the research tanks according to the research working guidelines. Before stocking the seeds in the tank's seeds were properly sorted and equally distributed.

### 3.5 Species Stocking Density

Juvenile freshwater prawn was equally distributed numerically in all tanks. In this study there were five tanks where one was used as controlled and others were used as treatments. There are 68 pieces juvenile *M. rosenbergii* were stocked in four tanks including one control and three treatment tanks. Double number of juvenile (140 pieces) was stocked in another treatment tanks (T5). It was done due to understand the growth and survival performance between low and high stocking density.

### 3.6 Post-stocking Management

The phase includes the activities or maintenance from stocking to harvesting defined as post -stocking management. In case of biofloc based culture system, many activities were similar to traditional system like feeding, sampling, chemical application and harvesting, etc.Besides, some different activities were performed during the study period.

#### 3.6.1 Feeding

Feeding is an important issue for any types of culture. For getting good results proper feeding should have to be maintained. Feeding was properly maintained on daily basis and two times (morning and evening). From the beginning of the study, feed was given at 1-2% rate of species body weight and after that it was increased upto 5% rate during the study period. Biofloc based culture helps to add natural food substance that helps to less feed demand. In the study, three types of extract (Ginger, Garlic and Amla ) were used with the feed for experimental purposes. Extract was mixed with feed (2 ml for 10 g) before two hours of feed given. As freshwater prawn is bottom feeder, feed was given at the bottom level by using sieving. In the culture time "duhuge feed" of renowned FishTech Company was used.

**Treatment of the experiement:**

Five different treatments were designed to feed the freshwater prawn. The treatment combination were given below;

T-1 (Control) = Only Commercial feed (No floc present)

T-2 (EG) = Commercial feed + Extract of Ginger (Floc present)

T-3 (EA) = Commercial feed + Extract of Amla (Floc present)

T-4 (EGa) = Commercial feed + Extract of Garlic (Floc present)

T-5 (EGa & HS) = Commercial feed + Extract of Garlic + Higher stocking (Floc present)

*(EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and EGa & HS= Extract of Garlic & Higher stocking, Commercial feed= Fish Tech Company)


**(A)** **(B)**

**Figure 09:** Feeding procedures during the study time. (A) Mixing extract with feed; (B) Giving feed by using sieving.

### 3.6.2 Water Quality Parameters

Prawn perform all their bodily functions in water. Because prawn are totally dependent upon water to breathe, feed and grow, excrete wastes, maintain a salt balance, and reproduce, understanding the physical and chemical qualities of water is critical to successful aquaculture. To a great extent water determines the success or failure of an aquaculture operation. The following parameters were considered for present study:

#### i) Temperature

Temperature is an important issue in case of biofloc based prawn culture. Many other water parameters depend on its balance. Good temperature range helps to grow prawn and other microbial community properly. Temperature range was measured everyday morning during the study period. Data was collected by using digital multimeter.

#### ii) pH (Puissance of Hydrogen)

pH is very important for prawn culture. pH defines the acid and base concentration of water. High and low pH levels are related with metal concentration, nutrients deficiencies and problems. In this study pH was measured everyday through using digital multimeter. For this, water was collected in mug from treatment tanks and put the pH sensor of multimeter into it. Concentration of pH is not suitable if it ranges in very low (< 4 ppm) or very high (> 9 ppm). In case of prawn culture pH changes can affect indirectly by altering other water parameters.


**Figure 10:** Measuring pH by using digital multimeter.

**iii) Ammonia ($NH_3$)**

Ammonia is the most important harmful factor for successful fish or prawn culture. In biofloc base prawn culture nitrogenous compound is a crucial factor for survival rate.

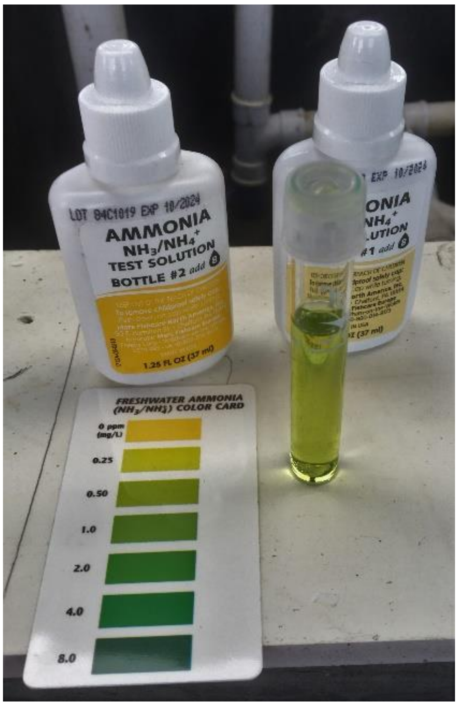



**Figure 11:** Measuring ammonia level by using API's kit.

Generally, ammonia is formed by the excreta of culture species, deposition of extra feed on the tank bottom level. The most important thing is ammonia is controlled in biofloc culture because of microbial accumulation in the tank water. Beneficial bacteria help to convert the ammonia into protein with the help of carbohydrate sources. Besides, carbon source was given regularly for maintaining ammonia concentration in the water according to ammonia level. Ammonia level was measured by API's ammonia kit.

**iv) Dissolve Oxygen (DO)**

For aquatic species dissolve oxygen is the most important factor for their survival. Prawn breathe oxygen just as we do, so dissolved oxygen (DO) is a critical environmental indicator in aquaculture. Depleted DO is the leading cause of prawn kills, and it is known that low-oxygen conditions are their worst enemy. In case of prawn culture at least 5.0 ppm DO level should be maintained. In this research work, an oxygen motor with 1 HP was used for maintaining DO level. In every tank, there were four air stone used for proper oxygen supply. DO level data was measured by Life Sonic's DO kit at daily basis.

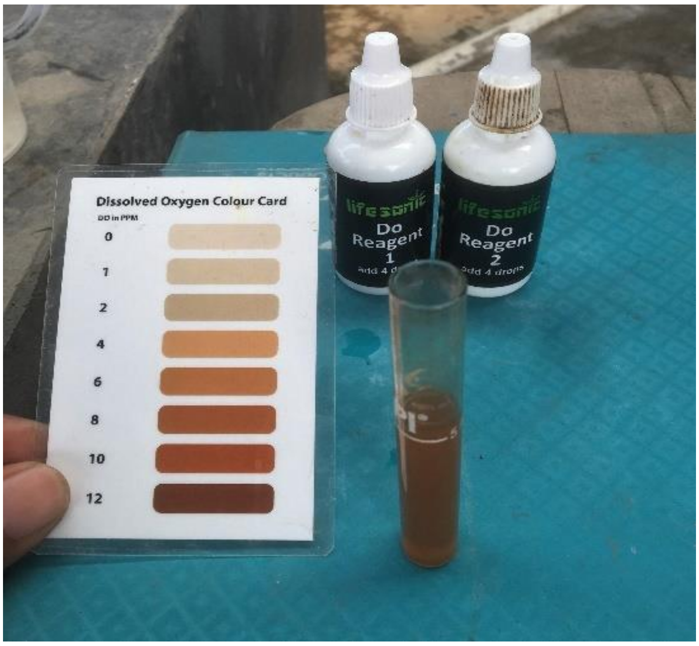



**Figure 12:** Measuring DO level by using Life Sonic's kit.

### v) Alkalinity

Alkalinity is the capacity of water to neutralize acids without an increase in pH. This parameter is a measure of the bases, bicarbonates ($HCO^{-3}$), carbonates ($CO^{--3}$) and, in rare instances, hydroxide ($OH^{-}$). Total alkalinity is the sum of the carbonate and bicarbonate alkalinities. Some waters may contain only bicarbonate alkalinity and no carbonate alkalinity. Alkalinity is important for fish and aquatic life because it defends or buffers against quick pH changes. If alkalinity is depleted, the pH of the water in the tanks can drop, causing extreme stress or death to the prawn and adversely affecting biofilter function. In this research work alkalinity was measured by using Alkalinity Reagent Kit of NICE at daily basis.


**Figure 13:** Measuring alkalinity level by using NICE's kit.

### vi) Hardness

Water hardness is similar to alkalinity but represents different measurements. Hardness is for the most part a proportion of calcium and magnesium, however different particles, for example, aluminium, iron, manganese, strontium, zinc, and hydrogen particles are likewise included. In case of *M. rosenbergii* culture water hardness is an important issue because it is related with prawn molting, hardening of shell and carapace, etc. Maintaining hardness is a crucial issue in case of prawn culture in biofloc based culture. In the research duration application EDTA and minerals were closely related with hardness controlling that was properly maintained. Hardness was measured by HANNA 3812 Hardness Test Kit.


**Figure 14:** Measuring hardness level by using HANNA's kit.

**vii) Total Dissolve Solid (TDS)**

Total dissolve solid means the concentration of mineral, salt and solid substances in water. In case of biofloc based culture, TDS remains higher than traditional culture. The importance of TDS in water for freshwater prawn is inevitable as it helps prawn body formation. In the culture period it was maintained properly and measured the level by using multimeter.

**3.6.3 C: N Ratio**

Carbon to Nitrogen ratio (C: N) is a ratio of the mass of carbon to the mass of nitrogen in a substance. For example, a C: N of 10:1 means there is ten units of carbon for each unit of nitrogen in the substance.


**Figure 15:** Addition of raw sugar in the tank water as carbon source.

The following procedures were followed for maintaining carbon nitrogen ration during the experiment.

Freshwater prawn was stocked in tank (kg/$m^3$)
↓
Amount of feed given per day
↓
Protein (%) contained in feed
↓
Amount of carbon in feed
↓
Amount of nitrogen in feed
↓
Total amount of nitrogen released in tank
↓
Total amount of carbon needed
↓
Amount of carbon derived from feed given
↓
Actual amount of carbon needed
↓
Amount of raw sugar needed to mitigate the actual amount

**Figure 16:** Flowchart of maintaining C:N ratio during the research.

### 3.6.4 Application of EDTA

Ethylenediaminetetraacetic acid (EDTA) is a chemical substance used to reduce the metal concentration in water. In biofloc based culture techniques huge amount of ionic substances are mixed with water for using various types of carbohydrates sources, salt and mineral, etc. In case of prawn culture in biofloc solid substances are deposited in the carapace and body shell due to the huge concentration heavy metal. That may result the growth degradation and sloth the molting process of juvenile prawn. For softening the water quality and helping to quick molting this chelator agent, EDTA was used at 10 mg/ L in this study. Freshwater prawn starts molting after full moon and new moon. For this, EDTA was mainly applied before day of full moon and new moon.


**Figure 17:** Titriplex III Pure used as EDTA in the experiment.

### 3.6.5 Application of Mineral

Mineral is an essential component for prawn culture. General function of minerals includes constituents of the exoskeleton, balance of osmotic pressure, structural constituents of tissues and transmission of nerve impulse and muscle contractions. Minerals serve as essential components for enzymes, vitamins, hormones, pigents, and co-factor in metabolism, catalysts, and enzyme activators. Shrimp can absorb or excrete minerals directly from the aquatic environment via gill and body surfaces. In this research work mineral was given to the tanks regularly. Generally, after completing sampling schedule and molting schedule mineral extract was given in all tanks at 20g per 1000 litre.

### 3.6.6 Sampling Schedule

Sampling is a process used in statistical analysis in which a predetermined number of observations are taken from a larger population. This tool describes the samples to take in order to quantify a system, process, issue or problem. Sampling saves time and the data can be collected and analysed more quickly with a sample than a complete count of the entire population. In the study sampling was done properly at 14 days of interval. Random sampling was done manually in every time with the help of scoop net.

### 3.6.7 Harvesting

Harvest means the number or weight of fish caught and retained from a given area over a given period of time. Harvesting is done to analyse the different parameters of any species through an experiment after a timeframe. In this research work, this procedure was accomplished at 70 days. This process was completed by draining 90 percent water of the tanks. The main purpose of harvesting was to assess the performance of overall growth, survival rate in this timeframe.

**Figure 18:** Harvesting freshwater prawn after completion the research duration.

## 3.7 Growth Parameters

Growth is an integrated physiological response encompassing external environmental conditions (food quality and quantity, temperature, water quality) and internal physiological status (health, stress, reproductive state). Growth parameters defines the assessment of subsequent growth and development and risk of disease from the starting level of any species culture considering different factors. The parameters are length, weight, size and shape, etc.

### i) Weight Gain

Weight gain was calculated by using this following formula;

$$\textbf{Weight gain} = \textbf{Mean final weight} - \textbf{Mean initial weight}$$

**ii) Percentage Weight Gain**

Percentage of weight gain was calculated by using this following formula;

$$\%\ \mathbf{Weight\ gain} = \frac{\mathbf{Mean\ final\ prawn\ weight - Mean\ initial\ prawn\ weight}}{\mathbf{Mean\ initial\ prawn\ weight}}$$

**iii) Survival Rate**

Survival rate was assessed by using this following formula;

$$\mathbf{Survival\ rate}\ (\%) = \frac{\mathbf{No.\ of\ total\ live\ prawn}}{\mathbf{No.\ of\ total\ prawn\ stocked}} \times \mathbf{100}$$

**iv) Average Daily Gain (ADG)**

Average daily weight gain was calculated by using this following formula;

$$\mathbf{ADG} = \frac{\mathbf{Final\ prawn\ weight - Initial\ prawn\ weight}}{\mathbf{No\ of\ Days}}$$

**v) Specific Growth Rate (SGR)**

Specific growth rate was calculated by using this following formula;

$$\mathbf{SGR}\ (\%\ \mathbf{per\ day}) = \frac{\mathbf{lnW_2 - lnW_1}}{\mathbf{T_2 - T_1}} \times \mathbf{100}$$

*($W_1$= Initial weight, $W_2$= Final weight,

$T_1$= Time of stock and $T_2$= Time of harvest)

**vi) Feed Conversion Ratio (FCR)**

Feed conversion ratio was calculated by using this following formula;

$$\mathbf{FCR} = \frac{\mathbf{Total\ feed\ consumed\ (g)}}{\mathbf{Live\ weight\ gain\ (g)}}$$

**vii) Net Production**

$$\textbf{Net Production (kg/m3)} = \frac{\textbf{SR}\ (\%) \times \textbf{Stocking density} \times \textbf{Weight gain}\ (\textbf{g})}{\textbf{100} \times \textbf{1000}}$$

*(SR= Survival Rate)

### 3.8 Evaluation of Nutritional Value

Nutritional value of the cultured prawn, juvenile prawn of hatchery sources, microbial floc produced during research work, commercial feed that was used in the time of study were evaluated from a government organization of Bangladesh called Animal Nutrition Section, Department of Livestock services.

### 3.9 Statistical Analysis

Statistical analysis was done for the purpose of getting better outcome of the research result. That was accomplished by using MS Excel, and SPSS (Statistical Package for Social Sciences). All non-repeatedly measured variables (prawn growth parameters) were analysed by one-way ANOVA Tukey HSD programme using SPSS software at 5% level of significance.

# CHAPTER IV

# RESULTS AND DISCUSSION

This chapter confined the results on the investigation accomplished during the research timeframe to fulfil the mentioned objectives earlier. Growth performance, different environmental and physiochemical parameters and nutritional value of freshwater prawn have been presented and discussed for obtaining the actual potentiality of Biofloc Technology which has been accompanied with microbial biotechnology. The experimental findings are given below:

## 4.1 Water Quality Parameters

Water quality maintenance and monitoring in aquaculture are the essential practices focusing on the achievement of the developing cycles. Temperature, dissolved oxygen (DO), pH (puissance of hydrogen), ammonia concentration ($NH_3$), salinity (for salt water species), TDS (total dissolve solids), alkalinity, hardness (quantity of $CaCO_3$) and orthophosphate are some examples of parameters that ought to be continuously checked and monitored, especially in Biofloc Technology (BFT). The comprehension and understanding of water quality parameters and its interactions in BFT are crucial to the correct development and maintenance of the production cycle. Water quality is significant in fish cultivating as low quality water can impact the wellbeing and development of the fish.

**Table 01:** Water quality parameters (Mean) of different treatments tanks.

| Para-Meters | T-1 (Control) | T-2 (EG) | T-3 (EA) | T-4 (EGa) | T-5 (EGa & HS) |
|---|---|---|---|---|---|
| **Temp.** | 29.3±1.03 | 29.3±1.01 | 29.2±0.95 | 29.2±1 | 29.4±0.9 |
| **pH** | 7.9±0.24 | 7.9±0.2 | 7.9±1.73 | 7.9±0.19 | 7.9±0.2 |
| **DO** | 6.6±0.51 | 6.5±0.58 | 6.5±0.67 | 6.5±0.67 | 6.2±0.56 |
| **$NH_3$** | 0.3±0.072 | 0.3±0.01 | 0.3±0.07 | 0.3±0.08 | 0.3±0.09 |
| **Alkali.** | 183.8±15.5 | 183.4±16.66 | 185.0±21.4 | 184.5±17.2 | 183.4±17.78 |
| **Hard.** | 115.5±7.46 | 112.3±8.77 | 112.9±7.63 | 113.9±7.19 | 115.8±6.65 |
| **TDS** | 974.3± 121.1 | 1062.8± 119.2 | 981.9± 169.9 | 974.7± 178.7 | 973.1± 156.9 |
| **FV** | 0.0 | 13.5±1.43 | 13.8±2.15 | 13.9±1.92 | 13.1±2.56 |

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic, HS= Higher stocking and FV=Floc volume; Unit of pH, and TDS=ppm; DO, $NH_3$, Alkalinity and Hardness= mg/l, Temperature = $^0$C, FV= ml/l.

In the present study, it was found that mean temperature range was more or less similar in every treatments ranged from 29.2 to 29.4 $^0$C (Table 01).The mean pH and $NH_3$ in different treatments was almost similar during the experiment that defines the positivity of good water quality for prawn culture. Range of mean dissolve oxygen (DO) was also more or less similar in every treatments ranged from 6.2 to 6.5 mg/l (Table 01). Alkalinity and hardness were more or less similar ranged from 183.4 to 185 and 112.3 to 115.8 mg/l respectively during the experiment (Table 01). Except Control treatment tanks, floc was found at satisfactory level in other treatments tanks.

### 4.1.1 Relationship Between Temperature and pH

**i) Treatment-1 (Control)**

Temperature is a crucial factor for growth and development in case of aquatic organisms. Water temperature is especially important to the growth and survival of aquatic animals because they are poikilothermic in nature. Optimum range of temperature helps fish to become more active in aquatic environment. Growth rate increased significantly with increased temperature (Niu *et al.*, 2003). Below temperature level (< 20$^0$C) affects the microbial growth (Emerenciano *et al.*, 2017). In this study, for treatment-1 (Control) tanks mean temperature range was (29.3$\pm$1.03)$^0$C (Table 01) where lower range was 24.8$^0$C and higher was 31.2$^0$C throughout the research duration (Figure 19).

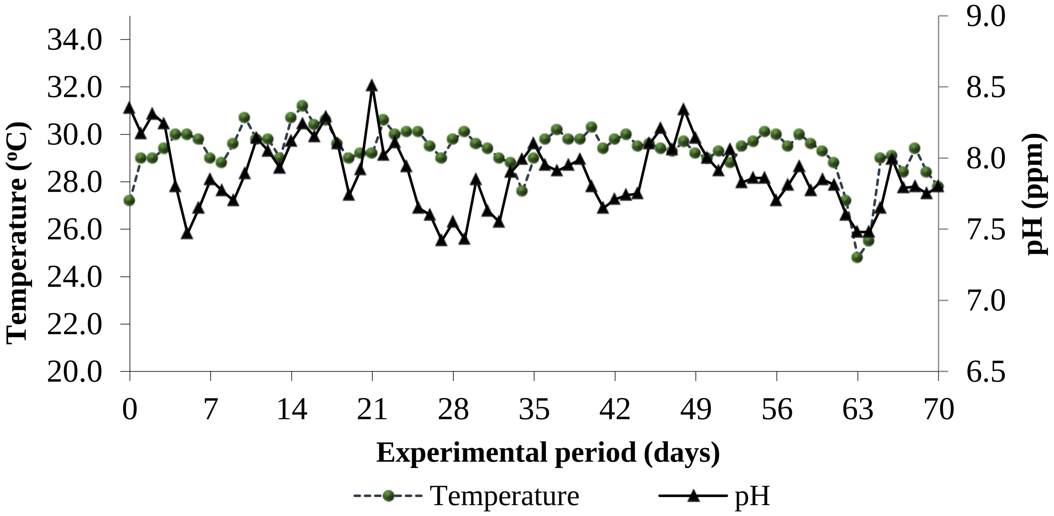



**Figure 19**: Relationship between temperature and pH during experimental periods in tanks of treatment-1 (Control).

The range of pH was balanced level (7.9±0.24) with temperature in T-1 tanks (Table 01). The highest range of pH was recorded 8.5 at day 21 and lowest was 7.4 at days 28 and 29 (Figure 19).

**ii) Treatment-2 (Extract of Ginger)**

In this study, for treatment -2 (EG) tanks mean temperature range was (29.3±1.01)$^0$C (Table 01) where lower range was 24.8$^0$C and higher was 31.2$^0$C throughout the research duration (Figure 20). The range of pH was balanced level (7.9±0.2) with temperature in T-2 tanks (Table 01). During the research period, lime was applied at a slight amount for maintain the pH ratio for proper growth and development. Lower range of pH tells the acidity of water that can be fatal for aquatic species. The highest range of pH was recorded 8.3 at days 8 and 31 and lowest was 7.5 at day 5 (Figure 20).

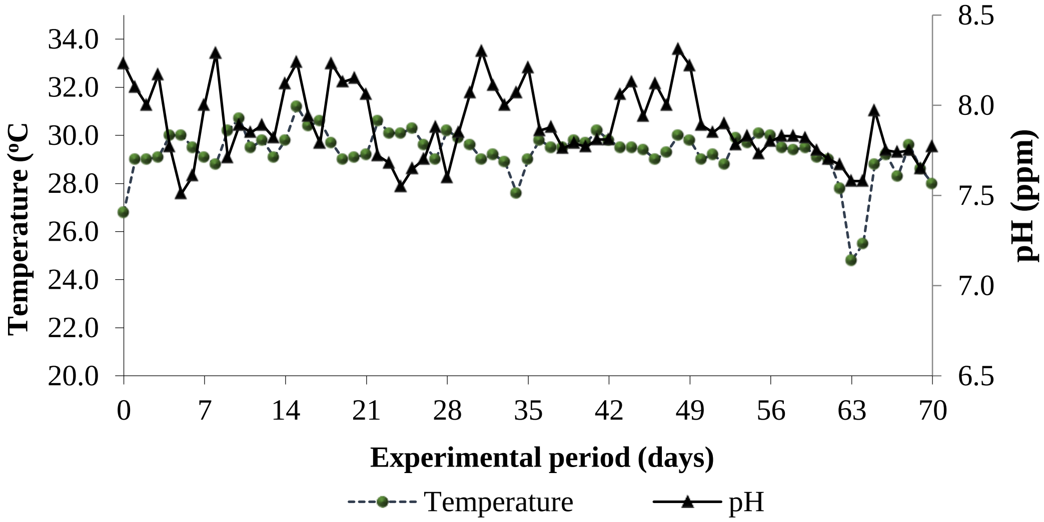



**Figure 20**: Relationship between temperature and pH during experimental periods in tanks of treatment-2 (EG).

**iii) Treatment-3 (Extract of Amla)**

From the study, for treatment -3 (EA) tanks mean temperature range was (29.3±1.01)$^0$C (Table 01) where lower range was 25$^0$C and higher was 31.4$^0$C throughout the research duration (Figure 21). The range of pH was balanced level (7.9±1.73) with temperature in T-3 tanks (Table 01). The highest range of pHwas recorded 8.2 at days 3, 23 and 43 and lowest was 7.3 at day 10 (Figure 21).

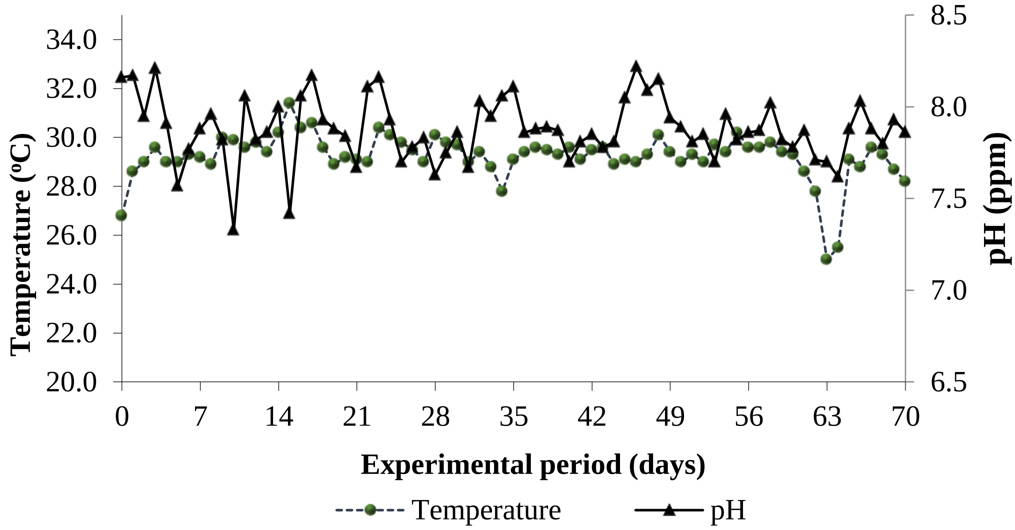



**Figure 21**: Relationship between temperature and pH during experimental periods in tanks of treatment- 3 (EA).

**iv) Treatment-4 (Extract of Garlic)**

In this study, for treatment -4 (EGa) tanks mean temperature range was (29.2±1)$^0$C (Table 01) where lower range was 24.8$^0$C and higher was 31.6$^0$C throughout the research duration (Figure 22). The range of pH was balanced level (7.9±0.19) with temperature in T-4 tanks (Table 01). Balanced pH range help to keep fish easier in aquatic environment. The highest range of pH was recorded 8.2 at days 3, 17, 23 and 35 and lowest was 7.3 at the day 10 (Figure 22).

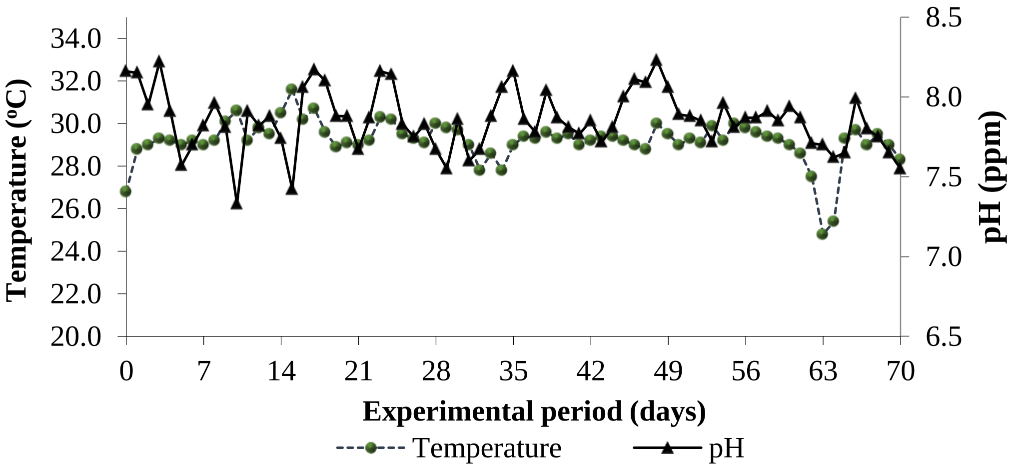



**Figure 22**: Relationship between temperature and pH during experimental periods in tanks of treatment-4 (EGa).

**v) Treatment-5 (Extract of Garlic & Higher stocking)**

In this study, for treatment -5 (EGa & HS) tanks temperature ranged $(29.4\pm0.9)^0$C (Table 01) where lower range was $24.8^0$C and higher was $31.4^0$C throughout the research duration (Figure 23). The range of pH was balanced level ($7.9\pm0.2$) with temperature in T-5 tanks (Table 01). The highest range of pH was recorded 8.4 at days 49 and 55 and lowest was 7.6 at days 16 and 65 (Figure 23). In case of freshwater prawn (*M. rosenbergii*) culture temperature range $28\text{-}33^0$C showed better food consumption rate (Niu *et al.*, 2003). Another researcher Mannan *et al.* (2012) gave a conclusion that $26\text{-}32^0$C temperature range help to growth and production of fish. pH of the water is inversely related to temperature but it doesn't mean that water becomes more acidic at higher range of temperature. Despite the slight deviation of temperature during the study period there was no such harmful effect in treatment tanks. So, this study findings fully support the actual range of temperature and its relation with pH.

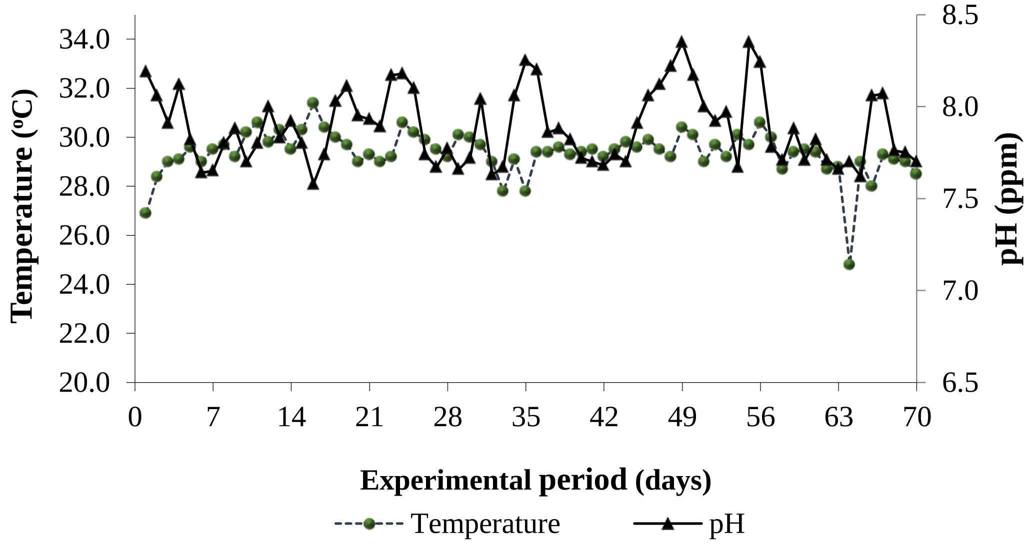



**Figure 23**: Relationship between temperature and pH during experimental periods in tanks of treatment-5 (EGa & HS).

**4.1.2 Relationship Between DO and pH**

**i) Treatment-1 (Control)**

Dissolve oxygen (DO) and puissance of hydrogen (pH) are important factors for fish growth and survival. In this study, for treatment-1 (Control) mean DO and pH were $6.6\pm0.51$ mg/l (ranged from 5.0 to 7.0) and $7.9\pm0.24$ (ranged from 7.4 to 8.5) (Table 01, Figure 24). A researcher stated that DO ranged from 6.8 to 11.2 mg/l help to incraese survival rate in case of *M. rosenbergii* culture (Chand, 2000). Lower range of pH could affect the nitrification process in BFT (Emerenciano *et al.*, 2017).

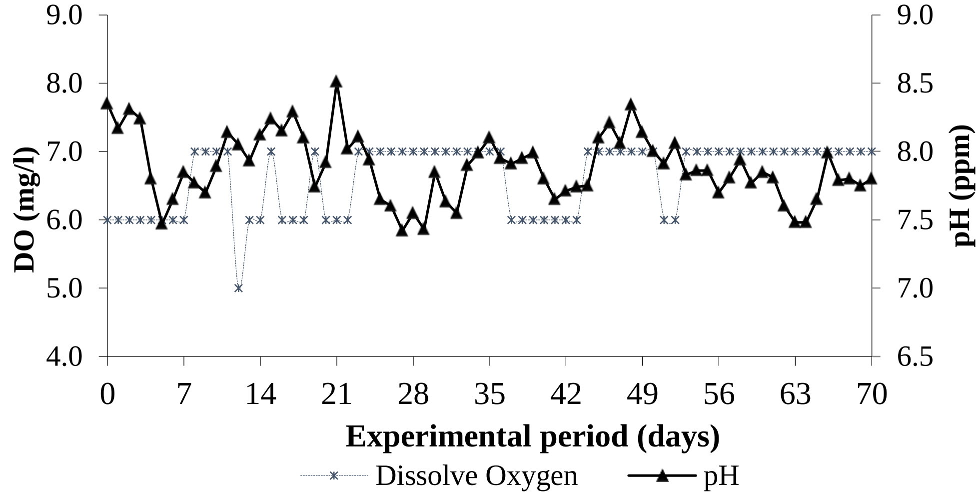



**Figure 24**: Relationship between DO and pH during experimental periods in tanks of treatment-1 (Control).

**ii) Treatment-2 (Extract of Ginger)**

For treatment-2 (EG) mean DO and pH were 6.5±0.58 mg/l (ranged from 6.0 to 7.0) and 7.9±0.2 (ranged from 7.5 to 8.3) (Table 01, Figure 25). DO is an essential factor for freshwater prawn because without sufficient DO level growth can be hampered due to decreased level of feed intake. It was seen from figure 25 that DO range was at a consequence level during the research.

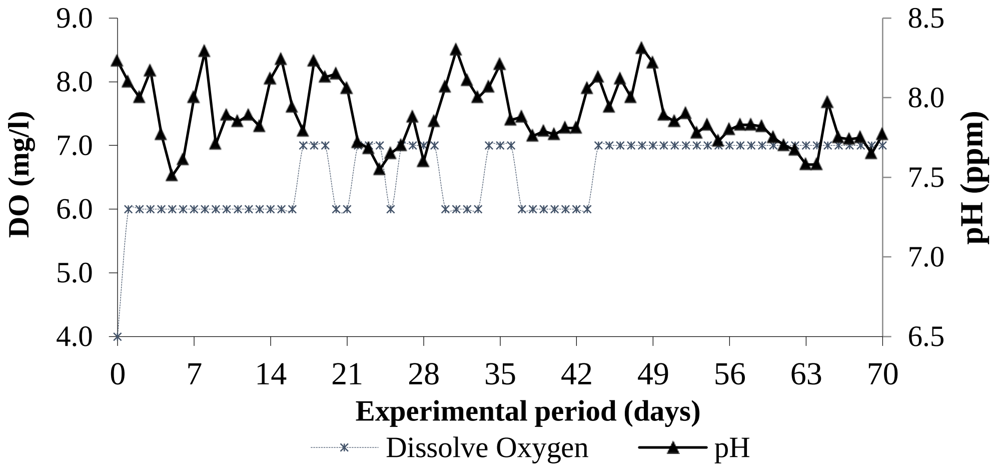



**Figure 25**: Relationship between DO and pH during experimental periods in tanks of treatment-2 (EG).

**iii) Treatment-3 (Extract of Amla)**

In this study, for treatment-3 (EA) mean DO and pH was 6.5±0.67 mg/l (ranged from 5.0 to 8.0) and 7.9±1.73 (ranged from 7.3 to 8.2) (Table 01, Figure 26). When DO and pH are in stable range it is said that fish are in suitable environment for their develpoment.

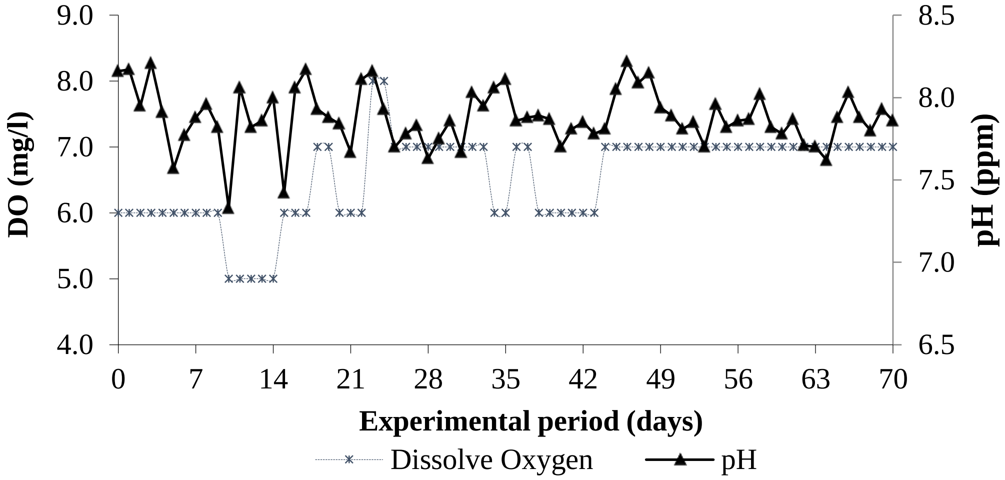



**Figure 26**: Relationship between DO and pH during experimental periods in tanks of treatment-3 (EA).

**iv) Treatment-4 (Extract of Garlic)**

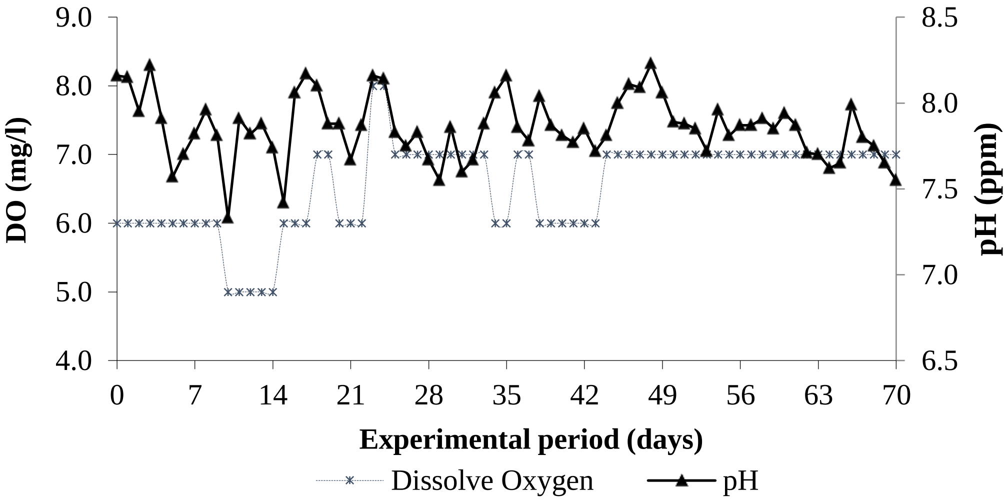



**Figure 27**: Relationship between DO and pH during experimental periods in tanks of treatment-4 (EGa).

For treatment-4 (EGa) mean DO and pH was 6.5±0.67 mg/l (ranged from 5.0 to 8.0) and 7.9±0.19 (ranged from 7.3 to 8.2) (Table 01). From the figure 27, it can be told that DO level was always in optimum range for *M. rosenbergii* culture. DO along with pH help to prawn more easier and faster in aquatic ambience.

**v) Treatment-5 (Extract of Garlic & Higher stocking)**

In this study, for treatment-5 (EGa & HS) mean DO and pH was 6.2±0.56 mg/l (ranged from 5.0 to 7.0) and 7.9±0.2 (ranged from 7.6 to 8.4) (Table 01, Figure 28). In biofloc technology (BFT), ideal DO and pH range was suggested above 4.0 mg/l and 6.8-8.0 for fish, prawn and microbiota respiration process (Emerenciano *et al.*, 2017). Another researcher stated that DO ranged from 6.8 to 11.2 mg/l help to incraese the production by improving survival rate and growth of *M. rosenbergii* culture at higher stocking (Chand, 2000). Lower range of pH could affect the nitrification process in BFT (Emerenciano *et al.*, 2017). The findings of the resaerch of DO an pH range for is fully supported the previously mentioned researchers suggestions.

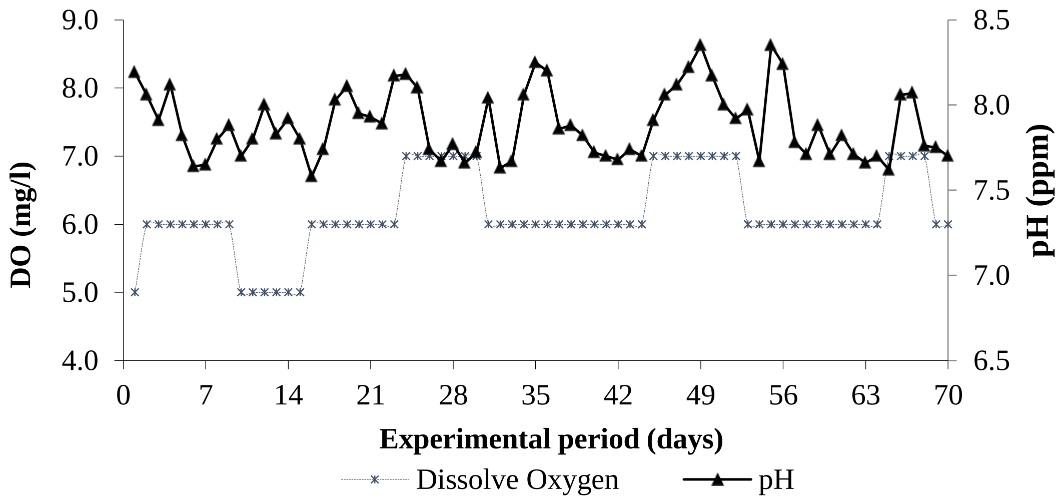



**Figure 28**: Relationship between DO and pH during experimental periods in tanks of treatment-5 (EGa & HS).

### 4.1.3 Ammonia Concentration

**i) Ammonia level in Treatment-1 (Control)**

In biofloc technology (BFT) concentration of $NH_3$ in cultured tank is regarded as critical parameter and should be maintained less than 1.0 mg/l (Emerenciano *et al.*, 2017). From the figure 29, the highest ammonia level (0.75 mg/l) was recored at day 19. The continuity was maintained in case of ammonia in T-1 round the research time.

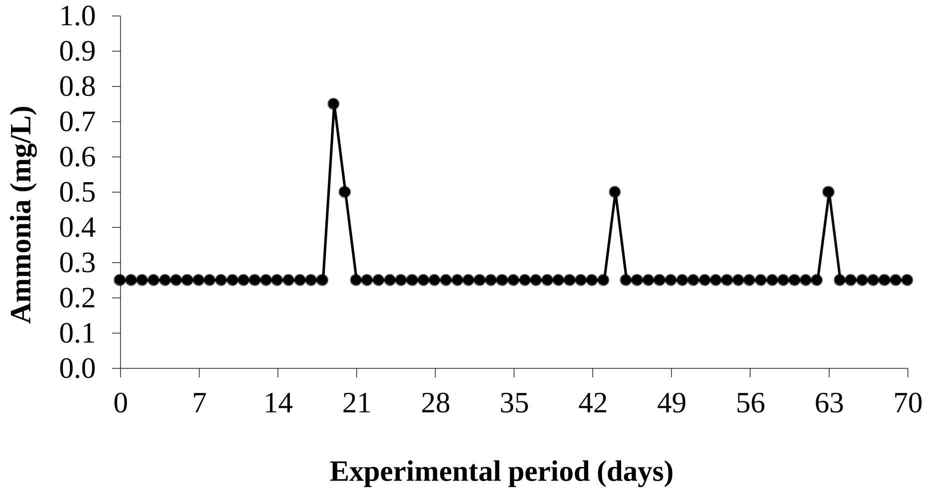



**Figure 29:** Ammonia concentration in tanks of treatment -1 (Control) during the research timeframe.

**ii) Ammonia level in Treatment-2 (Extract of Ginger)**

From the figure 30, the highest ammonia level (0.75 mg/l) was recored at days 19 and 25. The continuity was maintained in case of ammonia in T-2 round the research time. It is clearly noticed that ammonia level was convenient during the working period.

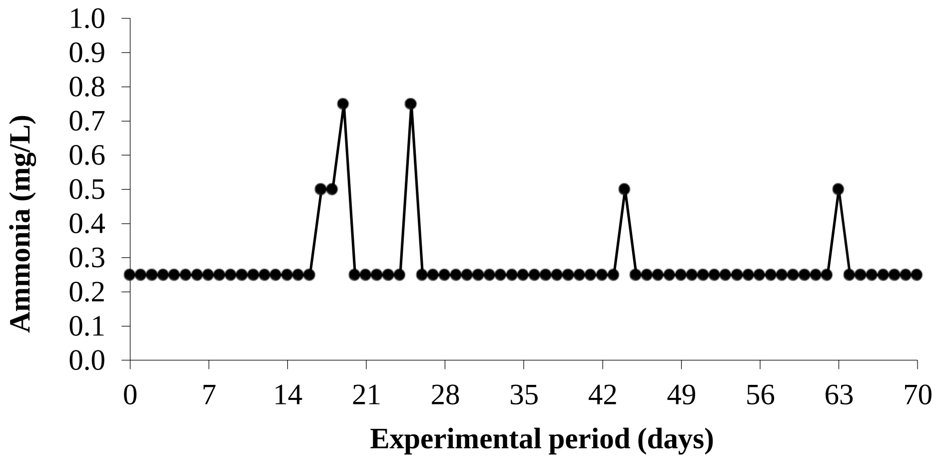



**Figure 30:** Ammonia concentration in tanks of treatment-2 (EG) during the research timeframe.

**iii) Ammonia level in Treatment-3 (Extract of Amla)**

From the figure 31, ammonia level ranged from 0.25 to 0.75 in treatment-3 (EA) tanks throughout the experimental days. It is understood that ammonia was in good condition in T-3. Some slight slippage of ammonia range during the experiment was not harmful for prawn. Because those range was not exceeded the tolerable range.

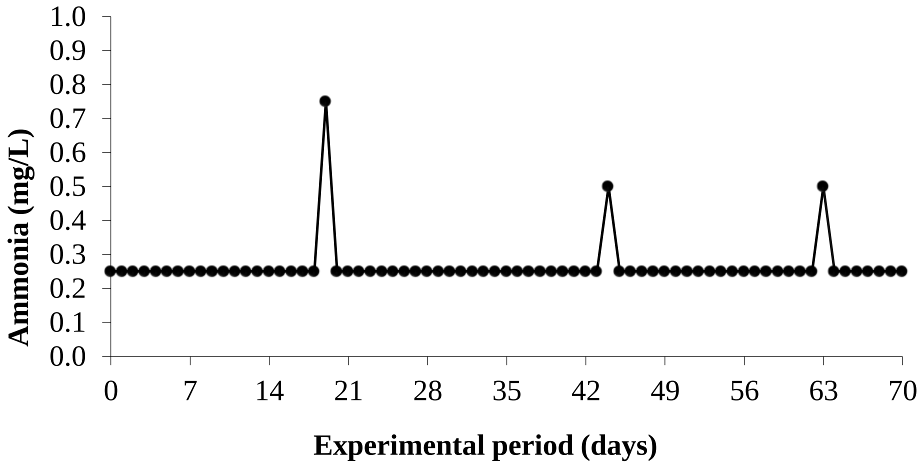



**Figure 31:** Ammonia concentration in tanks of treatment -3 (EA) during the research timeframe.

**iv) Ammonia level in Treatment-4 (Extract of Garlic)**

During the experiment, ammonia level ranged from 0.25 to 0.75 in treatment-4 (EG) tanks throughout the experimental days (Figure 32). Maintained ammonia level tells about the suitable level for prawn culture in biofloc technology.

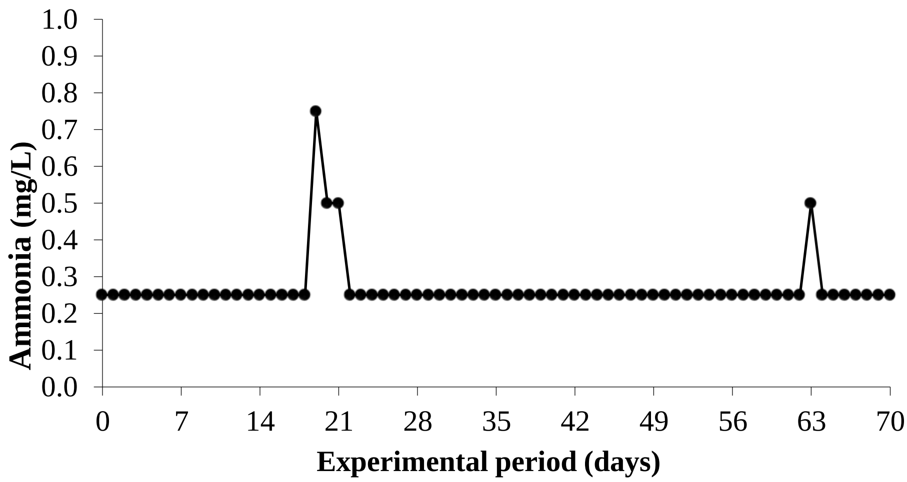



**Figure 32:** Ammonia concentration in tanks of treatment-4 (EGa) during the research timeframe.

**v) Ammonia level in Treatment-5 (Extract of Garlic & Higher stocking)**

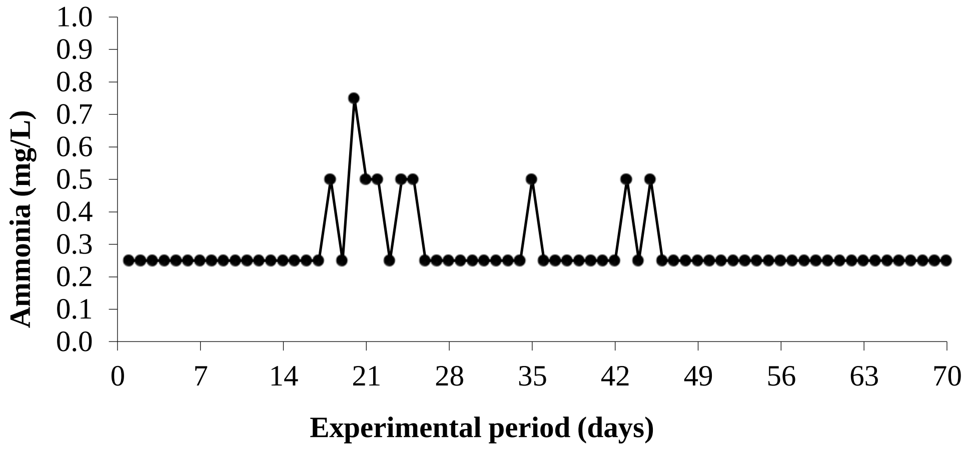



**Figure 33:** Ammonia concentration in tanks of treatment-5 (EGa & HS) during the research timeframe.

Figure 33 shows that ammonia level was maintained ( ranged from 0.25 to 0.75) in treatment-5 (EGa & HS) tanks throughout the experimental days. It also describes the frequent increase of ammonia level in this treatment compared to othe treatments that may be caused for higher stocking density of prawn in the tanks. Emerenciano *et al.* (2017) suggested to keep ammonia level below 1.0 mg/l for biofloc based culture. According to their suggestion it can be told that the ammonia level of all treatments tanks were in convenient level for avoiding any stress condition of prawn growth and development.

### 4.1.4 Comparison of Alkalinity

Alkalinity defines the level of $CaCO_3$ in the cultured tanks. Optimum level of alkalinity helps to nitrogen assimilation by heterotrophic bacteria and it is more than 100 mg/l (Emerenciano *et al.*, 2017). In this study, the highest (250 mg/l) and lowest (135 mg/l) alkalinity level were recorded in T-3 tanks at day 0 and 25 respectively. It is clearly shown that alkalinity level was near 150-200 mg/l in all treatments (Figure 34). This findings have similiarities with the final statement of Furtado *et al.* (2015) who stated that higher alkalinity level indiretly favors shrimp culutre by helping the biofloc formation.

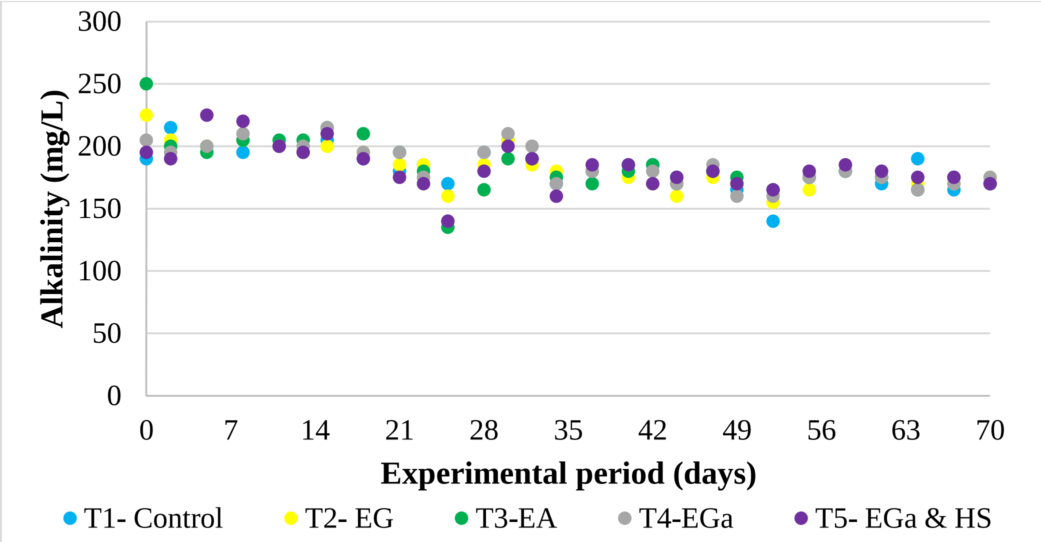



**Figure 34:** Concentration of alkalinity in all treatments tanks in biofloc based prawn culture (BFT) during research.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.1.5 Comparison of Hardness

Hardness means the amount of $CaCO_3$ dissolve in water that help to body formation of freshwater prawn.

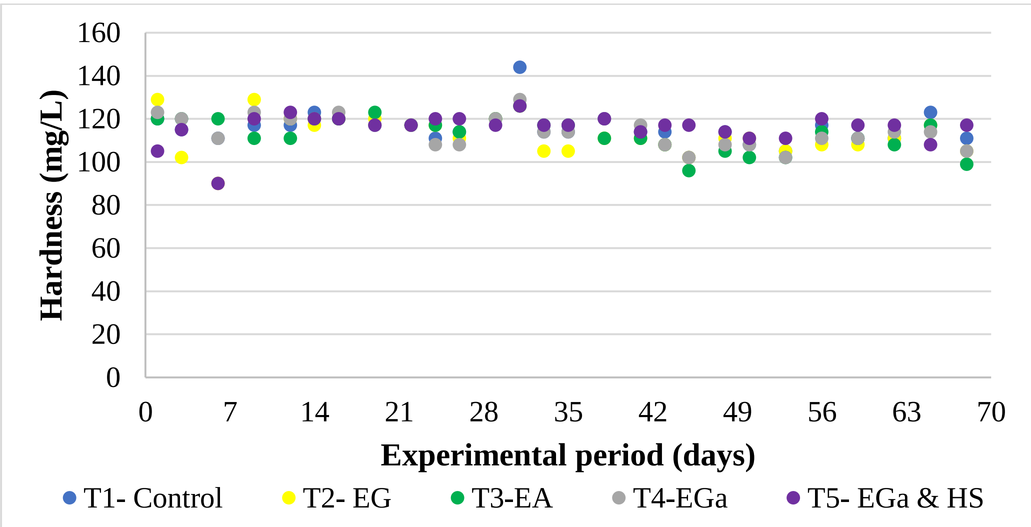



**Figure 35:** Concentration of hardness in all treatments tanks in biofloc based prawn culture (BFT) during research.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

In this study, the highest (144 mg/l) and lowest (90 mg/l) hardness level was recorded at day 30 and 6 in T-1 and T-5 treatmnets tanks. It is clearly shown that hardness level was near 100-120 mg/l in all treatments (Figure 35). In BFT, good results were obtained for *M. rosenbergii* culture when it was 97 mg/l (Ballester *et al.*, 2018). So, it is said that this range of hardness is supported by researcher's findings.

### 4.1.6 Comparison of Total Dissolve Solid

Total dissolved solids (TDS) is an important factor for *M. rosenbergii* culture in biofloc because it helps to exoskeleton formation. In this study it is shown that the highest (1276 mg/l) and lowest (722 mg/l) hardness level was recorded at day 14 and 42 in T-5 and T-3 treatmnets tanks. It is clearly shown that hardness level was near 800-1200 mg/l in all treatments (Figure 36). The optimum range for biofloc prawn farming is 1000-1500 mg/l (Chandan, 2020). Another research stated the annual range of TDS for shrimp was 1124 mg/l (Chowdhury & Ahmed, 2012). So, it can be told that the findings of this study was good that indicate the better development of prawn.

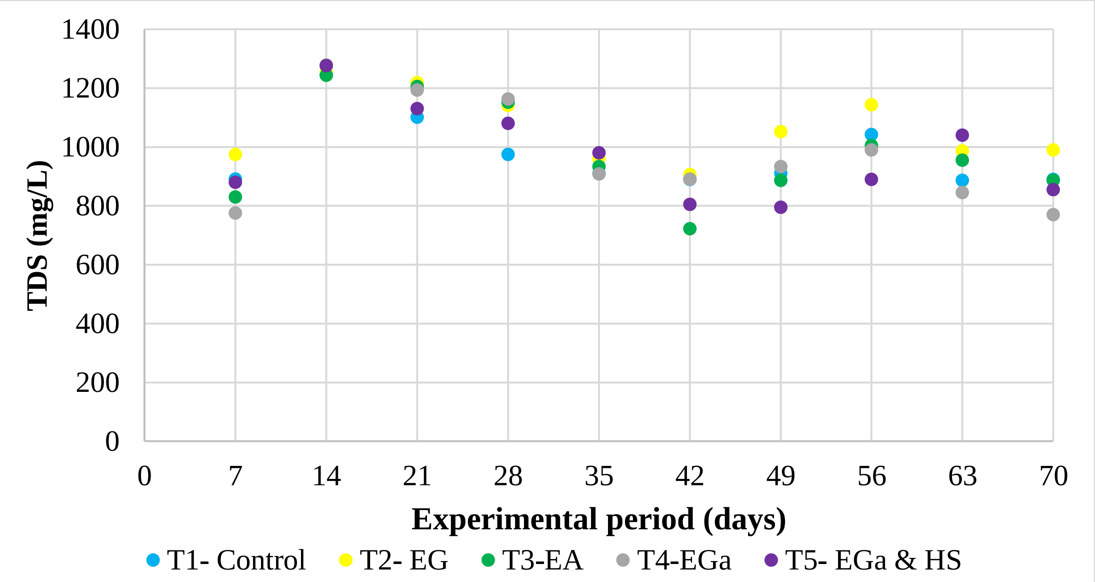



**Figure 36:** Concentration of total dissolved solids (TDS) in all treatments tanks in biofloc based prawn culture (BFT) during research.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.1.7 Comparison of Floc Volume

Biofloc means the biological floc including bacteria, algae, and fungi, etc. which are the main composition of biofloc based culture. In case of freshwater culture the optimal range of floc differs from species to species. In case of shrimp culture, 15.0 ml/l was considered as normal range (Rajkumar *et al.*, 2016). In this study, the highest (17 ml/l) and lowest (8 ml/l) hardness level was recorded at day 21 and 7 in T-3 and T-5 treatmnets tanks. It is clearly shown that hardness level was near 10-15 mg/l in all treatments (Figure 37). It can be told that floc voulme was in good level. Requirement of floc volume varies from species to sepcies and in case of prawn it was in optimum level that can be recommended by the aforementioned researchers.

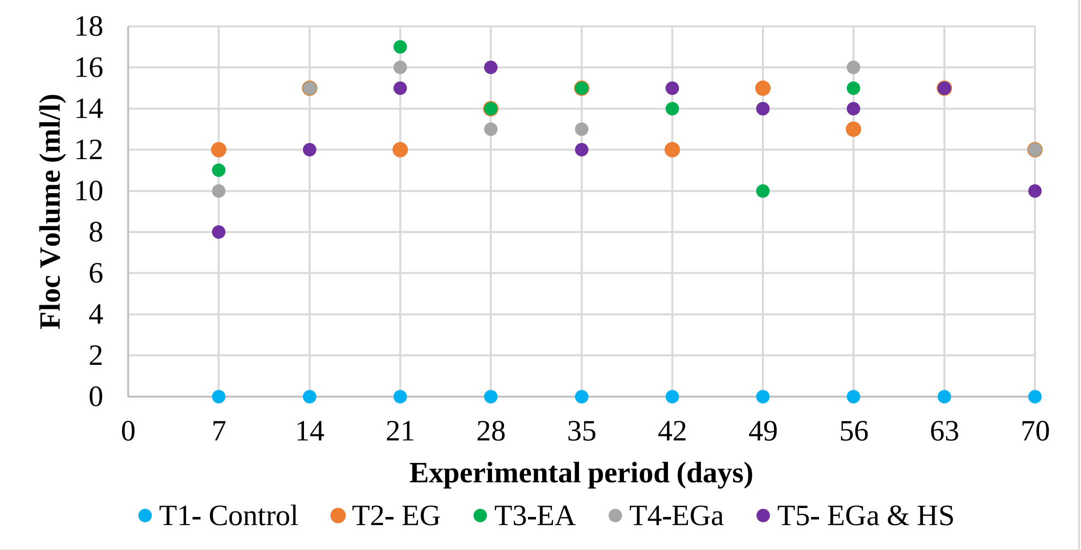



**Figure 37:** Floc volume coverage during research timeframe in all treatments tanks.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

## 4.2 Morphological Parameters of Freshwater Prawn

Growth parameters assessed at birth help predict subsequent growth and development. In this study, weekly data was recorded for assessing the progress of prawn growth and probability of disease infestation.

### 4.2.1 Mean Length of Prawn

Mean length of prawn were recorded after completing sampling at every two weeks of interval.

**Table 02:** Mean length of cultured prawn in experimental period (days).

| Treat-ments | Mean length (cm)/ Days | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 14 | 28 | 42 | 56 | 70 |
| **T-1** | 4.48±0.61 | 6.53±0.1 | 7.52±0.22 | 7.03±0.07 | 8.4±0.02 | 8.37±0.07 |
| **T-2** | 4.48±0.61 | 6.2±0.2 | 7.15±0.21 | 7.47±0.15 | 8.2±0.17 | 8.76±0.05 |
| **T-3** | 4.48±0.61 | 5.45±0.15 | 6.13±0.15 | 7.6±0.17 | 8.48±0.13 | 9.8±0.1 |
| **T-4** | 4.48±0.61 | 5.3±0.1 | 5.68±0.07 | 8.47±0.25 | 9±0.2 | 8.7±0.2 |
| **T-5** | 4.48±0.61 | 5±0.2 | 5.47±0.25 | 6.3±0.07 | 8.35±0.22 | 8.32±0.2 |

*[T-1 = Control, T-2 = EG, T-3= EA, T-4 = EGa, T-5 = EGa & HS]

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

From table 02, it is clearly stated that the initial mean length of prawn was 4.48 cm, stocked for conducting the experiment. After two weeks mean length was seen higher (6.5 cm) in treatment-1 tanks and that continued up to four weeks though sampling was done on random basis. After that, next four weeks treatment-4 gave better results (8.47 and 9.0 cm) than other treatments. It was observed that after two months mean length was higher in treatment-3 than others and after accomplishing harvest the highest mean (9.8 cm) was got in T-3 tanks.


**Plate 01:** Juvenile prawn at stocking .

**Plate 02:** The highest length found in T-1 (Control) after 14 days.


**Plate 03:** The highest length found in T-1 (Control) after 28 days.


**Plate 04:** The highest length found in T-4 (Extract of Garlic) after 42 days.


**Plate 05:** The highest length found in T-4 (Extract of Garlic) after 56 days.


**Plate 06:** The highest length found in T-3 (Extract of Amla) after 70 days.

### 4.2.2 Mean Weight of Prawn

The following mean weight was recorded on the basis of random sampling at two weeks interval in experimental duration. Total five sampling was done during experiment.

**Table 03**: Mean weight of cultured prawn in experimental period (days).

| Treat- | Mean weight (g)/ Days | | | | | |
|---|---|---|---|---|---|---|
| Ments | 0 | 14 | 28 | 42 | 56 | 70 |
| T-1 | 3.14±.19 | 7.88±.22 | 8.68±.28 | 9.83±.66 | 10.56±.46 | 11.05±.49 |
| T-2 | 3.04±.27 | 6.38±.48 | 7.50±.95 | 9.18±.84 | 10.50±.64 | 11.00±.6 |
| T-3 | 3.26±.16 | 6.42±.9 | 9.49±.52 | 10.28±1 | 13.76±.79 | 15.29±.5 |
| T-4 | 3.36±.06 | 7.06±.76 | 10.35±.79 | 11.04±.81 | 12.76±.66 | 13.75±.96 |
| T-5 | 3.02±.36 | 6.14±.94 | 8.57±.61 | 10.03±1.13 | 10.81±.51 | 11.14±.31 |

*[T-1 (Control), T-2 (EG), T-3 (EA), T-4 (EGa), T-5 (EGa & HS)]

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

At the beginning of the research approximately mean 3 g juvenile was stocked for culture. In first two weeks T-1 gave better results (7.88 g) but after that mean weight was better in T-4 tanks up to 42 days than other treatments (Table 03). It is clearly seen that after 42 days of experimental duration growth performance was satisfactory. But after three sampling the growth ratio was not as good as like in T-3 and T-4 tanks. Growth was slightly decreased in T-1, T-2 and T-5. In case of T-1 and T-2 it can be caused due to environmental adverse condition, decrease in feeding rate. In case of T-5, that might cause due to higher stocking. The minimum result was seen in treatment-5 tanks where stocking weight was 3.02±0.36g and final sampling weight was 11.14±0.31g (Table 03). In biofloc based culture of freshwater prawn a researcher got 13.13g (mean weight) where stocking density was 50/$m^2$ (Negrini *et al.*, 2017).

The comparison of mean weight of different treatments that was drawn on five sampling with an interval of fourteen days. In this present study, it was seen that treatment-3 tanks gave better result than control and other treatments tanks. It is cleared that mean weight was better in T-3 compared to other where after 42 days line was just jumped from equal level to higher. From initial day containing 3.26±0.16 g of *M. rosenbergii* juvenile reached into 15.29±0.5 g (Table 03). The rate of growth was less in T-3 at the beginning but after 42 days it crosses all record of other treatments. And finally the highest mean was observed in T-3 at among all sampling.

### 4.2.3 Liner Regression Line of Mean Weight

**i) Analysis of the treatment -1 (Control)**

Regression line predicts the change Y axis when X axis increase by one unit. $R^2$ is a measure of how close each data point fits to the regression line. From the following figure 38, it can be told that 81% mean weight was gained is accounted for its regression on experimental duration in T-1 (Control) experimental tanks. Positive regression line was found in treatment-1 tanks. And mean weight was found almost close according to its predicted time in figure 38.

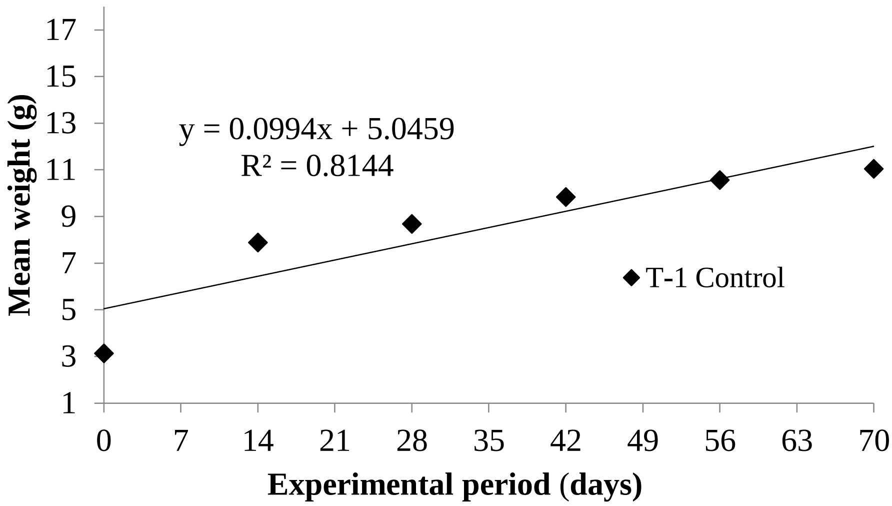



**Figure 38:** Linear regression line of mean weight in treatment-1 (Control) tanks.

**ii) Analysis of the treatment -2 (Extract of Ginger)**

From the following figure 39, it can be told that 93% mean weight was gained is accounted for its regression on experimental duration in T-2 (EG) experimental tanks. In treatment-2 positive line was found that means the growth was good level of progress.

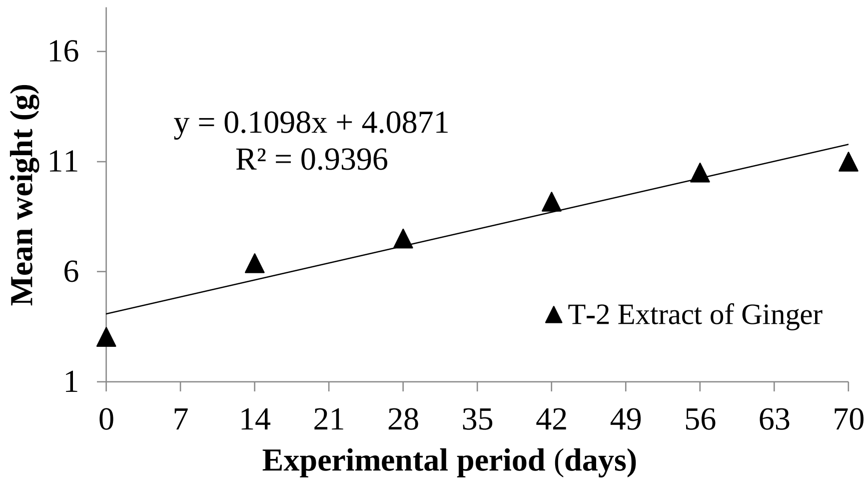



**Figure 39:** Linear regression line of mean weight in treatment-2 (EG) tanks.

**iii) Analysis of the treatment -3 (Extract of Amla)**

From the following figure 40, it can be told that 97% mean weight was gained is accounted for its regression on experimental duration in T-3 (EA) experimental tanks. In treatment-3 the best outcome was recorded during the experiment. Mean weight was at satisfactory level compared to other treatments including control. And the regression line means the positivity of the improvement of growth. In the figure 40, it can be told that mean weight was so close according to its predicted time.

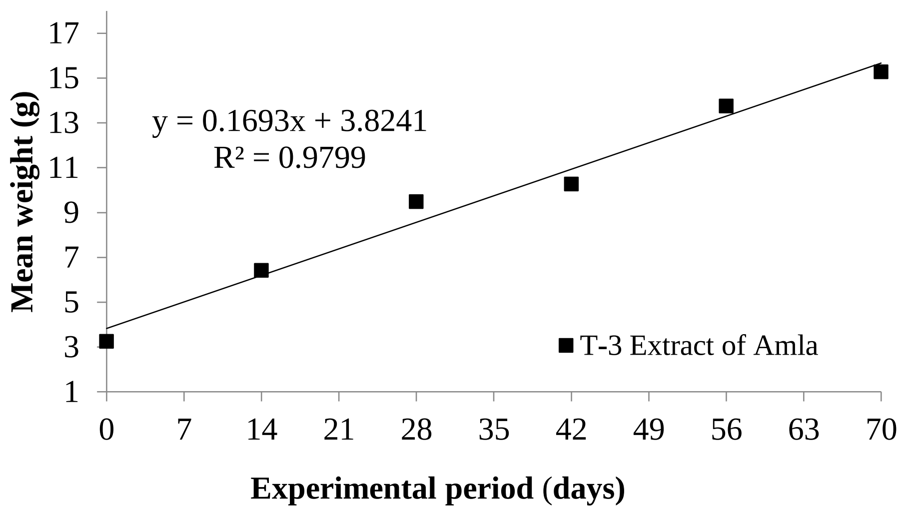



**Figure 40:** Linear regression line of mean weight in treatment-3 (EA) tanks.

**iv) Analysis of the treatment -4 (Extract of Garlic)**

From the following figure 41, it can be told that 92% mean weight gained was accounted for its regression on experimental duration in T-4 (EGa) experimental tanks. From figure 41, a positive line was formed against treatment-4 and actual value was so close to predicted timeframe. The result showed that T-4 gave better result than other treatments except T-3.

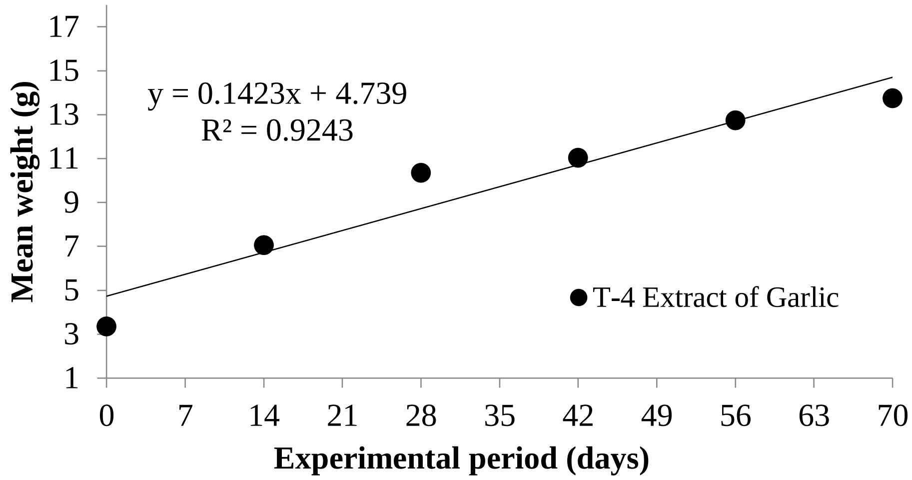



**Figure 41:** Linear regression line of mean weight in treatment-4 (EGa) tanks.

**v) Analysis of the treatment -5 (Extract of Garlic and higher stocking)**

In T-5 (EGa & HS) experimental tanks, there was a positive line found against the mean weight data and 81% mean weight was gained is accounted for its regression on experimental duration.

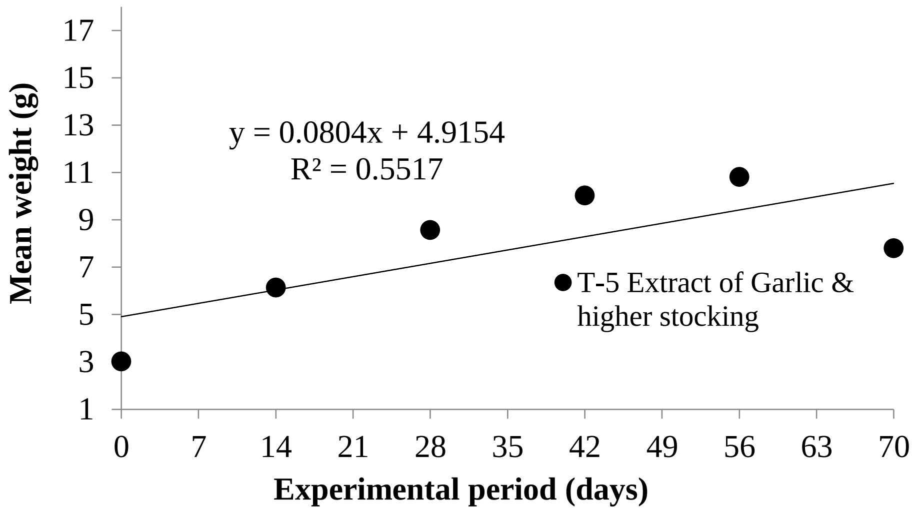



**Figure 42:** Linear regression line of mean weight in treatment -5 (EGa & HS) tanks.

### 4.2.4 Average Daily Weight Gain (ADG)

Daily weight gain fluctuation was normal. Generally weight was fluctuated for different reasons like feeding requirements, environmental factors and disease infestation possibilities. In case of optimum level of water quality factors and fish health condition growth progress depends on feeding rate. Good feeding rate will give better outcome. From the figure 43, the highest weight gain (0.34 g) was found in T-1 after 14 days where it decreased after that day. In the study period, T-4 gave better weight gain (0.24 g) after 28 days but it was observed the least weight at day 40 sampling time. After completing 42 days, the rate of weight gain was seen in better condition in T-3 (0.25 g) and T-4 (0.12 g). The fluctuation of weight gain was clearly seen in different treatments where T-3 gave better result compared to other treatments.

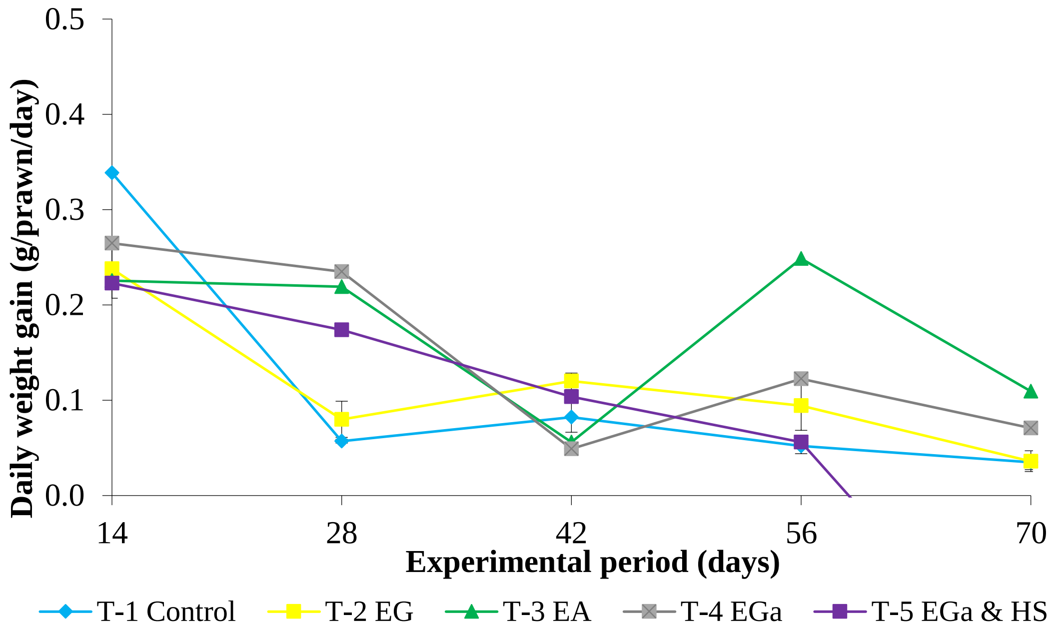



**Figure 43:** Average daily weight gain (ADG) of *M. rosenbergii* at experimental duration.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.2.5 Weekly Specific Growth Rate (SGR)

The following figure shows the specific growth rate of *M. rosenbergii* at weekly basis. From the figure 44, it can be told that SGR rate was good (6.57, 5.29, 4.80, 5.29 and 5.05 in T-1, T-2, T-3, T-4 and T-5 respectively) at the beginning stage of culture. But with the increasing of time, the rate was decreased than previous rate. After 28 days of experiment the rate was increased at a consequent level. And after 56 days of experiment it was recorded that T-3 gave better result (2.10) than other treatments.

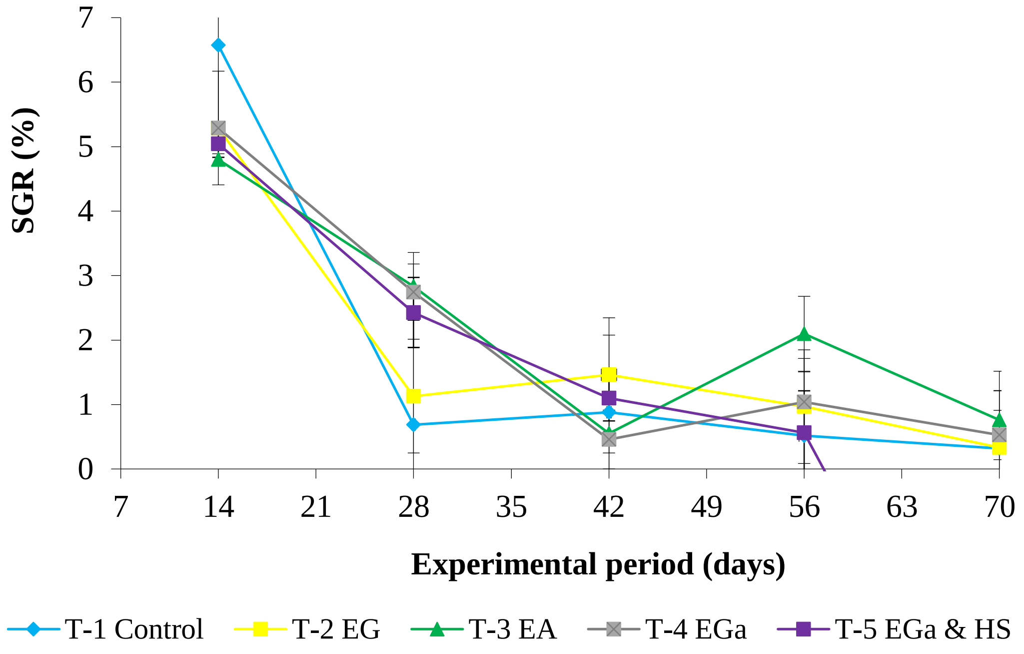



**Figure 44:** Specific growth rate (SGR) of freshwater prawn at research duration.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.2.6 Weekly Nutrition Intake

Nutritional requirements are prerequisite for fish growth and development. In freshwater prawn culture, better protein containing feed helps to grow quickly, complete molting and increase survival rate. Anand *et al.* (2014) found that 35-37% protein containing feed was necessary for juvenile prawn.

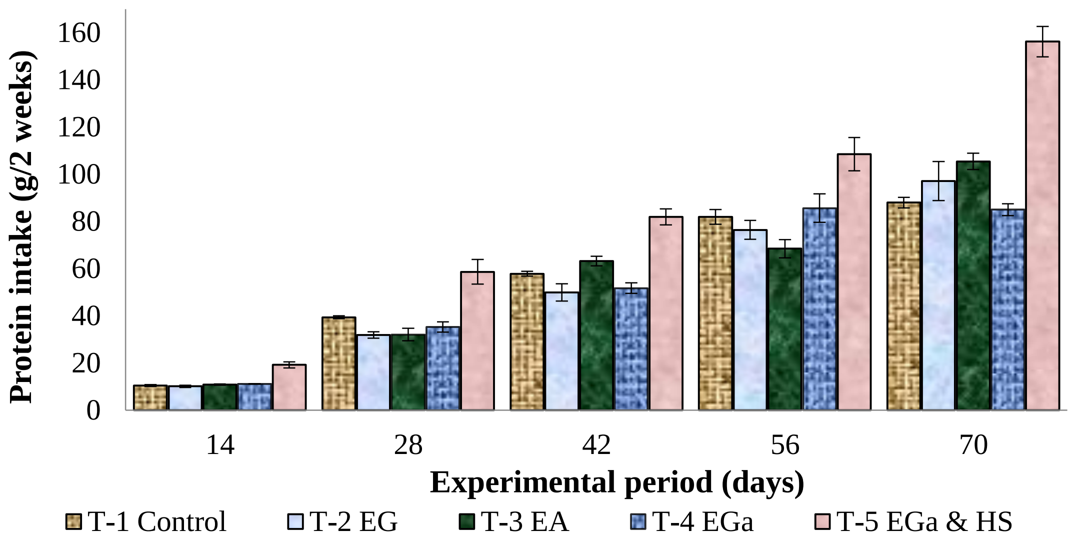



**Figure 45:** Protein intake (g/per 2 weeks) by *M. rosenbergii*. Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

The following figure 46 highlights the lipid intake quantity in every two weeks.

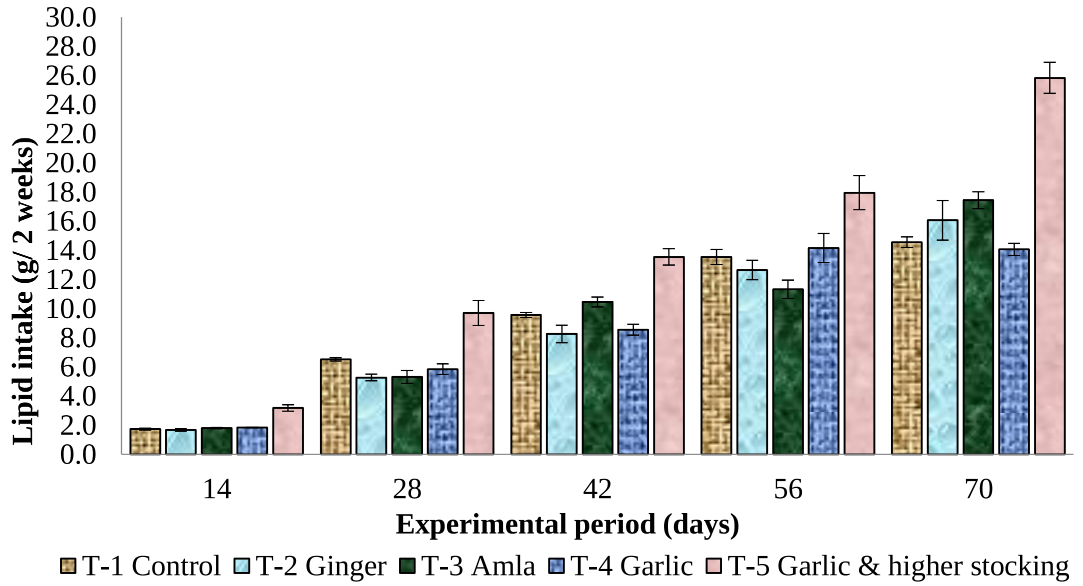



**Figure 46:** Lipid intake (g/per 2 weeks) by *M. rosenbergii*.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

From the figure 45, it is estimated that protein and lipid intake were at constant level. The main reason of the same intake ratio was giving same quality feed in every treatments tanks. In biofloc culture system, less amount of feed is required. But it doesn't hamper on nutritional requirements because fish take protein, lipid by uptaking floccules. The highest rate of protein and lipid intake were in T-5 tanks because feed was applied at higher rate for double stocking density than other treatments. The rate of protein and lipid intake were higher in T-3 tanks than other three treatments tanks except T-5.

### 4.2.7 Growth Curve of *M. rosenbergii*

The exponential growth curves help to understand the growth efficiency for different treatments. The curves show the mean weight increasing level with the increasing of experimental duration. From the figure 47 it is showed that the highest mean weight was derived from treatment-3 tanks where it was 91% for its experimental duration. The lowest was found from treatment-5 where it was 68%. The main reason of the lowest outcome in treatment-5 tanks was higher stocking density than other treatments tanks. Besides that treatment-5, other treatments had satisfactory results.

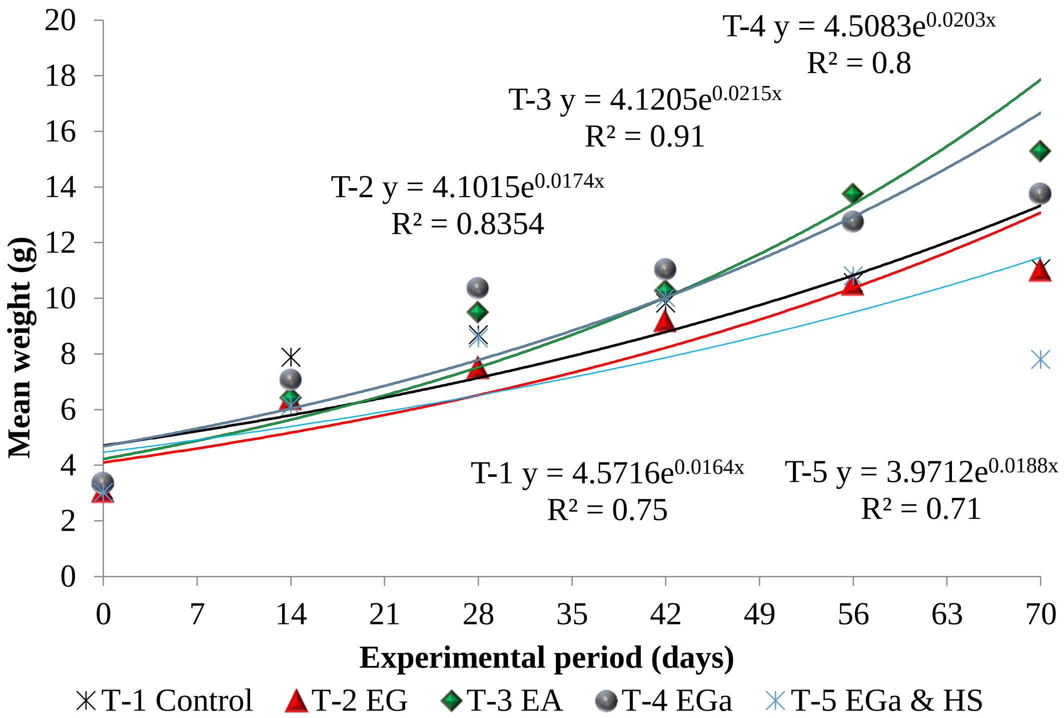



**Figure 47:** Growth curve of *M. rosenbergii* in biofloc culture in different treatments at 70 days.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.3 Growth Parameters of Freshwater Prawn (*M. rosenbergii*)

Growth is an integral biological response including external ecological and internal physiological status. In case of fish, weight gain, feed conversion ratio, survival rate, specific growth rate, protein intake, etc. are considered as growth parameters. In this study, freshwater prawn was harvested after three days of study duration. And the following data were recordetd from *M. rosenbergii* is given below;

**Table 04:** Growth parameters (Mean) of *M. rosenbergii* in biofloc technology.

| **Parameters** | **T-1 Control** | **T-2 EG** | **T-3 EA** | **T-4 EGa** | **T-5 EGa & HS** |
|---|---|---|---|---|---|
| **MIW** (g) | 3.14±0.19 | 3.04±0.27 | 3.26±0.16 | 3.36±0.06 | 3.02±0.36 |
| **MFW** (g) | 10.77±0.03$^{a}$ | 11.29±0.2$^{a}$ | 14.14±0.5$^{b}$ | 12.4±0.3$^{c}$ | 10.2±0.3$^{d}$ |
| **TWG** (g/m$^3$) | 515.3±16.5$^{a}$ | 481.7±14.9$^{b}$ | 659±7.9$^{c}$ | 603.7±8.5$^{d}$ | 780.4±25$^{e}$ |
| **MWG** (g) | 7.6±0.2$^{a}$ | 8.2±0.4$^{b}$ | 10.9±0.3$^{c}$ | 9.1±0.2$^{d}$ | 7.2±0.4$^{a}$ |
| **WG (%)** | 242±22.2$^{a}$ | 234.4±27.9$^{ad}$ | 297.8±16.2$^{c}$ | 264.6±6.6$^{ac}$ | 200.9±27.2$^{bd}$ |
| **SR (%)** | 99.5±0.8$^{a}$ | 89.7±1.4$^{b}$ | 91.6±2.2$^{b}$ | 98.5±1.4$^{a}$ | 88.7±1.9$^{b}$ |
| **SGR (%)** | 1.8±0.1$^{a}$ | 1.9±0.1$^{a}$ | 2.1±0$^{b}$ | 1.9±0$^{a}$ | 1.7±0.2$^{a}$ |
| **FCR** | 1.5±0.1$^{a}$ | 1.6±0.1$^{a}$ | 1.2±0.1$^{b}$ | 1.3±0.1$^{b}$ | 1.6±0.2$^{ac}$ |
| **RGR (%)** | 343.5±19.9 | 373±34.3 | 433±10.1 | 370±5.9 | 339.7±36.7 |
| **PI** (70 days) | 277.6±12.8 | 265.5±26.1 | 280.1±19.7 | 268.9±14 | 422.6±21.9 |
| **LI** (70 days) | 45.9±2.1 | 43.9±4.3 | 46.3±3.3 | 44.5±2.3 | 70.2±3.6 |
| **GP** (g/m$^3$ /70day) | 729±3.61 | 688.7±3.51 | 880.7±9.29 | 832±8 | 1132±34.6 |
| **GP** (kg/m$^3$/crop) | 1.8±0.09$^{a}$ | 1.76±0.09$^{b}$ | 2.26±0.2$^{c}$ | 2.14±0.2$^{d}$ | 2.9±0.9$^{e}$ |
| **NP** (g/m$^3$/70 days) | 515.3±16.5 | 481.7±14.9 | 659±7.9 | 603.7±8.5 | 780.4±25 |
| **NP** (kg/m$^3$/crop) | 1.3±0.04 | 1.25±0.03 | 1.7±0.02 | 1.5±0.02 | 2.03±0.06 |
| **NP** (kg/m$^3$/year) | 2.6±0.08 | 2.51±0.07 | 3.4±0.04 | 3.1±0.04 | 4.06±0.13 |

(EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic, HS= Higher stocking)

*[MIW=Mean Initial Weight, MFW= Mean Final Weight, MWG=Mean Weight Gain, TWG=Total Weight Gain SR= Survival Rate, SGR= Specific Growth Rate, FCR= Feed Conversion Ratio, RGR= Reactive Growth Rate, PI= Protein Intake, LI= Lipid Intake, GP= Gross Production, NP= Net Production]

From figure 04, it was found that the highest mean weight gain (10.9 g) and FCR value was found in T-3 (Extract of Amla) and lowest was found in T-5 (Extract of Garlic and Higher stocking). Protein and lipid intake were higher in T-5 tanks comparatively than others. In case of same stocking density of prawn, the highest gross production (880.7 g) was found in T-3. In T-5 gross production was higher (1132 g) than T-3 because stocking density was doubled in that treatment tanks but not higher proportionally. That will visualize in net production where T-3 gave the best outcome (659 g) in case of normal stocking density. Higher stocking desity decrease the rate of survivality of prawn in biofloc based culture. And from the calculation, it is estimated that by following this culture tactics net production of freshwater prawn could be attained approximately 3.4 kg/m$^3$ per year (Figure 04).


**Plate 07:** Biomass weight of prawn in T-3 after harvest.

Growth parameters of *M. rosenbergii* at final harvesting time are discussed below:

### 4.3.1 Mean Final weight

The present study was started with 70 days duration of freshwater prawn culture in biofloc technology. The research was started with juvenile *M. rosenbergii* comprising the initial weight was 3.14±0.19, 3.04±0.27, 3.26±0.16, 3.36±0.06 and 3.06±0.36g and after completing the research final weight were 10.77±0.03, 11.29±0.2, 14.14±0.5, 12.4±0.3 and 10.2±0.3g in T-1, T-2, T-3, T-4 and T-5 treatments respectively (Table 04). The mean final weight was significantly higher in T-3 than control and other treatments ($P<0.05$). Negrini *et al*. (2017) conducted a research where they got 13.13 g mean weight after 60 days. Another research accomplished on juvenile prawn culture started with 1.87 g mean weight and after 60 days they got 13.33-17.63g mean weight for different treatments. So, it can be said that the present observations is quite similar to their findings.

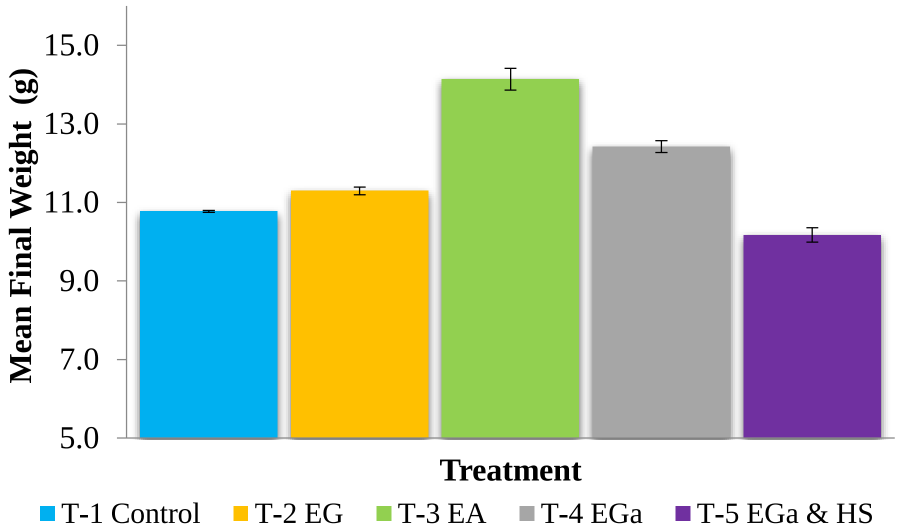



**Figure 48:** Mean final weight of freshwater prawn (*M. rosenbergii*) in different treatments.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.3.2 Mean Weight Gain

The mean weight gain of cultured freshwater prawn were 7.6±0.2, 8.2±0.4, 10.9±0.3, 9.1±0.2 and 7.2±0.4 g in T-1, T-2, T-3, T-4 and T-5 treatments respectively (Table 04). From figure 49, it is clearly said that T-3 (EA) gave significantly better outcome (10.9 g) than control and other treatments ($P<0.05$). In case of T-4 (EGa), the outcome was satisfactory than control and other treatments. The least outcome (7.2 g) was found in T-5 where stocking density was double than other treatments.

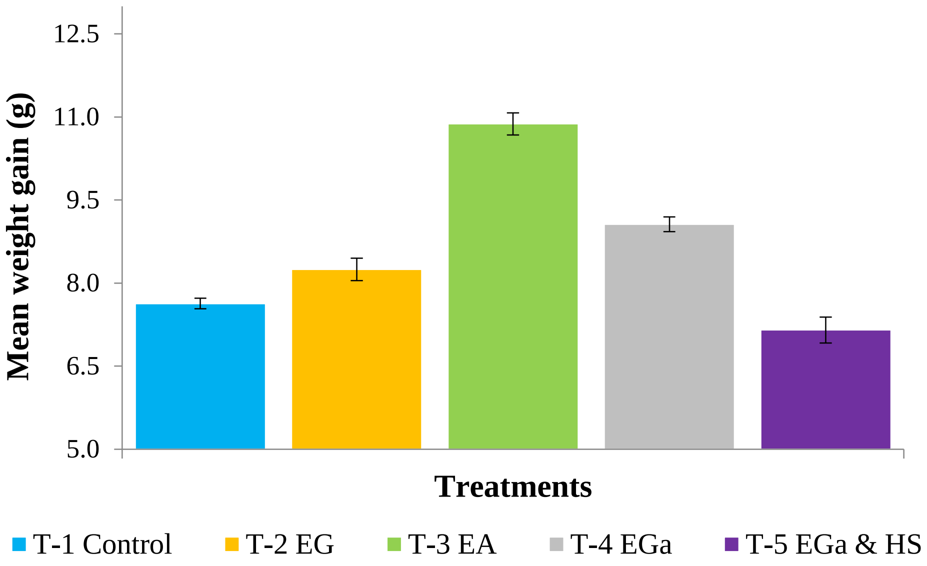



**Figure 49:** Mean final weight gain of freshwater prawn (*M. rosenbergii*) in different treatments.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.3.3 Total Weight Gain

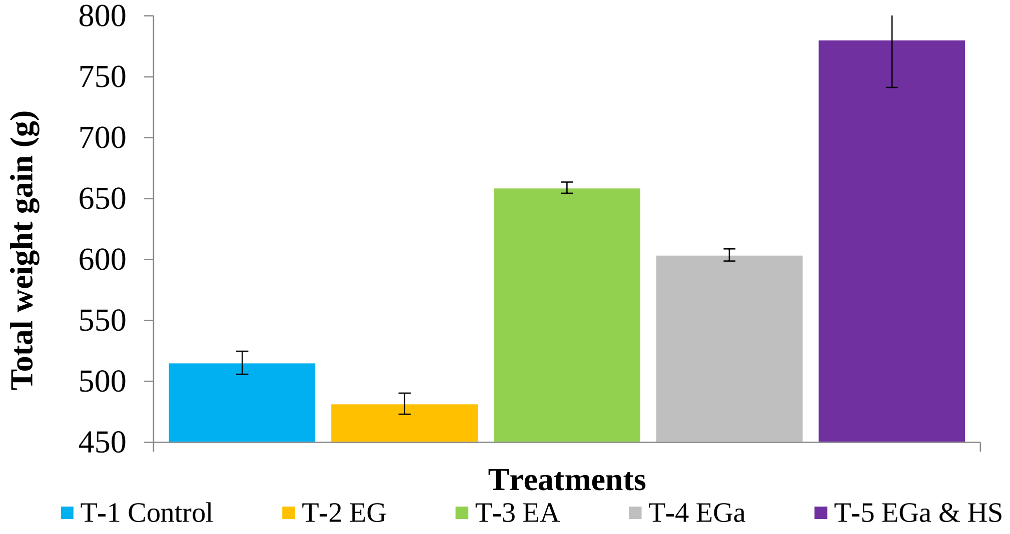



**Figure 50:** Total weight gain of freshwater prawn (*M. rosenbergii*) in different treatments.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

The total weight gain of cultured freshwater prawn were 515.25±16.5, 481.72±14.9, 658.99±7.9, 603.75±8.5 and 780.4±25 g in T-1, T-2, T-3, T-4 and T-5 treatments respectively (Table 04). From the figure 50, it is clearly said that T-3 (EA) gave best outcome (659 g) among all treatments of same stocking density. In case of T-4 (EGa), the outcome was satisfactory than control and other treatments. The least outcome (780 g) was found in T-5 where stocking density was double than other treatments but total weight gain was comparatively less than all treatments.

### 4.3.4 Percentage Weight Gain

The percentage weight gain (%) of cultured freshwater prawn were 242±22.2%, 234.4±27.9%, 297.8±16.2%, 264.6±6.6% and 200.9±27.2% in T-1, T-2, T-3, T-4 and T-5 treatments respectively (Table 04). In this present study best weight gain percentage (297 %) was in T-3 and least (200 %) was in T-5. Extract of Amla gave significantly better than control, T-2 and T-5 ($P<0.05$). Anand *et al.*(2014) performed a research in biofloc on *P. monodon* over a 60 day indoor trial and got maximum 133.71% weight gain. The percentage weight gain is said to be better comparatively aforementioned outcome.

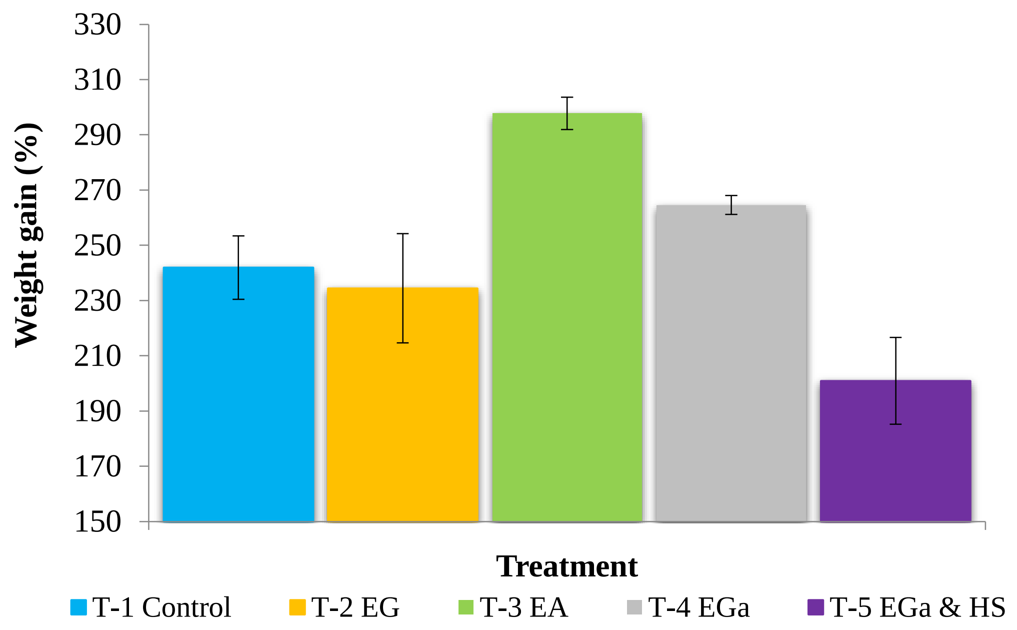



**Figure 51:** Percentage weight gain (%) of freshwater prawn (*M. rosenbergii*) in different treatments. (Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking).

### 4.3.5 Survival Rate (%)

Survival rate is an important parameters for fish culture. It defines the live organism's percentage after completing research timeframe. In this research it was found at 99.5±0.85%, 89.7±1.47%, 91.67±2.24%, 98.5±1.47% and 88.7±1.94% survival rate in T-1, T-2, T-3, T-4 and T-5 treatments respectively (Table 04). Survival rate was significantly better in T-4 and control than other treatments ($P<0.05$). Ballester *et al.* (2017) and Pérez-Fuentes *et al.* (2013) conducted a research in biofloc system on juvenile prawn and found 85.32% rate of survival. Another research conducted by Paul & Rahman (2016) where they got highest 86% survivality in different treatments. In case of normal (50 /$m^2$) and higher stocking (100/$m^2$) survivality were found 73% and 52% respectively (Negrini *et al*., 2017). Another research of stocking densities where 90.83% and 47.92% survivality were revealed for 40 and 80 pieces PL stocking per decimal in pond (Paul *et al.*, 2016). So, it can be said that the findings of this research is fully supported by another different research in biofloc and traditional culture of *M. rosenbergii*.

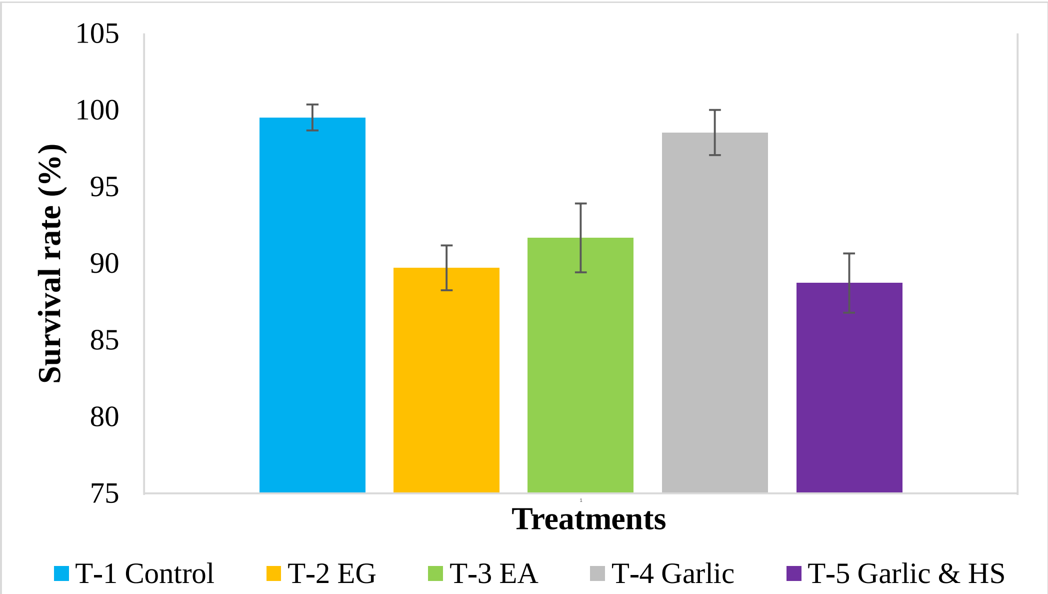



**Figure 52:** Survival rate (%) of freshwater prawn (*M. rosenbergii*) in different treatments.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.3.6 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) is an important parameters for fish culture. It is simply the amount of feed it takes to grow a kilogram (kg) of fish. It helps to estimate the quantity of feed that will be required in the growing cycle. Considering the ratio a farmer can determine the profitability of an aquaculture enterprise. In this research it was found that FCR were 1.5±0.1, 1.6±0.1, 1.2±0.1, 1.3±0.1 and 1.6±0.2 in T-1, T-2, T-3, T-4 and T-5 treatments respectively (Table 04). T-3 (Extract of Amla) gave significantly better feedback than control and other treatments ($P<0.05$). Ballester *et al.* (2017) observed FCR value at 2.25 for *M. rosenbergii* culture in biofloc technolgy. In case of normal (50 /$m^2$) and higher stocking (100/$m^2$) of prawn FCR were found 1.28 and 1.77 respectively (Negrini *et al.*, 2017). The FCR value is better than their findings and that will help to encourage biofloc based culture in farmers and commercial level. The best FCR value (1.2) was found in T-3 and lowest (1.6) was in T-2 and T-5.

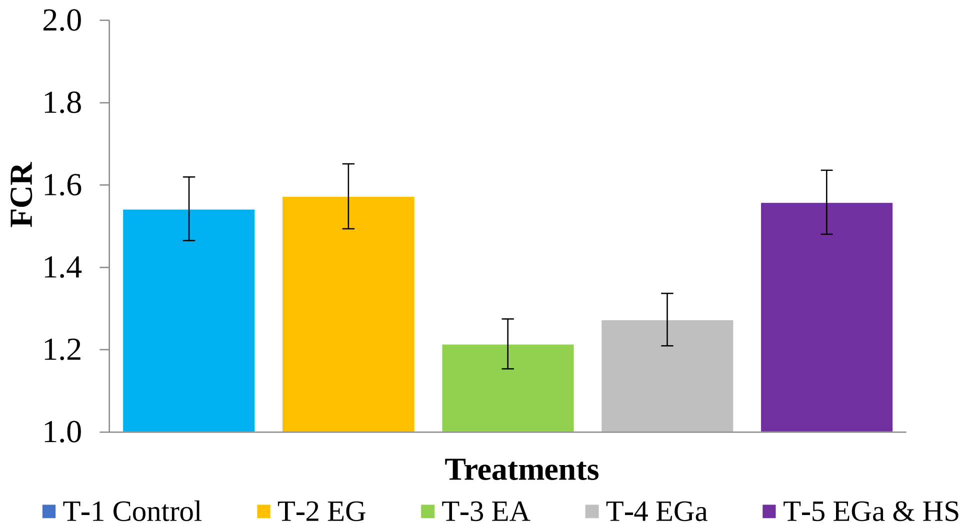



**Figure 53:** Feed conversion ratio of freshwater prawn (*M. rosenbergii*) in different treatments.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.3.7 Specific Growth Rate (SGR)

The specific growth rate of cultured freshwater prawn were 1.8±0.1%, 1.9±0.1%, 2.1%, 1.9% and 1.7±0.2% in T-1, T-2, T-3, T-4 and T-5 treatments respectively (Table

04). Specific growth rate was significantly higher in T-3 than control and other treatments ($P<0.05$). *M. rosenbergii* culture in biofloc technology can be fruitful for national development that we can understand the many more advantage of this environmentally viable technology. The findings of this study is fully supported by Ballester *et al.* (2017) and Paul & Rahman (2016) where SGR were 1.21 and 2.19 (highest in different treatments). In case of normal (50 /$m^2$) and higher stocking (100/$m^2$) of prawn SGR were found 3.28 and 3.1 respectively (Negrini *et al.*, 2017) and partially supports the present results. From the figure we can understand the best SGR value showed in treatment-3 (EA).

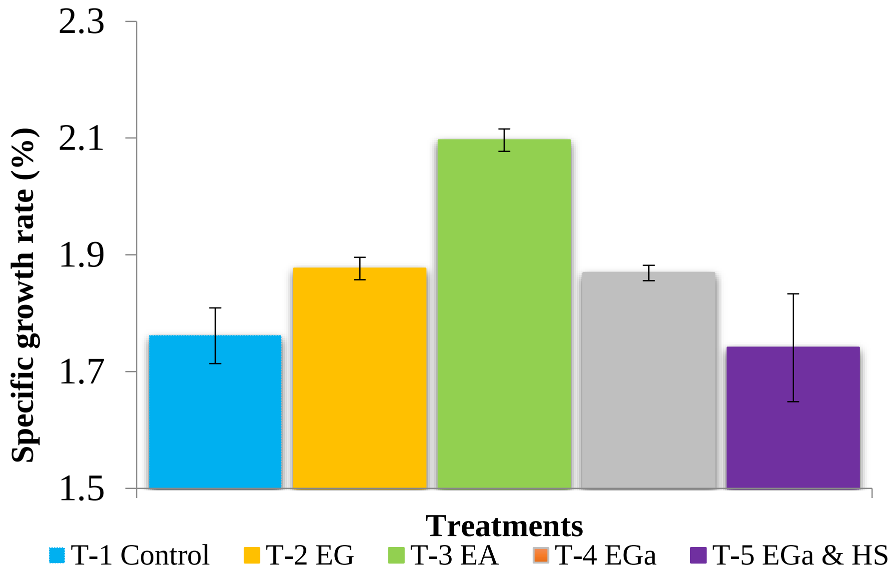



**Figure 54:** Specific growth rate of freshwater prawn (*M. rosenbergii*) in different treatments.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

#### 4.3.8 Reactive Growth Rate (RGR)

The reactive growth rate of cultured freshwater prawn were 343.5±19.9%, 373±34.3%, 433.9±10.1%, 370±5.9% and 339.7±36.7% in T-1, T-2, T-3, T-4 and T-5 treatments respectively (Table 04). The highest result was obtained in treatment-3 and lowest was in treatment-5. The lowest was observed in T-5 that may be caused for higher stocking density. From figure 55, reactive growth rate was close at rate in T-2 and T-4 tanks.

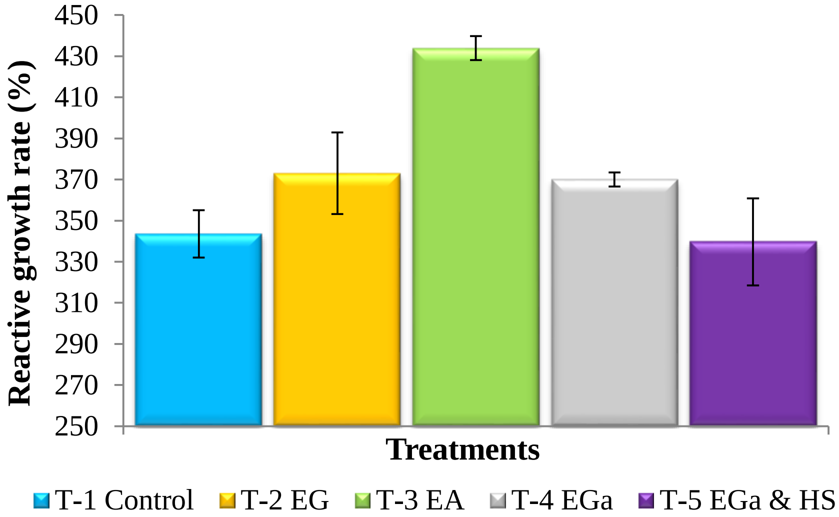



**Figure 55:** Reactive growth rate of freshwater prawn (*M. rosenbergii*) in different treatments.

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic and HS= Higher stocking.

### 4.4 Nutritional Value

Nutritional value refers contents of food and the impact of constituents on body. It relates carbohydrates, fat, protein, mineral, additives, enzymes, vitamins, etc. Nutritional value as part of food quality is the measure of well-balanced ratio of the essential nutrients.

#### 4.4.1 Proximate Composition of Floc and Feed

After analysis it was revealed that commercial feed has nutritional value including 35% crude protein, 41.51% carbohydrate, 10.48% ash, 0.42% acid insoluble ash, 5.79% crude fat and 6.8% crude fiber (Table 05). Cortés *et al.* (2018) performed a study and suggested 35% protein containing commercial feed for getting good results in biofloc production. Sarman *et al.* (2018) told about juvenile nutritional requirements of *M. rosenbergii* in their diet and stated that 35-37% proetin containing feed sholud be provided. Another reseacher got best results in growth performance by using 37% protein containing artificial diet in shrimp culture (Anand *et al.*, 2014).

Proximate analysis of applied feed in the culture period and floc produced in the research duration are given below:

**Table 05:** Proximate composition (% on DM basis) of microbial feed.

| Features | Floc |
|---|---|
| **Moisture** | 11.02 |
| **Dry Matter** | 88.98 |
| **Crude Protein** | 35 |
| **Total Ash** | 10.48 |
| **Acid Insoluble Ash** | 0.42 |
| **Crude Fiber (CF)** | 6.8 |
| **Crude Fat** | 5.79 |
| **Carbohydrate** | 41.51 |

Microbial floc is the main component of biofloc technology. In this study it was found that around 13.1- 13.9 ml/l floc was maintained in the experimental duration (Table 01). After analysis it was revealed that the floc has nutritional value including 21.7% crude protein, 69.1% carbohydrate, 6.77% ash, 0.7% crude fat and 1.7% crude fiber (Figure 56). A research was done on shrimp culture in biofloc and after proximate analysis they concluded that 25.29% crude protein (Rajkumar *et al.*, 2016) was found in floc composition. Anand *et al.* (2014) got this result for P. monodon culture and it was 24.30% crude protein contained in floc. The percentage of crude protein help to enhace the growth and fulfill the demand of daily nutritional requirements.

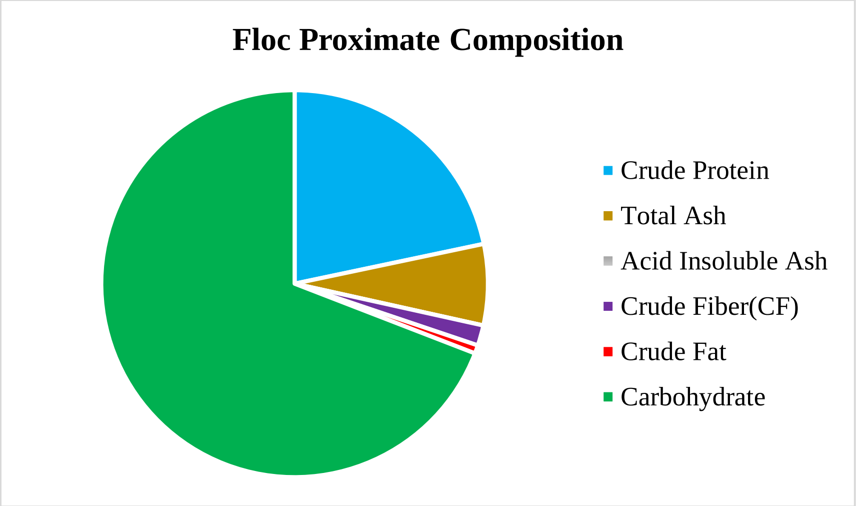



**Figure 56:** Proximate composition of floc produced in the research tanks.

### 4.4.2 Proximate Composition of Cultured Prawn

In a nutritional point of view, the chemical composition of any edible organism is very crucial. The nutritional values of crustaceans rely on their body biochemical ingredients. The proximate composition of the fish varied from species to species and even within the same species. The proximate composition of the present study is given below:

**Table 06:** Proximate composition (% on DM basis) of *M. rosenbergii* cultured in biofloc (Mean).

| Features | Initial | T-1 Control | T-2 EG | T-3 EA | T-4 EGa | T-5 EGa & HS |
|---|---|---|---|---|---|---|
| **Moist-ure** | 77.1 | 77.03 ±1.29 | 81.6 ±2.3 | 79.12 ±.35 | 77.86 ±0.81 | 75.05 ±0.5 |
| **Dry Matter** | 22.83 | 22.97 ±1.29 | 18.4 ±2.3 | 20.88 ±.35 | 22.14 ±0.81 | 24.95 ±0.5 |
| **Crude Protein** | 73.21 | 66.77 ±1.92 | 56.73 ±1.39 | 74.13 ±.68 | 64.1 ±1.34 | 64.35±1.77 |
| **Total Ash** | 14.79 | 22.29 ±0.92 | 23.75 ±0.6 | 17.37 ±1.19 | 21.35 ±1.05 | 22.23±0.75 |
| **AIA** | Nil | Nil | Nil | Nil | Nil | Nil |
| **Crude Fiber** | 0.58 | 0.51 ±0.08 | 0.6 ±0.02 | 0.4 ±.11 | 0.61 ±0.06 | 0.94 ±0.05 |
| **Crude Fat** | 3.15 | 3.28 ±0.27 | 1.72 ±0.29 | 2.63 ±0.25 | 3.05 ±0.26 | 3.38 ±0.33 |
| **Carbo-hydr.** | 8.27 | 7.14 ±2.64 | 17.2 ±1.1 | 5.46 ±0.88 | 10.89 ±0.15 | 9.11 ±0.69 |

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic, HS= Higher stocking and AIA= Acid Insoluble Ash.

Proximate analysis was done on dry matter basis. In the present study, the biochemical analysis of cultured prawn helps to understand the difference of nutritional value from traditional and biofloc culture methods. In this study it was evaluated that highest crude protein was found 74.13±0.68% in T-3 (EA) and lowest was 56.73±1.39% in T-2 (EG) treatment tanks (Table 06). Highest carbohydrate, crude fat, crude fibre and ash were 17.2±1.1 in T-2, 3.38±0.33 in T-5, 0.94±0.05 in T-5 and 23.75±0.6% in T-2 respectively (Table 06). Lowest carbohydrate, crude fat, crude fibre and ash were 7.14±2.64 in T-1, 1.72±0.29 in T-2, 0.41±0.11 in T-3 and 17.37±1.19% in T-3 respectively (Table 06). Asaikkutti *et al.* (2016) evaluated a study on freshwater prawn nutritional value where they found 54.39% crude protein in cultured prawn and another research was performed by Ferdose & Hossain (2011) where they got 74.85% crude protein in cultured *M. rosenbergii*.

It can be concluded that among the culutred prawn with different treatments T-3 tanks prawn were better in protein percentage than other treatments including control environments. So, from the analysis it can be told that there was no very different in protein percentage in normal and biofloc culture. Besides, the highest range of protein content was found in biofloc based cultured prawn for T-3 (EA) tanks.

# CHAPTER V

## SUMMARY AND CONCLUSION

Fish is a vital component of food security and livelihood especially in developing countries of the world. As the world population grow, the need for more food and more fish correspondingly increases. Freshwater prawn have become an important component of global aquaculture both in terms of quantity and value. It is one of the most important species for culture due to superior cultivable attributes such as very fast growth rate, high market demand, hardiness, and its compatibility to grow with cultivable fin fishes. As freshwater prawn culture becomes more intensive, it also becomes less dependent on natural food and more on prepared feeds. Freshwater prawn farming is carried out by small and marginal farmers who are employing a low input level. The research was performed in “Biofloc Lab” at Sher-e-Bangla Agricultural University. The experiment was accomplished to assess the growth performance potentiality of freshwater prawn (*M. rosenbergii*) and evaluate the nutritional value after completing the rearing through biofloc technology.

There were five treatments used in this research with three replications. Treatment-1 was used as control where no microbial floc was observed throughout the research duration. In treatment-2, 3 and 4 stocking density was similar as control tanks but different extracts were applied with feed like Ginger, Amla and Garlic respectively. The extracts were derived from raw products by using biotechnological process.

The most important thing of biofloc based culture is maintaining the water quality parameters properly. The crucial parameters of water quality like Temperature, DO, pH and ammonia ranged from $29.2\pm1$ to $29.4\pm0.9^0$C, $6.2\pm0.56$ to $6.6\pm0.51$ mg/l, $7.9\pm0.2$ to $7.9\pm1.73$, $0.3\pm0.01$ to $0.3\pm0.09$ mg/l respectively among the treatments tanks were considered to optimum level. The remaining parameters of water quality like alkalinity, hardness and TDS were also in optimum level and ranged from $183.4\pm16.66$ to $185.0\pm21.4$ mg/l, $112.9\pm7.63$ to $115.8\pm6.65$ mg/l and $973.1\pm156.9$ to $1062.8\pm119.2$mg/l. In biofloc based culture, volume of microbial floc requirement varies from species to species. In this study on freshwater prawn culture no floc was observed in control tanks and ranged from $13.1\pm2.56$ to $13.9\pm1.92$ ml/l among rest of the treatments.

The highest mean final weight 14.14±0.5g and mean weight gain 10.9±0.3g were noticed in T-3 (EA) tanks. In case of survival rate, the highest rate was observed in T-1 (Control) treatment tanks and lowest was got in T-5 (EGa & HS). Treatment-3 (EA) gave the best specific growth rate (2.1%) and reactive growth rate (433±10.1%). It was found the lowest value for SGR at 1.7±0.2% and RGR at 339.7±36.7% in T-5 treatment tanks.

Feed conversion ratio is an important factor for fish culture. It helps to estimate the quantity of feed that will be required in the growing cycle. The highest FCR was calculated 1.2±0.1 in T-3 (EA) tanks and lowest was in T-2 (EG) and T-5 (Ginger and higher stocking) tanks. The highest protein intake (422.6±21.9g) was seen in T-5 and lowest (265.5±26.1g) was in T-2 tanks.

The highest crude protein 74.13± 0.68% was noticed in T-3 (EA) and lowest 56.73±1.39% was in T-2 (EG) experimental units. Treatment-2 (EG) gave the best percentage in case of total ash (23.75±0.6%) and carbohydrate (17.2±1.1%) contents. Treatment-5 (EGa & HS) gave the best percentage in case of crude fat (3.38±.33%) and crude fiber (0.94±.05%) contents. The lowest rate of crude fiber 0.41±0.11% was found in T-3 (EA) tanks. It's good to say that no acid insoluble ash was noticed in any treatments unit. In biofloc based culture it was revealed that microbial floc have 21.7% crude protein, 69.1% carbohydrate, 6.77% ash, 0.7% crude fat and 1.7% crude fiber that helped meet that nutritional requirements of prawn.

At last, it can be concluded that freshwater prawn (*M. rosenbergii*) culture could be performed in super intensive biofloc technology for getting higher production and meeting the demand of animal protein. Application of Amla with feed (T-3) provided the best outcome in case of growth performance and nutritional value. It can be told that prawn and other fish species can be cultured in this advanced culture system with the help of microbial biotechnology. The results of the research may become an instruction for upcoming researcher and a recommendation for farmer who are interested in this technology in Bangladesh and other countries in the world as well.

# RECOMMENDATIONS

The study was performed on juvenile freshwater prawn and its potentiality on growth performance through biofloc based culture technique. The outcome of the research triggers to move forward with biofloc technology in modern aquaculture era. The methodology and findings may use as a guideline for forthcoming researcher. Based on the results and conclusion the following recommendation might be considered:

- ❖ As freshwater prawn is bottom feeder, so poly or mix culture with freshwater prawn could be practiced for culture.
- ❖ Different strains genotypes of prawn can be used for assessing the growth parameter in biofloc technology.
- ❖ Suitable microbial strain may be identified for rapid growth and production of prawn.
- ❖ Male and female prawn growth can be separately studied for screening of better yield performance.
- ❖ Post-larvae (PL) of *M. rosenbergii* can be used as seed for trial in biofloc technology.
- ❖ Microbial research should be conducted for getting suitable microorganism (probiotic) in biofloc based culture.

# CHAPTER VI

# REFERENCES


Abila, R. O. (2003). Fish trade and food security: Are they reconcilable in lake victoria. Kenya Marine and Fisheries Research Institute. Kisumu, Kenya.

Aftab Uddin, S., Siddique, M. A., Sein, A., Dey, P. K., Nabi, M. U., & Haque, M. A. (2020). First use of biofloc technology for *Penaeus monodon* culture in Bangladesh: Effects of stocking density on growth performance of shrimp, water quality and bacterial growth. *Aquaculture Reports*. **18**.

Ahmad, I., Rani, A. B., Verma, A. K., & Maqsood, M. (2017). Biofloc technology: An emerging avenue in aquatic animal healthcare and nutrition. *Aquacult. Int*. pp.7.

Ahmed, N., Ahammed, F., & Brakel, M. V. (2008). An economic analysis of freshwater prawn (*Macrobrachium rosenbergii*) farming in Mymensingh, Bangladesh. *Journal of the World Aquaculture Society*. pp. 1-22.

Anand, P. S., Kohli, M., Sundaray, J. K., Roy, S. D., Venkateshwarlu, G., Sinha, A., & Pailan, G. H. (2014). Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture*. **418-419**:108-115.

Asaikkutti, A., Bhavan, P. S., Vimala, K., Karthik, M., Cheruparambath, P., & Rajkumar, G. (2016). Comparative studies of the proximate composition of three body parts of wild, cultured and frozen prawn *Macrobrachium rosenbergii* (De Man, 1879). *International Journal of Pure and Applied Zoology*. **4**(1): 85-91.

Ballester, E. L., Marzarotto, S. A., Castro, C. S., Frozza, A., Pastore, I., & Abreu, P. C. (2017). Productive performance of juvenile freshwater prawns *Macrobrachium rosenbergii* in biofloc system. *Aquaculture Research*. pp. 1-8.

Ballester, E. L., Maurente, L. P., Heldt, A., & Dutra, F. M. (2018). Vitamin and mineral supplementation for *Macrobrachium rosenbergii* in biofloc system. *Lat. Am. J. Aquat. Res*. **46**(4): 855-859.

Banu, M. R., Christianus, A., Siraj, S. S., Ikhsan, N. F., & Rajaee, A. H. (2016). Effects of stocking density on growth performance and survival of three male morphotypes in all-male culture of *Macrobrachium rosenbergii* (De Man). *Iranian Journal of Fisheries Sciences*. **15**(2): 738-750.

CABI. (2018). *Macrobrachium rosenbergii* (giant freshwater prawn) (original text by J. Brown). In Invasive Species Compendium. Retrieved from https://www.cabi.org/isc/datasheet/96269

Castro-Nieto, L. M., Castro–Barrera, T., De Lara-Andrade, R., Castro-Mejía, J., & Castro-Mejía, G. (2012). Biofloc systems: A technological breakthrough in aquaculture. *Revista Digital del Departamento El Hombre y su Ambiente*. **1**(1): 1-5.

Chand, B. K. (2000). Effect of aeration on water quality, growth and production of prawn in commercial farming. *Indian J. of Anim. Hlth*. **39**(1): 43-47.

Chandan. (2020, July 3). *Biofloc Fish Farming*. Retrieved from http://www. bioflocfishfarming.com/what-is-tds-in-biofloc-fishfarming/#:~:text=Should%20bring%20TDS%20of%20water,farming%20is%201000%20to%201500.

Cheng, W., & Chen, J. C. (1998). Enterococcus-like infections in *Macrobrachium rosenbergii* are exacerbated by high pH and temperature but reduced by low salinity. *Diseases of Aquatic Organisms*. **34**: 103-108.

Chowdhury, A. H., & Ahmed, R. (2012). Water, sediment and macrophyte quality of some shrimp culture ponds and freshwater ecosystems of Koyra. *Bangladesh J. Bot*. **41**(1): 35-41.

Cortés, D. B., Dosta, M. D., Emerenciano, M. G., Mejía, G. C., Bermúdez, B. S., & Correa, G. V. (2018). Effect on nutritional composition of produced bioflocs with different carbon sources (molasses, coffee waste and rice bran) in biofloc system. *International Journal of Fisheries and Aquatic Studies*. **6**(2): 541-547.

Das, S. K., & Mandal, A. (2018). Biofloc Technology (BFT): An effective tool for remediation of environmental issues and cost effective novel technology in aquaculture. *International Journal of Oceanography & Aquaculture*. **2**(2): 1-8.

Denga, M., Chena, J., Goua, J., Hou, J., Li, D., & Hea, X. (2018). The effect of different carbon sources on water quality, microbial community and structure of biofloc systems. *Aquaculture*. **482**: 103-110.

Devi C. A., & Kurup, B. (2015). Biofloc Technology: An Overview and its application in animal food industry. *International Journal of Fisheries and Aquaculture Sciences*. **5**(1): 1-20.

DoF. (2016). National fish week compendium (In Bengali). Department of Fisheries, Dhaka. Ministry of Fisheries and Livestock, Government of Bangladesh.

DoF. (2018). Yearbook of Fisheries Statistics of Bangladesh 2017-18. Department of Fisheries, Bangladesh, Ministry of Fisheries.

El-Sayed, A. F. M. (2020). Technological Innovations. **In**: Tilapia Culture (Second ed., pp. 297-328). Academic Press. Retrieved from http://doi.org/10.1016/ B978-0-12-816509-6.00013-6.

Emerenciano, M. G., Martínez-Córdova, L. R., Martínez-Porchas, M., & Miranda-Baeza, A. (2017). Biofloc Technology (BFT): A tool for water management in aquaculture. Intech. Retrieved from http://dx.doi.org/10.5772/66416

Emerenciano, M., Cuzon, G., Lopez-Aguiar, K., Norena- Bauoso, E., Mascaro, M., & Gaxiola, G. (2011). Biofloc meal pellet and plant based diet as an alternative nutrion for shrimp under limited water exchange systems. World Aquaculture Society Meeting 2011. Natal, RN, Brazil.

FAO. (2018). The state of world fisheries and aquaculture (opportunities and challenges). Rome, Italy: Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/3/I9553EN/i9553en.pdf

FAO. (2020). The state of world fisheries and aquaculture (sustainability in action). Rome, Italy: Food and Agriculture Organization of the United Nations. Retrieved from https://doi.org/10.4060/ca9229en

Fatema, K., Wahab, M. A., Pervin, R., Khan, M. S., & Roy, H. C. (2011). Comparison of Growth and Production Performance between Male and Female Giant Freshwater Prawn in Combination with Mola. *J. Environ. Sci. & Natural Resources*. **4**(2): 137-142.

Ferdose, A., & Hossain, M. B. (2011). Nutritional value of wild, cultured and frozen prawn Macrobrachium rosenbergii (De Man, 1879). *International Journal of Natural Sciences*. **1**(2): 52-55.

Furtado, P. S., Poersch, L. H., & Wasielesky Jr, W. (2015). The effect of different alkalinity levels on *Litopenaeus vannamei* reared with biofloc technology (BFT). *Aquacult. Int*. **23**: 345-358.

Gain, D., Mistri, N. A., Mahfuj, M. E., & Sohag, M. H. (2015). A comparative study on freshwater prawn ( *Macrobrachium rosenbergii* ) farming using wild source and hatchery produced post larvae (PL). *International Journal of Fisheries and Aquatic Studies*. **2**(5): 137-141.

Halima, M. A., Nahar, S., & Nabi, M. M. (2019). Biofloc technology in aquaculture and its potentiality: A review. *Int. J. Fish Aquat. Stud*. **7**(5): 260-266.

Hossain, M. A., & Islam, M. S. (2006). Optimization of stocking density of freshwater prawn *Macrobrachium rosenbergii* (De Man) in carp polyculture in Bangladesh. *Aquaculture Research*. **37**: 994-1000.

Hussain, M. G. (2010). Freshwater fishes of Bangladesh: Fisheries, biodiversity and habitat. *Aquatic Ecosystem Health & Management*. **13**(1): 85-93.

Jaganmohan, P., & Kumari, L. (2018). Production of decreased calcium waters for shrimp (*Litopenaeus vannamei*) farming using EDTA. *International Journal of Recent Scientific Research*. **9**(6): 27336-27339.

Karim, M. F., Zhang, X., & Li, R. (2019). Dynamics of shrimp farming in the southwestern coastal districts of Bangladesh using a shrimp yield dataset (syd) and landsat satellite archives. *Sustainability*. **11**.

Kunda, M., Dewan, S., Uddin, M. J., Kabir, S., & Uddin, M. S. (2008). Length-weight relationship, condition factor and relative condition factor of *Macrobrachium rosenbergii* in Rice Fields. *Asian Fisheries Science*. **21**: 451-456.

Lalrinsanga, P. L., Pillai, B. R., Patra, G., Mohanty, S., Naik, N. K., & Sahu, S. (2012). Length weight relationship and condition factor of giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879) based on developmental stages, culture stages and sex. *Turkish Journal of Fisheries and Aquatic Sciences*. **12**: 917-924.

Manan, H., Moh, J. H., Kasan, N. A., Suratman, S., & Ikhwanuddin, M. (2017). Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system. *Applied Water Science*. **7**: 2437–2446.

Mannan, M., Islam, M. S., Rimi, R. H., Suravi, & Meghla, N. T. (2012). Impact of water quality on fish growth and production in semi-intensively managed aquaculture farm. *Bangladesh J. Environ. Sci*. **23**: 108-113.

Martínez, K. C., Monroy-Dosta, M. C., Partida, A. H., Hernández-Vergara, M. P., Cortés, D. B., & García2, E. L. (2020). A review of the use of probiotics in freshwater prawn (*Macrobrachium* sp.) culture in biofloc systems. *Latin American Journal of Aquatic Research*. **48**(4):518-528.

Motoh, H., & Kuronuma, K. (1980). Field Guide For The Edible Crustacea Of The Philippines. Iloilo, Philippines. Southeast Asian Fisheries Development Centre (SEAFDEC). Retrieved from https://web.archive.org/web/ 20171201072729/ https://repository.seafdec.org.ph/bitstream/handle/10862/152/ediblecrustacea. pdf;jsessionid=BFAC47AB199C35EDB0062CE423639508.jvm1?sequence= 1

Negrini, C., Castro, C. S., Guimarães, A. B., Frozza, A., Kracizy, R. O., & Ballester, E. L. (2017). Stocking density for freshwater prawn *Macrobrachium rosenbergii* (Decapoda, Palaemonidae) in biofloc system. *Latin American Journal of Aquatic Research*. **45**(5): 1-17.

Negrini, C., Castro, C. S., Guimarães, A. T., Frozza, A., Kracizy, R. O., & Ballester, E. L. (2017). Stocking density for freshwater prawn *Macrobrachium rosenbergii* (Decapoda, Palaemonidae) in biofloc system. *Lat. Am. J. Aquat. Res*. **45**(5): 891-899.

New, M. B. (1995). Status of freshwater prawn farming : A review. *Aquaculture Research*. pp. 1-54.

Niu, C., Lee, D., Goshima, S., & Nakao, S. (2003). Effect of temperature on food consumption, growth and oxygen consumption of freshwater prawn (*Macrobrachium rosenbergii*) postlarvae. *Aquaculture research*. **34**: 501-506.

Ogello, E. O., Musa, S. M., Aura, C. M., Abwao, J. O., & Munguti, J. M. (2014). An appraisal of the feasibility of tilapia production in ponds using biofloc technology: A review. *International Journal of Aquatic Science*. **5**(1): 21-39.

Paul, P., & Rahman, M. A. (2016). Growth performance of fresh water prawn *Macrobrachium rosenbergii* under different supplemental feeding options. *International Journal of Fisheries and Aquatic Studies*. **4**(2): 203-207.

Paul, P., Rahman, M. A., Hossain, M. M., Islam, M. S., Mondal, S., & Haq, M. M. (2016). Effect of stocking density on the growth and production of freshwater prawn (*Macrobrachium rosenbergii*). *International Journal of Fisheries and Aquaculture Sciences*. **6**(1): 77-86.

Pérez-Fuentes, J. A., Pérez-Rostro, C. I., & Hernández-Vergara, M. P. (2013). Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with the biofloc system. *Aquaculture*. pp. 105–110.

Pérez-Rostro, C. I., Pérez-Fuentes, J. A., & Hernández-Vergara, M. P. (2014). Biofloc, a technical alternative for culturing Malaysian prawn *Macrobrachium rosenbergii*. **In**: Sustainable Aquacultue Techniques (pp. 87-104). Retrieved from http://dx.doi.org/10.5772/57501

Prajith, K. K. (2011). Application of biofloc technology (BFT) in the nursery rearing and farming of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man). Ph. D. thesis, Cochin University of Science and Technology, Kochi, India.

Rajeevan, R., Edirisinghe, U., & Athauda, A. (2018). Length-weight relationships and condition factor of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879) in five perennial reservoirs in Northern Province, Sri Lanka. *International Journal of Fisheries and Aquatic Studies*. **6**(5): 283-287.

Rajkumar, M., Pandey, P. K., Aravind, R., Vennila, A., Bharti, V., & Purushothaman, C. S. (2016). Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). *Aquaculture Research*. **47**: 3432–3444.

Rosenberry, B. (2010). Controlling pH in biofloc ponds. Retrieved from http:// www.shrimp news.com/ free reports folder/ pH control biofloc ponds.

Sarman, V., Vishal, R., Mahavadiya, D., & Sapra, D. (2018). Nutritional aspect for freshwater prawn (*Macrobrachium rosenbergii*) farming. *International Journal of Fauna and Biological Studies*. **5**(2): 172-175.

Serra, F. P., Gaona, C. P., Furtado, P. S., Poersch, L. H., & Wasielesky Jr, W. (2015). Use of different carbon sources for the biofloc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. *Aquacult. Int*. **23**(6).

Shamsuzzaman, M. M., Xiangin, X., & Islam, M. M. (2016). Legal status of Bangladesh fisheries: Issue and Responses. *Indian Journal of Geo Marine Sciences*. **45**(11): 1474-1480.

Sontakke, R., & Haridas, H. (2018). Economic viability of biofloc based system for the nursery rearing of Milkfish (*Chanos chanos*). *International Journal of Current Microbiology and Applied Sciences*. **7**(8): 2960-2970.

# APPENDICES

**Appendix I:** Weight of cultured *M. rosenbergii* in biofloc at 14 days (2 weeks) interval.

| Treat-ments | Rep. | Weight (g) at Sampling Schedule (per 2 weeks) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **21/08/2020** | **04/09/2020** | **18/09/2020** | **02/10/2020** | **16/10/2020** | **30/10/2020** |
| T-1 (Control) | 1 | 3.13 | 7.90 | 8.70 | 9.80 | 10.50 | 11.20 |
| T-1 (Control) | 2 | 3.34 | 8.10 | 8.95 | 10.50 | 11.05 | 11.45 |
| T-1 (Control) | 3 | 2.96 | 7.65 | 8.40 | 9.20 | 10.14 | 10.50 |
| T-2 (EG) | 1 | 3.03 | 6.40 | 7.50 | 8.98 | 10.93 | 11.60 |
| T-2 (Ginger | 2 | 3.32 | 5.88 | 6.55 | 8.45 | 9.76 | 10.40 |
| T-2 (EG) | 3 | 2.78 | 6.85 | 8.44 | 10.10 | 10.80 | 11.00 |
| T-3 (EA) | 1 | 3.23 | 6.40 | 9.60 | 9.80 | 13.80 | 15.65 |
| T-3 (EA) | 2 | 3.12 | 7.34 | 9.95 | 11.43 | 14.52 | 14.72 |
| T-3 (EA) | 3 | 3.43 | 5.52 | 8.92 | 9.60 | 12.95 | 15.50 |
| T-4 (EGa) | 1 | 3.32 | 7.00 | 10.20 | 11.40 | 13.50 | 14.85 |
| T-4 (EGa) | 2 | 3.42 | 7.85 | 11.21 | 11.61 | 12.23 | 13.35 |
| T-4 (EGa) | 3 | 3.33 | 6.34 | 9.65 | 10.11 | 12.54 | 13.05 |
| T-5 (EGa & HS) | 1 | 2.72 | 6.10 | 8.90 | 10.80 | 11.00 | 11.22 |
| T-5 (EGa & HS) | 2 | 3.42 | 7.10 | 8.95 | 10.55 | 11.20 | 11.40 |
| T-5 (EGa & HS) | 3 | 2.92 | 5.22 | 7.87 | 8.73 | 10.23 | 10.80 |

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic, HS= Higher stocking, Rep.= Replication

**Appendix II:** Proximate composition of stocking juvenile *M. rosenbergii* and cultured *M. rosenbergii* in biofloc technology.

| **Features** | **Rep** | **Mois.** | **DM** | **CP** | **Total Ash** | **AIA** | **Crude Fiber** | **Crude Fat** | **Carb.** |
|---|---|---|---|---|---|---|---|---|---|
| Stocking | | 77.17 | 22.83 | 73.21 | 14.79 | Nil | 0.58 | 3.15 | 8.27 |
| T-1 (Control) | 1 | 77.03 | 22.97 | 66.77 | 22.03 | Nil | 0.51 | 3.28 | 7.41 |
| | 2 | 78.32 | 21.68 | 64.85 | 21.53 | Nil | 0.43 | 3.55 | 9.64 |
| | 3 | 75.74 | 24.26 | 68.69 | 23.33 | Nil | 0.59 | 3.01 | 4.38 |
| T-2 (EG) | 1 | 81.6 | 18.4 | 56.73 | 23.74 | Nil | 0.6 | 1.72 | 17.21 |
| | 2 | 79.3 | 20.7 | 58.12 | 23.15 | Nil | 0.62 | 2.01 | 16.1 |
| | 3 | 83.9 | 16.1 | 55.34 | 24.35 | Nil | 0.58 | 1.43 | 18.3 |
| T-3 (EA) | 1 | 79.12 | 20.88 | 74.13 | 17.71 | Nil | 0.41 | 2.63 | 5.12 |
| | 2 | 78.77 | 21.23 | 73.45 | 18.35 | Nil | 0.51 | 2.88 | 4.81 |
| | 3 | 79.47 | 20.53 | 74.81 | 16.05 | Nil | 0.3 | 2.38 | 6.46 |
| T-4 (EGa) | 1 | 77.86 | 22.14 | 64.1 | 21.52 | Nil | 0.61 | 3.05 | 10.72 |
| | 2 | 78.67 | 21.33 | 65.44 | 20.23 | Nil | 0.55 | 2.79 | 10.99 |
| | 3 | 77.05 | 22.95 | 62.76 | 22.31 | Nil | 0.67 | 3.31 | 10.95 |
| T-5 (EGa & HS) | 1 | 75.05 | 24.95 | 64.35 | 22.39 | Nil | 0.98 | 3.38 | 8.9 |
| | 2 | 75.55 | 24.45 | 66.12 | 21.41 | Nil | 0.88 | 3.05 | 8.54 |
| | 3 | 74.55 | 25.45 | 62.58 | 22.88 | Nil | 0.95 | 3.71 | 9.88 |

*[Rep.= Replication, DM= Dry Matter, CP= Crude Protein, AIA= Acid Insoluble Ash, Carb.= Carbohydrate]

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic, HS= Higher stocking, Rep.= Replication

**Appendix III:** Feed given and rearranging schedule of freshwater prawn at two weeks interval.

| **Treatments** | **Feed given to Prawn (g) weekly basis** | | | | |
|---|---|---|---|---|---|
| | **1.0%** | **1.5%** | **1.5-2%** | **1.7-2.5%** | **2.3-2.5%** |
| | **04/09/20** | **18/09/20** | **02/10/20** | **16/10/20** | **30/10/20** |
| T-1 (Control) | 29.80 | 112.81 | 165.65 | 233.24 | 249.90 |
| T-1 (Control) | 31.80 | 115.67 | 170.41 | 249.90 | 262.99 |
| T-1 (Control) | 28.18 | 109.24 | 159.94 | 218.96 | 241.33 |
| T-2 (EG) | 28.85 | 91.39 | 142.80 | 213.72 | 312.16 |
| T-2 (EG) | 31.61 | 83.97 | 124.71 | 201.11 | 232.29 |
| T-2 (EG) | 26.47 | 97.82 | 160.70 | 240.38 | 287.88 |
| T-3 (EA) | 30.75 | 91.39 | 182.78 | 186.59 | 302.16 |
| T-3 (EA) | 29.70 | 104.82 | 189.45 | 217.63 | 317.93 |
| T-3 (EA) | 32.65 | 78.83 | 169.84 | 182.78 | 283.55 |
| T-4 (EGa) | 31.61 | 99.96 | 145.66 | 217.06 | 257.04 |
| T-4 (EGa) | 32.56 | 112.10 | 160.08 | 276.32 | 232.86 |
| T-4 (EGa) | 31.70 | 90.54 | 137.80 | 240.62 | 238.76 |
| T-5 (EGa & HS) | 49.50 | 166.53 | 242.97 | 334.15 | 460.46 |
| T-5 (EGa & HS) | 62.24 | 193.83 | 244.34 | 326.42 | 468.83 |
| T-5 (EGa & HS) | 53.14 | 142.51 | 214.85 | 270.11 | 409.61 |

**Appendix IV:** Protein intake (g) by *M. rosenbergii* at weekly basis during experiment.

| **Treatments** | **P % in feed** | **Protein intake (g) during research** | | | | |
|---|---|---|---|---|---|---|
| | | **14** | **28** | **42** | **56** | **70 days** |
| T-1 (Control) | 35 | 10.4 | 39.5 | 58.0 | 81.6 | 87.5 |
| T-1 (Control) | 35 | 11.1 | 40.5 | 59.6 | 87.5 | 92.0 |
| T-1 (Control) | 35 | 9.9 | 38.2 | 56.0 | 76.6 | 84.5 |
| T-2 (EG) | 35 | 10.1 | 32.0 | 50.0 | 74.8 | 109.3 |
| T-2 (EG) | 35 | 11.1 | 29.4 | 43.6 | 70.4 | 81.3 |
| T-2 (EG) | 35 | 9.3 | 34.2 | 56.2 | 84.1 | 100.8 |
| T-3 (EA) | 35 | 10.8 | 32.0 | 64.0 | 65.3 | 105.8 |
| T-3 (EA) | 35 | 10.4 | 36.7 | 66.3 | 76.2 | 111.3 |
| T-3 (EA) | 35 | 11.4 | 27.6 | 59.4 | 64.0 | 99.2 |
| T-4 (EGa) | 35 | 11.1 | 35.0 | 51.0 | 76.0 | 90.0 |
| T-4 (EGa) | 35 | 11.4 | 39.2 | 56.0 | 96.7 | 81.5 |
| T-4 (EGa) | 35 | 11.1 | 31.7 | 48.2 | 84.2 | 83.6 |
| T-5 (EGa & HS) | 35 | 17.3 | 58.3 | 85.0 | 117.0 | 161.2 |
| T-5 (EGa & HS) | 35 | 21.8 | 67.8 | 85.5 | 114.2 | 164.1 |
| T-5 (EGa & HS) | 35 | 18.6 | 49.9 | 75.2 | 94.5 | 143.4 |

**Appendix V:** Lipid intake (g) by *M. rosenbergii* at weekly basis during experiment.

| Treatments | L%in feed | Protein intake (g) during research | | | | |
|---|---|---|---|---|---|---|
| | | 14 | 28 | 42 | 56 | 70 days |
| T-1 (Control) | 5.79 | 1.7 | 6.5 | 9.6 | 13.5 | 14.5 |
| T-1 (Control) | 5.79 | 1.8 | 6.7 | 9.9 | 14.5 | 15.2 |
| T-1 (Control) | 5.79 | 1.6 | 6.3 | 9.3 | 12.7 | 14.0 |
| T-2 (EG) | 5.79 | 1.7 | 5.3 | 8.3 | 12.4 | 18.1 |
| T-2 (EG) | 5.79 | 1.8 | 4.9 | 7.2 | 11.6 | 13.4 |
| T-2 (EG) | 5.79 | 1.5 | 5.7 | 9.3 | 13.9 | 16.7 |
| T-3 (EA) | 5.79 | 1.8 | 5.3 | 10.6 | 10.8 | 17.5 |
| T-3 (EA) | 5.79 | 1.7 | 6.1 | 11.0 | 12.6 | 18.4 |
| T-3 (EA) | 5.79 | 1.9 | 4.6 | 9.8 | 10.6 | 16.4 |
| T-4 (EGa) | 5.79 | 1.8 | 5.8 | 8.4 | 12.6 | 14.9 |
| T-4 (EGa) | 5.79 | 1.9 | 6.5 | 9.3 | 16.0 | 13.5 |
| T-4 (EGa) | 5.79 | 1.8 | 5.2 | 8.0 | 13.9 | 13.8 |
| T-5 (EGa & HS) | 5.79 | 2.9 | 9.6 | 14.1 | 19.3 | 26.7 |
| T-5 (EGa & HS) | 5.79 | 3.6 | 11.2 | 14.1 | 18.9 | 27.1 |
| T-5 (EGa & HS) | 5.79 | 3.1 | 8.3 | 12.4 | 15.6 | 23.7 |

Here, EG= Extract of Ginger, EA= Extract of Amla, EGa= Extract of Garlic, HS= Higher stocking, Rep.= Replication.