

ANTI BIOFOULING EFFECT OF PLANT EXTRACTS AGAINST PRAWN CULTURE POND ASSOCIATED BIOFOULING

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Biotechnology

By

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Ponna Monika Venkata Sivadurga Nagavalli (38770012) who carried out the project entitled “**Anti biofouling effect of plant extracts against prawn culture pond associated biofouling**” under our supervision from December 2020 to March 2021.

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I, Ponna Monika Venkata Sivadurga Nagavalli (38770012), hereby declare that the project report entitled “**Anti biofouling effect of plant extracts against prawn culture pond associated biofouling**” done by me under the guidance of **Dr. S. Karthick Raja Namasivayam** at **Sathyabama Institute Of Science and Technology, Chennai** is submitted in partial fulfillment of the requirement for the award of Bachelor of Science degree in Biotechnology.

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ABSTRACT

Biofouling or Biofilm is a dense mass of heterogenous group of cells adhere on the solid surface known to cause severe environmental and health effects. Various chemical agents as anti biofouling agents are utilized to control biofouling associated with various sources. Due to poor efficacy and adverse toxicity, biofouling controlling agents of chemical origin, biological based anti biofouling agents are new gained recent attention.

In this present study anti biofouling agents derived from herbal plants neem and turmeric were tested against biofouling or biofilm network associated prawn culture ponds. Water sample collected from prawn culture pond reveals more bacterial population and coliform count. Biofouling efficacy represented as percentage (%) was also found to be more. Anti biofouling efficacy using respective plant extracts shows notable reduction of biofouling development. Further studies will be helpful to utilize the herbal plant based materials as an effective anti biofouling efficacy.

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LIST OF ABBREVIATIONS

D.O	- Dissolved Oxygen
ppm	- Parts per Million
ppt	- Precipitate
PAO1	- <i>Pseudomonas aeruginosa</i>
AgNPs	- Silver Nano particles
CO ₃	- Carbonate
HCO ₃	- Bicarbonate
K	- Potassium
Ca	- Calcium
Mg	- Magnesium
NH ₄	- Ammonium
NO ₂	- Nitrite
NO ₃	- Nitrate
H ₂ S	- Hydrogen Sulphide
SiO ₂	- Silicate
PO ₄	- Phosphate
Fe	- Iron
Cl ₂	- Chlorine
TVC	- Total Viable Count

- MPN - Most Probable Number
- CFU - Colony Forming Unit
- EMB Agar - Eosin Methylene Blue Agar
- ELISA - Enzyme Linked Immuno Sorbent Assay
- SEM - Scanning Electron Microscopy
- GCMS - Gas Chromatography Mass Spectrometry
- HPLC - High Performance Liquid Chromatography

CHAPTER 1

INTRODUCTION

1.1 AQUACULTURE

Aquaculture is breeding, raising, and harvesting fish, shellfish, and aquatic plants. Basically, it's farming in water. Aquaculture is additionally referred to as aqua farming, is that the farming of fish, crustaceans, mollusks, aquatic plants, algae, and other organisms. Aquaculture involves cultivating freshwater and saltwater populations under controlled conditions, and should be contrasted with commercial fishing. Aquaculture is that the controlled process of cultivating aquatic organisms, especially for human consumption. It's an identical concept to agriculture, but with fish rather than plants or livestock. The seafood that you simply find at your local grocery is perhaps going labeled as farmed fish. Aquaculture can happen everywhere in the planet, and it does: in coastal ocean waters, freshwater ponds and rivers, and even ashore in tanks. Aquaculture has the potential to reinforce the health of our planet and therefore the health of our population, as long because it is completely during a manner that's environmentally friendly, socially responsible, and considers food safety and animal welfare.

1.2 IMPORTANCE OF AQUACULTURE

Given that overfishing of our oceans and other natural resources is continuously increasing year over year, humans need alternate sources for seafood to feed the planet's ever-growing population. "Unfortunately, the days of the ocean's natural productivity providing for the planet is over. Wild fish are exploited for generations. Some estimate that the annual catch of edible marine protein has already passed its peak. The oceans cannot naturally provide the demand for seafood" (Positive Aquaculture Awareness). Aquaculture is that the tool to fill within the gap of seafood supply. Farming fish responsibly and sustainably is that the solution to

providing future generations with access to healthy and environmentally friendly protein options.

Not only is aquaculture necessary, it's also a sustainable option for consumers, especially as compared to other farmed proteins. Seafood is extreme resource efficient — it's the absolute best protein retention compared to chicken, pork and beef. It also has rock bottom feed conversion ratio among an equivalent sorts of protein. Aquaculture has lower greenhouse emissions than other sorts of farming.

1.3 IMPORTANCE OF WATER QUALITY IN AQUACULTURE

Water carries nutrients to all or any cells in our body and oxygen to our brain.

Water allows the body to soak up and assimilate minerals, vitamins, amino acids, glucose and other substances. Water flushes out toxins and waste. Water helps to manage body heat.

Water carries nutrients and oxygen to all cells. Water quality is that the most vital factor that affects prawn health and performance in aquaculture production systems. Different Vannamei species have different and specific range of water quality aspects like pH, oxygen concentration, temperature, salinity, hardness, etc. within which they will survive, grow and reproduce.

The fish perform all its physiological activities within the water – breathing, excretion of waste, feeding, maintaining salt balance and reproduction.

Thus, water quality is that the determining factor on the success or failure of an aquaculture operation.

Water quality may be a critical factor when culturing any aquatic organism.

Optimal water quality varies by species and must be monitored to make sure growth and survival. The standard of the water within the production systems can significantly affect the organism's health and thus the prices associated with getting a product to the market. Water quality parameters that are commonly monitored within the aquaculture industry include temperature, dissolved oxygen,

pH, alkalinity, hardness, ammonia, and nitrites. Counting on the culture system, CO₂, chlorides, and salinity can also be monitored. Some parameters like alkalinity and hardness are fairly stable, but others like dissolved oxygen and pH fluctuate daily. It is important to determine a uniform water quality testing protocol for your particular situation. Know the tolerance range for your culture species, establish critical levels, and be prepared to act if a drag occurs.

Dissolved oxygen is one among the foremost important parameters in aquaculture. Maintaining good levels of Dissolved Oxygen in the water is essential for successful production since oxygen features an immediate influence on feed intake, disease resistance and metabolism.

A sub-optimal level of dissolved oxygen is extremely stressful for shrimp. Lower levels (3ppm) of DO end in slower growth and decreased immune reaction and levels below 1ppm are often lethal. It is therefore important to keep DO levels in aquaculture systems above 4ppm.

1.4 BIOFOULING

Biofouling or biological fouling is the undesirable aggregation of microbes, plants, algae and animals on submerged structures. If biofouling occurs on the surfaces of living organisms it is called epibiosis. A biofilm forms when certain microorganisms adhere to the surface of some object during a moist environment and start to breed. The microorganisms form an attachment to the surface of the object by secreting a slimy, glue-like substance.

1.5 PRAWN CULTURE

In terms of value the prawn culture of India rank highest. Prawns are considerable value and play a serious role in exchange. India has always been among the topmost prawn producing countries within the world. Freshwater prawn farm is an aquaculture business designed to boost and produce freshwater prawn for human

consumption. All farmed freshwater prawns today belong to the genus *Macrobrachium*.

1.5.1 PRAWN CULTURE POND

There is no standard design for a shrimp rearing pond. Present day farming practices still heavily believe on the experience of individual farmers, financial capability prevailing at the location.

An ideal shrimp farm may be a complex establishment consisting of:

- (a) Various size ponds for nursery and grow-out.
- (b) Water control structures including embankments, supply and drainage canals and sluice gates
- (c) Support facilities like roads, bridges, quarters, workshops and warehouses etc.

Careful layout of the described facilities and appropriate structural design in reference to the physical features of the area to make sure the smooth and effective operational management.

1.5.1.1 Size and Shape of Culture ponds

Rectangular or square ponds are appropriate for shrimp culture. The longest axis of a pond should be parallel to the prevailing wind direction. This facilitates water movement generated by wind action thereby increasing dissolved oxygen within the water and minimising water temperature fluctuations in summer or warmer months.

The breadth of a pond depends largely on the aim and therefore the operational system employed.

The rearing pond must have a minimum depth of 1.0 meter. Most traditional brackishwater ponds for shrimp farming are relatively shallow. To satisfy depth requirement, a ditch is made

along the dike or a central canal between two opposite sides of the pond. The average depth is 1.0-1.2 meters and depth of the platform is 30-60cm.

Such pond design with peripheral ditches and central platform affords several benefits:

1. The ditch provide better living conditions during hot weather.
2. The shallow, centrally located platform serves as growth area for the natural food organisms.
3. The ditch also serves as harvesting canal.

1.5.1.2 Dikes

Dikes don't only function as boundaries to point pond size and shape but also function to carry water within the pond also as protecting other farm facilities from flood. Diking materials must preferably be tested for load bearing capabilities and compatability. In some cases where the quality of the soil is inferior for diking, other materials, viz concrete or clay must be used as core materials to be placed at the pond bottom. Design and construction of embankment must be based on sound engineering principles and economic feasibility. A typical design of perimeter dikes facing the ocean or a river.

1.5.1.2.1 Height of dike

Coastal soil used as diking material usually shrinks initially. As such, the height of perimeter dike should have a free board of 0.6-0.7 meter above the desired water depth. Free board allowance is determined from the occurrence and frequency of flood levels over a period from 5-15 years at the farm site.

1.5.1.2.2 Slope

The slope of perimeter dike is maintained at average ratio of 2:1 to 3:1. Dikes with steep slopes are always subjected to erosion and require higher maintenance cost. Slope of a dike also highly depends on soil quality.

1.5.1.3 Supply and drainage canal

Not all shrimp farms are located on the brink of the coast or estuaries. For those that are located far away from the water sources, it is necessary to construct supply and drainage canals.

Conceptually, a shrimp pond must possess separate canals for drainage and provide and for avoiding probable contamination of the water system. Both supply and drainage canals would likewise function as water level control within the pond and as temporary holding areas for shrimps. It is important that the siting of the canal systems takes advantage of the natural waterways within the proposed site.

1.5.1.4 Water control gate

When designing a sluice gate, it's essential to think about tidal fluctuations and gravity so as to make sure the effective control of the inflow and outflow of water within a given period of time.

The water gates are classified consistent with function as primary (main) gate or secondary gate. Main gates are strategically situated at the perimeter and are usually constructed or reinforced concrete. These are the main structure controlling the quantity of water for distribution to shrimp farm.

Irrespective of the material to be used to construct for the water gates, the following requirements should be met

- a. A gate should have adequate capacity for the required amount of water to be taken in or drained out
- b. A gate should be constructed in a position that water can be totally discharged
- c. A gate should have sufficient grooves for placement of filter screens, slabs and harvest nets
- d. A gate should be firmly placed at the pond bottom and properly linked with dikes to prevent seepage and possible collapse.

Figure.1. Sampling Site



Name: Shrimp Culture Pond (Rectangular)

Area: 2 Acres

Depth: 6 ft

Height of Dike: 10 ft

CHAPTER 2

REVIEW OF LITERATURE

Namasivayam *et.al* : Biofilm inhibition study revealed both free and chitosan coated plant extracts inhibited biofilm formation, enhanced effect on biofilm inhibition was recorded in polymer coated extracts of the all the tested plants. Biochemical composition of biofilm matrix mainly total carbohydrates and total protein was highly reduced in all the tested concentration of polymer coated plant extracts.

S.Mousavi *et.al* : Prodigiosin extracted from *S. marcescens* can be used as a bioactive compound to design and manufacture of anti-biofouling and anti-biofilm formation products to use extensively for industrial applications as a natural color in marine industries, food industry, cosmetics and textile productions.

Manying Zhang *et.al* : Silver nanoparticles (AgNPs) have excellent antimicrobial properties and are considered to be a promising anti-biofouling agent. A modified method was used to produce small sized and well-dispersed biogenic silver nanoparticles with a mean size of ~6 nm (Bio-Ag0-6) using *Lactobacillus fermentum*. Determination of minimal inhibitory and bactericidal concentration results revealed that biogenic silver Bio-Ag⁰⁻⁶ can effectively suppress the growth of the test bacteria.

K. Ponnusamy *et.al* : Reverse Osmosis (RO) membranes were used to learn about the anti-biofouling properties of vanillin affecting the membrane performances. Vanillin has been recognized as a potential quorum quenching compound for *Aeromonas hydrophila* biofilms. Vanillin addition was effective in the prevention of biofilm formation on the tested membrane surfaces. Among the membranes, RO membranes made with cellulose acetate showed the most substantial reduction of biofilm formation by addition of vanillin.

MF Siddiqui *et.al* : A number of natural compounds in plants have an anti-biofouling effect, reducing the formation of biofilm. This study addressed the feasibility of *Piper betle* extract (PBE) as anti-biofouling agent against the model organism

Pseudomonas aeruginosa PAO1 and bacterial consortium. a number of natural compounds in plants have an anti-biofouling effect, reducing the formation of biofilm. This study addressed the feasibility of *Piper betle* extract (PBE) as anti-biofouling agent against the model organism *Pseudomonas aeruginosa* PAO1 and bacterial consortium.

RS Kumar *et.al* : Nanocurcumin (CCM) was prepared by sonication-assisted wet-milling technique and then incorporated in polyethersulfone (PES) membrane to enhance the anti-biofouling property. Zone inhibition studies revealed that PES/CCM nanocomposite membranes exhibited the better anti-biofouling propensity against *Escherichia coli* and *Pseudomonas aeruginosa*.

V. Sendamangalam *et.al* : Some natural phenolic compounds found in plants and vegetables have an antibiofouling effect, reducing formation of biofilm by Gram-negative bacteria. In this study, *Streptococcus mutans*, a Gram-positive bacterium was investigated for the antibiofouling effect of polyphenols.

Kang-Ju Kim *et.al*: Biofilm formation inhibition results by addition of subinhibitory concentrations (75, 50, and 25% of MBC) of plant extracts against *E. coli* and *S. aureus* indicated that the obtained effect was dose-dependent. The best biofilm reduction is observed in higher concentrations of the extracts (75% of WBC).

Vaseeharan *et.al* : AgNps were synthesized by a simple procedure using tea leaf extract as the reducing agent and were effective against *V. harveyi* isolated from *F. indicus*. The combined results of long- and short-term treatment of AgNps synthesized by tea leaf extract showed a 71% reduction in accumulated mortality. The long-term administration of AgNps synthesized by tea leaf extracts at the concentration of 10 µg significantly reduced the mortalities in *F. indicus* from *V. harveyi* infections.

M. Aghazadeh *et.al*: Ginger extract successfully inhibited biofilm formation by *A. baumannii*, *B. cereus*, *C. krusei*, and *C. albicans*. MTT assay revealed no significant reduction in cell viability after 24 hours. The minimum inhibitory biofilm concentrations

(MIBCs) of ginger extract for fungi strains (*C. krusei* and *C. albicans*) were greater than those of fluconazole and nystatin.

F. Blando *et.al*: The results obtained in this work indicate that cladodes of *Opuntia ficus-indica* are rich in phenolic compounds, particularly *p*-hydroxybenzoic acid derivatives, and a significant inhibition of the biofilm formation were shown by the cladodes at both developmental stages.

C.L. Quave *et.al*: Extracts from 10 plants exhibited an $IC_{50} \leq 32 \mu\text{g/ml}$ for biofilm inhibition: *Lonicera alpigena*, *Castanea sativa*, *Juglans regia*, *Ballota nigra*, *Rosmarinus officinalis*, *Leopoldia comosa*, *Malva sylvestris*, *Cyclamen hederifolium*, *Rosa canina*, and *Rubus ulmifolius*. Limited bacteriostatic activity was evident.

K Alam *et.al*: Solvents play a vital role in extraction of plant components and it was found that the plants in various solvents exhibit different activity against the PAO1 biofilm. Comparatively, 1% methanolic extract of *B. ciliata* (rhizome with skin), showed more than 80% inhibition of biofilm formation without effecting on the growth of the bacterium. Significant correlation between flavonoids content and antibiofilm activity in methanolic extract revealed the contribution of secondary metabolites in *P. aeruginosa* (PAO1) biofilm inhibition.

M.C. Moura *et.al* : WSMoL inhibited biofilm formation by *S. marcescens* at concentrations lower than $2.6 \mu\text{g ml}^{-1}$ and impaired bacterial growth at higher concentrations, avoiding biofilm formation. For *Bacillus* sp., the lectin inhibited bacterial growth at all concentrations. The antibiofilm action of WSMoL is associated with damage to bacterial cells. WSMoL was able to control biofilm formation when immobilized on glass surface ($116 \mu\text{g cm}^{-2}$), damaging *S. marcescens* cells and avoiding adherence of *Bacillus* sp. cells on glass.

CHAPTER 3

AIM AND SCOPE

3.1 AIM:

Control of biofouling using synthetic biofouling agents are known to cause adverse effects due to their poor efficacy and non target toxicity.

3.2 Objectives:

1. To evaluate microbiological parameters and biofouling associated with prawn culture pond.
2. To check the anti biofouling efficacy of neem and turmeric extracts.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Equipments used

1. Test tubes
2. Conical flask
3. Durham tubes
4. Pippettes
5. Incubator
6. Laminar air flow
7. Petri plates
8. Microtitre plate
9. Scanning electron microscope
10. Media

4.2 METHODOLOGY

4.2 Sample collection

Sample collection is a very important part of river study because conclusions drawn are base only on the testing of collected samples. Grab sampling procedure was adopted as recommended by Standard Method for microbiological analysis. Samples were collected from sampling locations Prawn culture ponds. Water samples for microbiological examination, were collected in non-reactive borosilicate glass bottles of 500 ml capacity each that had been cleansed and rinsed carefully, given a final rinse with distilled water and sterilized. Samples were taken from the river by holding the bottle near its base in the hand and plunging it, neck downward, below the surface. Then turning the bottle until neck points slightly upward and mouth is directed toward the current. The sampling bottle was not filled up to the brim and 20 mm to 30 mm space was left for

effective shaking of the bottle (APHA, 1998).The water samples was brought to the lab in a sterile container and the Microbiological analysis of water samples was started as soon as possible.

4.3 Total viable count

Serial dilution was made in sterile distilled water upto and then from the serial diluted tubes 1ml of the water sample was taken and Pour plated, and the plates were kept in inverted position for incubation at 37°C for 24hours. After 24hours the plate was taken and the colonies were counted. Based on the morphology structure a few bacterial isolates was isolated and streaked and kept for incubation at 37°C for 24hours.

4.4 Isolations of bacteria with differential media

The isolated bacterial strains were streaked on Mc Conkey Agar, Salmonella Shigella Agar, Milk agar, Simmons Citrate Agar, Deoxycholate Citrate Agar, Bile Esculin Agar, Violet Red Bile Agar, and Starch Agar and kept for incubation at 37°C for 24hours. The medium was prepared by the Composition.

4.5 Detection of total coliform by MPN method

4.5.1 Double strength broth

71.2 g of lauryl sulphate broth was dissolved in 1 litre distilled water. After the medium got dissolved, 10 ml of the water sample was dispensed into each test tube containing inverted Durham-tubes. The tubes was Sterilized by autoclaving at 121°C for 15 minutes. The tube was allowed to cool down slowly to prevent bubbles in Durham-tubes.

4.5.2 Single strength broth

35.6 g of lauryl sulphate broth was dissolved in 1 litre distilled water. After the medium got dissolved, 10 ml of water sample was dispensed into each test tube containing inverted Durham-tubes. The tubes was Sterilized by

autoclaving at 121°C for 15 minutes. The tube was allowed to cool down slowly to prevent bubbles in Durham-tubes.

4.5.3 Total Coliforms

Three rows of 3 (3) tubes was arranged each in a test-tube rack. With a sterile pipette, 10 ml of the water sample was added to each of the 3 (3) tubes of the first row. With a sterile pipette, 1 ml of the water sample was added to each of the 3 (3) tubes of the second row. With a sterile pipette, 0.1 ml of the water sample was added to each of the 3 (3) tubes of the third row. And the tubes was incubated at 37°C for 24 hours. The change in turbidity and appearance of gas in Durham tubes in bottles was observed. The tube showing fermentation (primary fermentation, presumptive coliforms), was inoculated in new tube of brilliant green bile broth, from the tube showing primary fermentation, and the tubes was incubated at 44°C respectively. The tube in which fermentation was observed was streaked on Eosin methylene blue agar (EMB agar) to isolate coliforms.

4.6 Evaluation of biofilm formation:

The water sample was added in 96 well plate and was incubated at 37°C for 72hours. Then the water was removed from the 96well plate and was washed with saline water then again crystal violet to coat the biofilm and was incubated for 30mins at room temperature. Then after incubation the 96well plate was washed with distilled water and saline water and then finally the biofilm was fixed to the surface by ethanol wash and then the reading was taken at 570nm in the ELISA reader.

4.7 Evaluation of anti biofilm or biofouling effect

4.7.1 Selection of plant material

Rhizome extract of turmeric and aerial parts of neem were selected, Respective plant material were extracted with distilled water and the collected extract was concentrated using rota evaporator.

4.7.2 Phytochemical Analysis

The Phytochemical examination was carried out for all three crude extracts as per the standard methods (Evans, & Trease., 1994)

4.7.2.1 Alkaloids

Mayers Test: 1ml extract was taken and few drops of Mayer's reagent were added. Formation of cream color precipitate was observed. It indicates presence of alkaloids.

4.7.2.2 Carbohydrates and glycosides

Fehling Test: To 1ml extract few drops of Fehling solution were added and the formation of a red precipitate was observed. Red precipitate showed the presence of Carbohydrates and glycosides.

4.7.2.3 Phenolic compounds & tannins

Ferric Chloride Test: Few drops of Ferric chloride solution (5%w/v) were added to 1ml of extract and the formation of a bluish black coloration was observed. Bluish colour indicated the presence Phenolic compounds & tannins.

4.7.2.4 Proteins & Amino acids

Ninhydrin test: To 1ml extract few drops of Ninhydrin were added and the Formation of a purplish pink coloration was observed. It indicates presence Proteins & Amino acids.

4.7.2.5 Flavonoids

Shinoda test: Add small piece of magnesium ribbon to extract (5 mL) plant material. Then add concentrated hydrochloric acid drop-wise. Formation of pink colour indicates presence of flavonoids.

4.7.2.6 Saponins

Froth Test: 2gms of extracts were boiled in 20ml of distilled water in water bath and filtered. To 10ml of filtrate 5ml of distilled water was added and shaken vigorously. Froth indicated the presence of saponins.

4.7.2.7 Terpenoids

Salkowski test: To 5ml of each extract, 2mL of chloroform was added followed by addition of concentrated H₂SO₄ (3mL). A reddish brown coloration of the interface showed presence of terpenoids.

4.8 Evaluation of anti biofouling effect of plant extracts

The water sample was added in 96 well plate followed by the addition of plant extracts (0.1%) and was incubated at 37°C for 72hours. then the water was removed from the 96well plate and was washed with saline water then again crystal violet to coat the biofilm and was incubated for 30mins at room temperature. Then after incubation the 96well plate washed with distilled water and saline water and then finally the biofilm was fixed to the surface by ethanol wash and then the reading was taken at 570nm in the ELISA reader.

CHAPTER 5

RESULTS AND DISCUSSION

Since the industrial revolution, industries have been booming and, consequently, millions of anthropogenic compounds have entered our environment. Persistent organic pollutants have been found even in remote areas of the world. Pollution may be defined as the presence of undesired natural or anthropogenic substances in our environment or a chemical that exceeds normal background level and has the potential to cause harm. Harm is taken to include biochemical or physiological changes that adversely affect an individual, organism's ability to breed, grow or survive. Water is the renewable natural resource essential for all life sustaining systems on the earth. Majority of water available on earth is saline in nature. Only a small quantity exists as fresh water. Fresh water becomes a scarce commodity due to over exploitation and pollution of water. Increasing pollution and its necessities lead to the deprivation of surface and sub-surface water. The causative factors for the pollution of water are industries, agriculture and domestic activities. Further, the industrial growth and consequent pollution let into the freshwater system are a challenge to this fragile ecosystem. The ability of water bodies to clean themselves has been affected by the sheer quantity of waste generated by ever increasing population. The quantity of utilizable water decreases due to over exploitation and also by pollution. Wise and restrained use of the earth's resources, especially water being the most vital one is emphasized. For the quantification of the effects of aquatic habitats contamination, analysis of the composition of aquatic constituents, physicochemical and biological parameters are needed.

5.1 Physicochemical parameters of prawn culture pond

Various physicochemical parameters of water samples collected from prawn culture pond were presented in table.

5.1 Table 1. Physicochemical parameters of water samples

S.No	Parameters	Unit	Optimum	I	II	III
			Range			
1.	pH	-	7.5-8.5	7.69	8.26	8.60
2.	Salinity	ppt	5-25	5	13	13
3.	Alkalinity	ppm	80-300	150	196	2.74
4.	Carbonate	CO ₃ /ppm	0-32	0	12	52
5.	Bicarbonate	HCO ₃ /ppm	80-300	150	184	222
6.	Hardness	ppm	750-3000	1400	3930	4040
7.	Ca Hardness	Ca H/ppm	> 150	400	1320	1520
8.	Mg Hardness	Mg H/ppm	> 200	1000	2610	2520
9.	Calcium	Ca/ppm	> 60	160	528	608
10.	Magnesium	Mg/ppm	> 190	243	634	612
11.	Potassium	K/ppm	> 50	-	-	-
12.	Sodium	Na/ppm	> 1500	-	-	-
13.	Ammonium	NH ₄ /ppm	< 1.0	Nil	0.19	0.25
14.	Ammonia	NH ₃ /ppm	< 0.1	Nil	0.01	0.04
15.	Nitrite	NO ₂ /ppm	< 0.25	0.09	Nil	Nil
16.	Nitrate	NO ₃ /ppm	0.2-0.5	-	-	-
17.	Hydrogen Sulphide	H ₂ S/ppm	<0.25	Nil	Nil	Nil

18.	Phosphate	PO ₄ /ppm	0.05-0.3	-	-	-
19.	Silicate	SiO ₂ /ppm	2-20	-	-	-
20.	Dissolved Oxygen	D.O/ppm	> 4	4.8	4.0	4.0
21.	Carbon dioxide	CO ₂ /ppm	< 10	-	-	-
22.	Iron	Fe/ppm	< 1.0	-	-	-
23.	Chloride	Cl ₂ /ppm	< 0.001	Nil	Nil	0.001

5.2 Total bacterial count

Microbial analysis of water samples were studied by determination of total viable count (TVC) and total including faecal coliforms using most probable number (MPN) test. The results clearly reveals bacterial population was found to be more in all tested time periods. A gradual increase in bacterial population was recorded in increasing time intervals.

5.2 Table 2. Total bacterial count

S.No	Sampling Time	Total viable count (CFU.ml)
1.	I	55×10 ⁵
2.	II	63×10 ⁵
3.	III	77×10 ⁵

5.3 Total faecal coliform count by MPN test

MPN technique was used to study the bacteriological quality which also indicates that MPN index was found to increased in all the tested time periods.

5.3 Table 3. Total faecal coliform count by MPN test

S.No	Sampling time	MPN Index
1.	I	500
2.	II	650
3.	III	700

5.4 Anti biofouling effect of plant extracts

Biofouling is a noteworthy issue in water treatment as it exceedingly bargains the effectiveness of the treatment forms. Chemical antifouling agents are the most regularly utilized course to control biofilms However; microorganisms in biofilms are commonly impervious to numerous antimicrobial materials that are viable against planktonic cells and the successive high utilization of such concoction specialists known to cause destructive non-target impacts (Klasen et al 2000). Eco amicable, powerful dependable biofouling control procedures are required for the sterile supply of water. Successful, multifunctional high productive nanotechnology

forms are giving reasonable answers for water/wastewater treatment without the utilization of high complex auxiliary materials by restraining biofouling (Melaiye and Youngs 2005). Control of biofouling components has demonstrated that nanotechnology approaches including the use of nanoparticles may give powerful options in contrast to utilizing chemical antibiofoulants (Sharma et al 2008). Among the various agents, biological based compounds are found to be highly effective against biofouling associated with various sources. In this study, traditional plant extracts like turmeric and neem were tested against biofouling associated with prawn culture ponds.

5.5 Phytochemical analysis of plant extracts

Prepared plant extract was tested qualitatively to determine the presence of various bioactive phytoconstituents. Table shows the presence of various bioactive phytochemical constituents like alkaloids, flavonoids, phenolics and tannins.

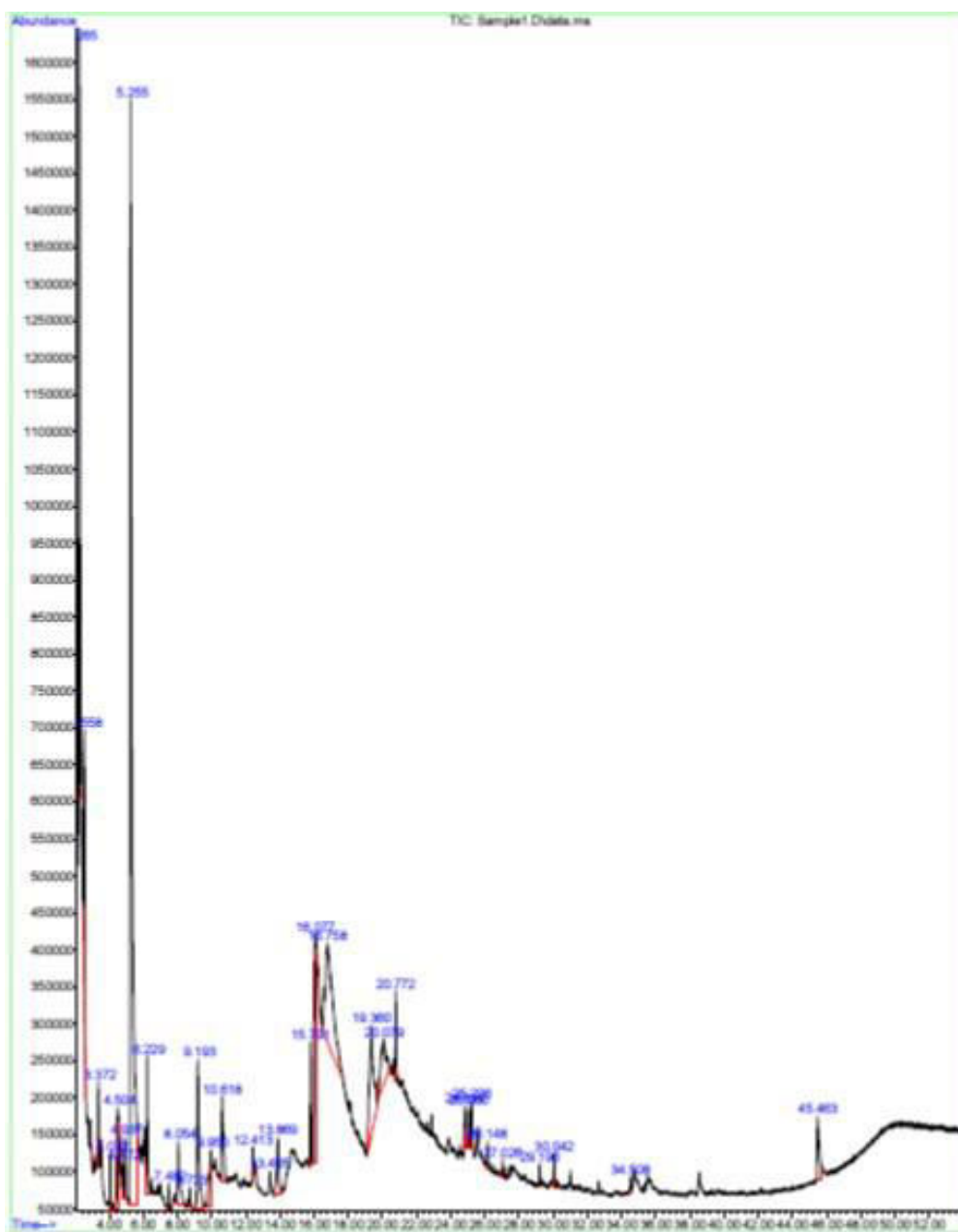
S.No	Phytochemicals	Status
1.	Carbohydrates and Glycosides	+
2.	Alkaloids	+
3.	Phenolic compounds and tannins	+
4.	Protein and Amino acids	+
5.	Flavanoids	+
6.	Saponins	+
7.	Terpenoids	+

+ present

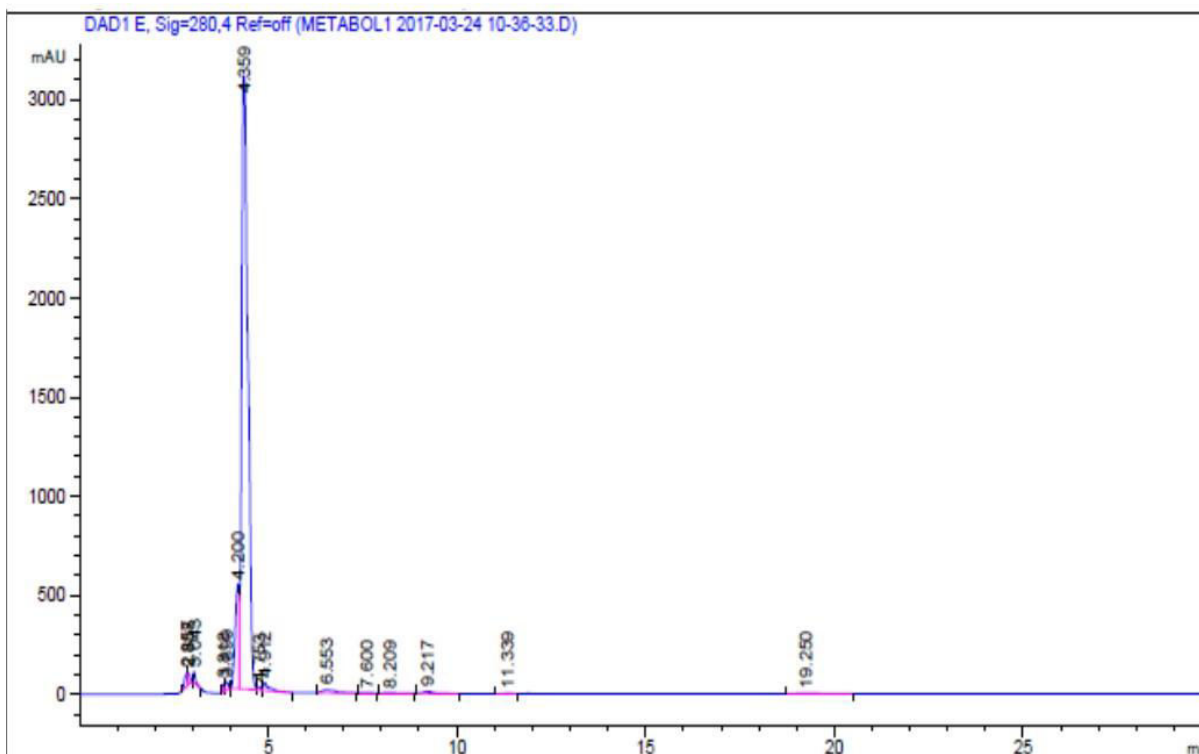
5.6 Anti biofouling efficacy

Microtitre plate spectrophotometric assay was used to study anti biofouling effect.

GCMS – Gas Chromatography Mass Spectrometry



HPLC – High Performance Liquid Chromatography



5.7 Scanning Electron Microscopy (SEM) Analysis:

5.7.1 Methodology

Scanning electron microscopy analysis was also to study the effect of nanoparticles on biofilm. The disrupted biofilm aggregates were fixed with glutaraldehyde (2.5%) followed by distilled water washing. Processed samples were finally coated with gold and palladium, viewed under a Carl Zeiss subra (Germany) scanning electron microscope.

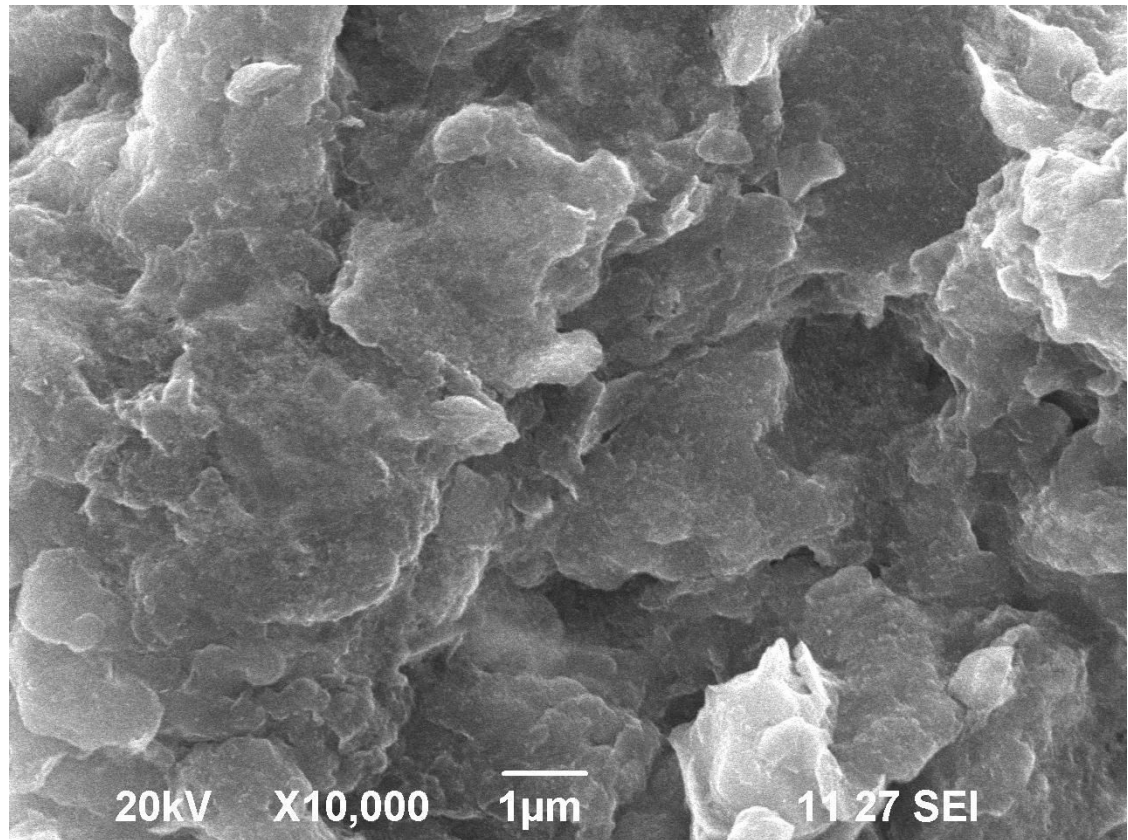
5.7.2 Results

Biofilms - adherent communities of bacteria surrounded by a matrix of extracellular polymeric substance. Some bacterial biofilms have been reported to have useful effects on food chains, sewage treatment plants, to eliminate petroleum oil/hydrocarbon spillage from the oceans. Now the biofilm is considered as major target for the pharmacological development of drugs. A biofilm serves to promote

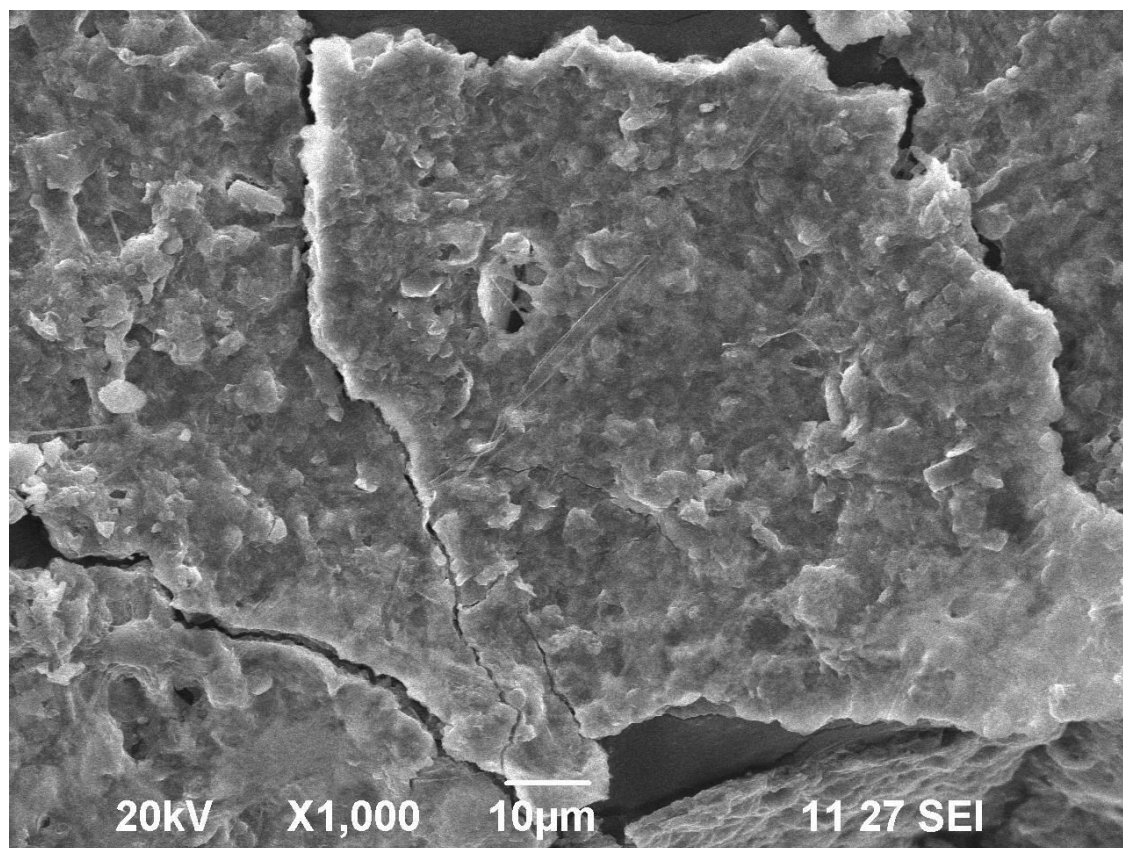
bacteria persistence by resisting antibiotic treatment and host immune responses. Antibiotics are rendered ineffective when biofilms form due to their relative impermeability, the variable physiological status of microorganisms, subpopulations of persistent strains, and variations of phenotypes present. Biofilms have been reported to show increased resistance to antimicrobial agents including antibiotics compared to free-floating cells. Plant extracts and other biologically active compounds isolated from plants have gained widespread interest in this regard as they have been known to cure diseases and illnesses since ancient times. Modern science and technological advances are accelerating the discovery and development of innovative pharmaceuticals with improved therapeutic activity and reduced side-effects from plants. Plant compounds are widely accepted due to the perception that they are safe and they have a long history of use in folk medicine as immune boosters and for the prevention and treatment of several diseases. Over the years, the use of medicinal plants, which forms the backbone of traditional medicine, has grown with an estimated 80% of the populations, mostly in developing countries, relying on traditional medicines for their primary health care. Plant-derived substances under intensive research for possible applications in the pharmaceutical industry include crude extracts of leaves, roots, stems and individual compounds isolated from these, essential oils and essential oil components. Although a lot of research on plants and the active constituents is currently underway, the focus is mainly on the antimicrobial properties against planktonic or biofilm forming bacteria. In the present study, anti biofilm effect of plant extracts was discussed. SEM analysis shows the anti biofouling effect. Plant extracts mediated effect on the biofouling was further confirmed by SEM analysis of adherent biofilm which revealed both the extracts damaged the biofilm and weakened the cell aggregates of biofilm in contrast to the tightly packed cell masses of control groups.

5.8 SEM MICROGRAPH OF BIOFOULING TREATED PLANT EXTRACTS

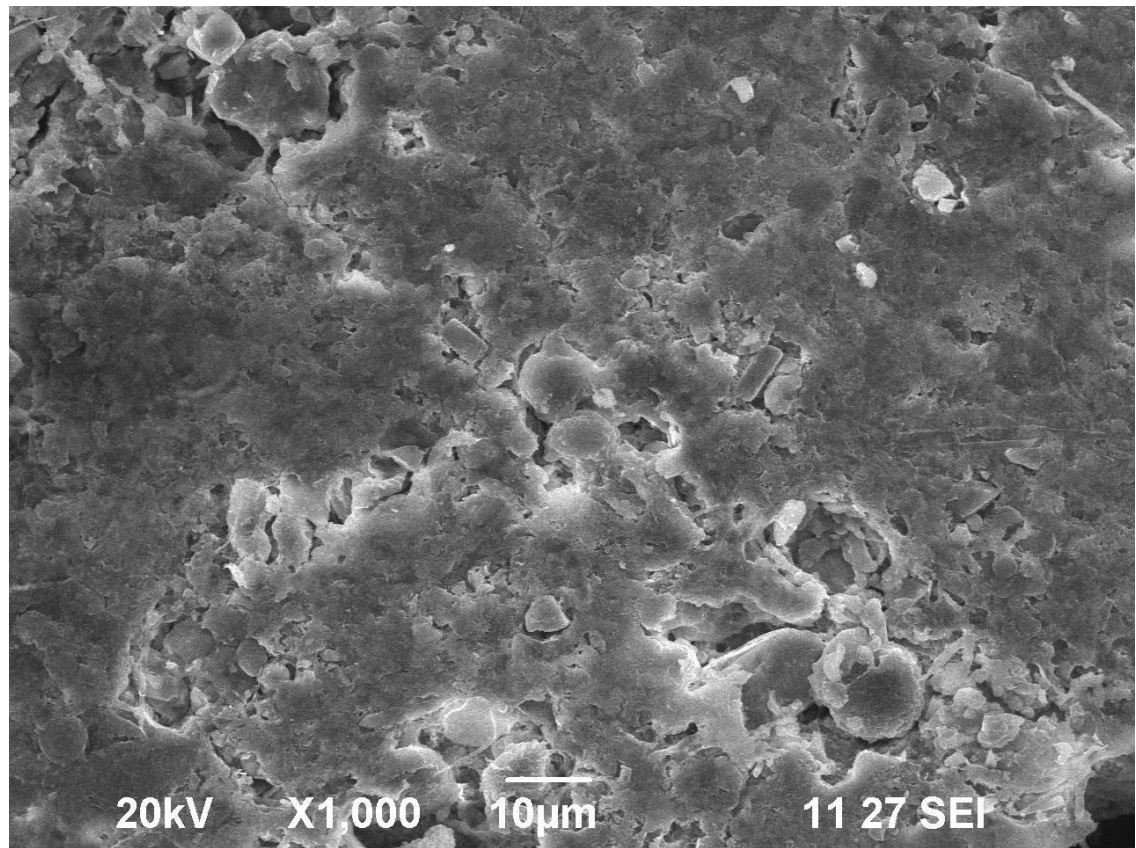
A. Control sampling site (1) - 0th day



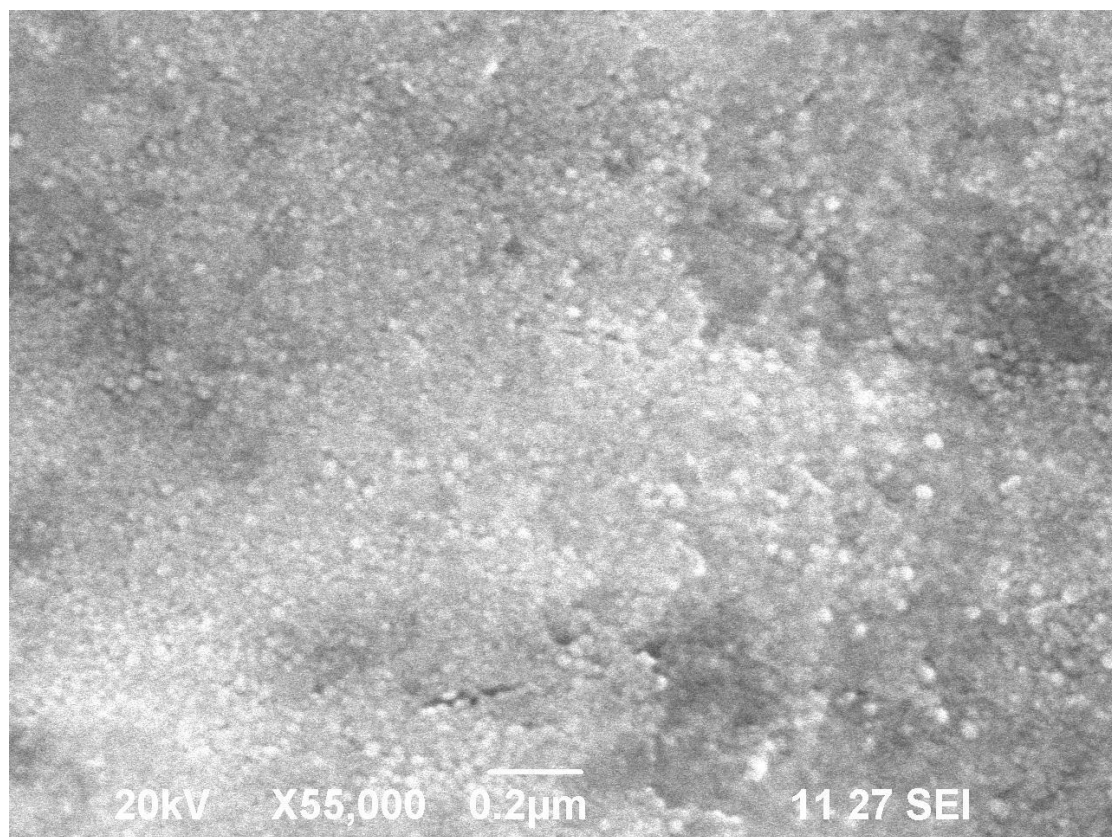
B. Control sampling site (1) - 10th day



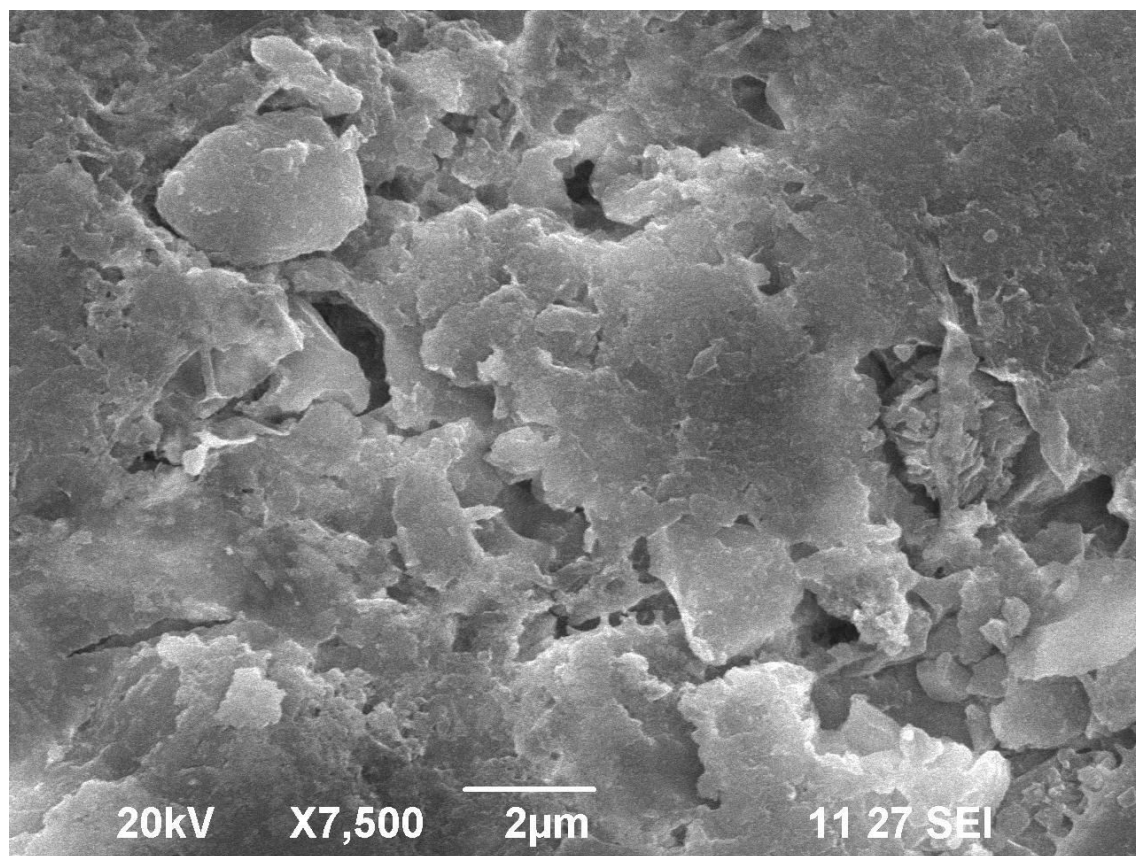
C. Neem sampling site (1) - 0th day



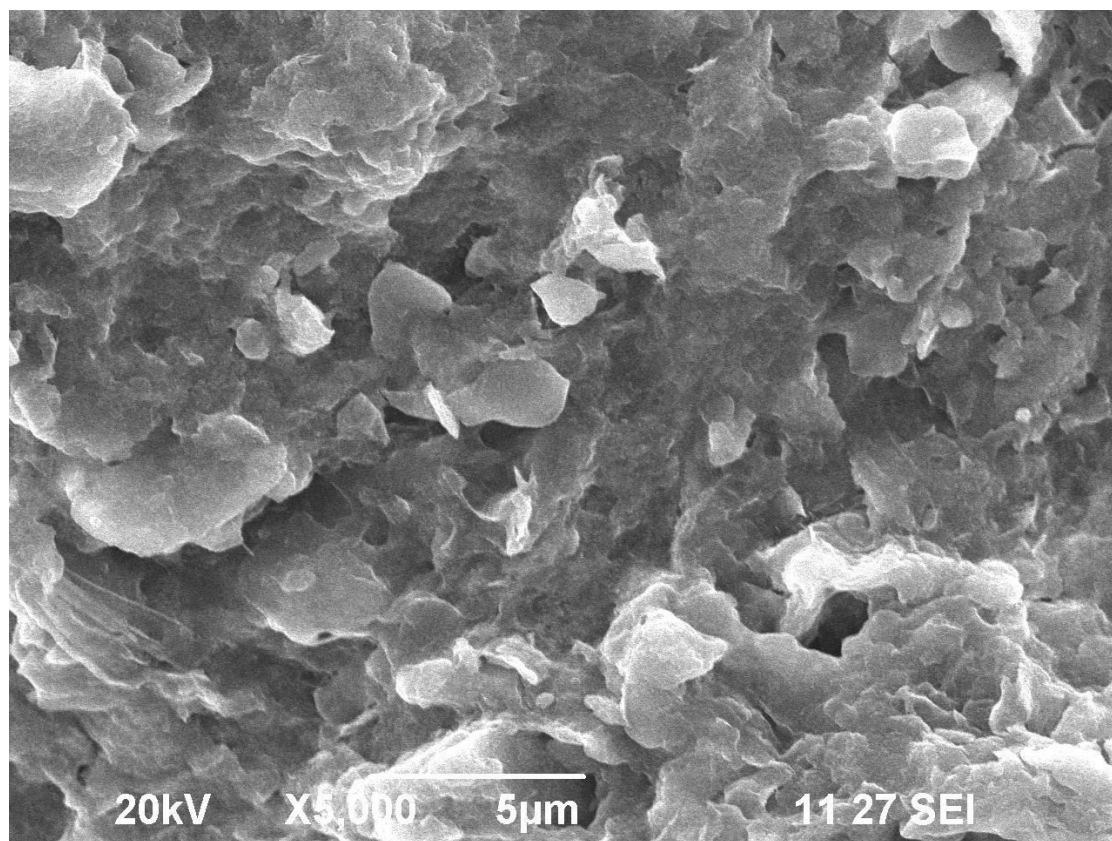
D. Neem sampling time (1) - 10th day



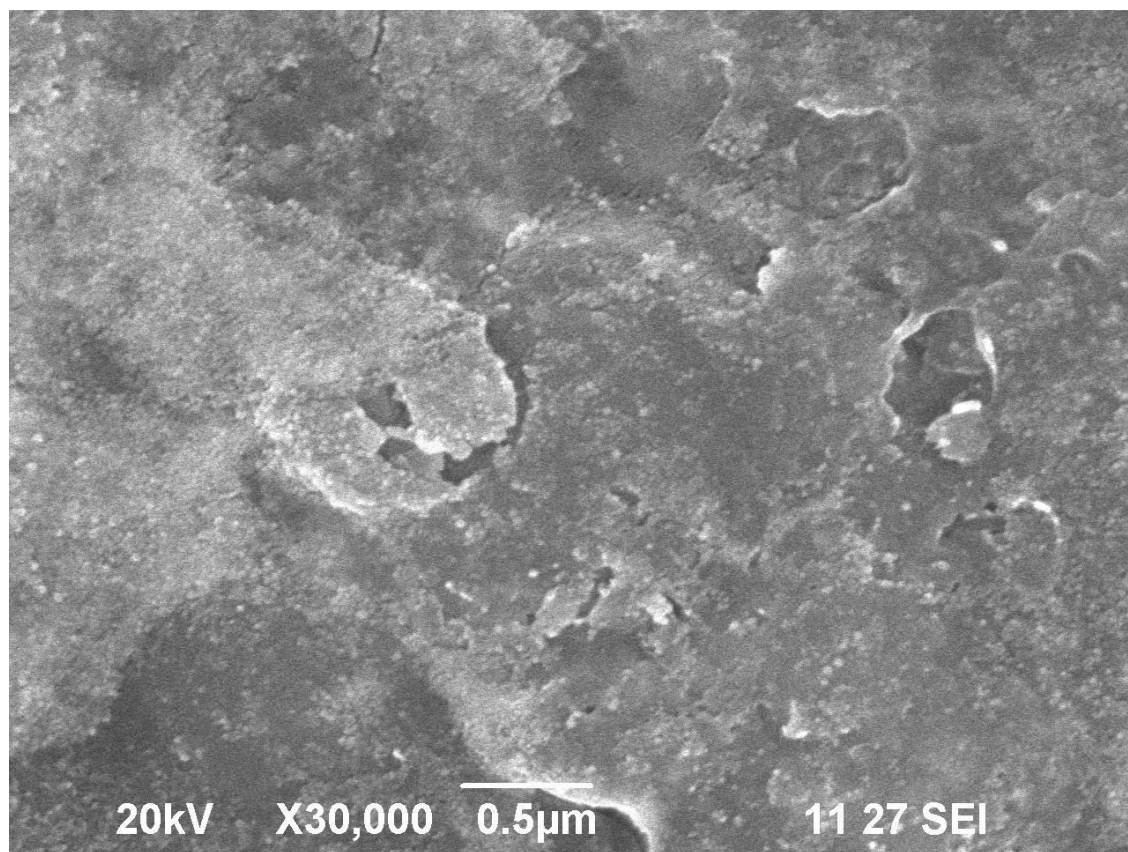
E. Control sampling site (2) - 0th day



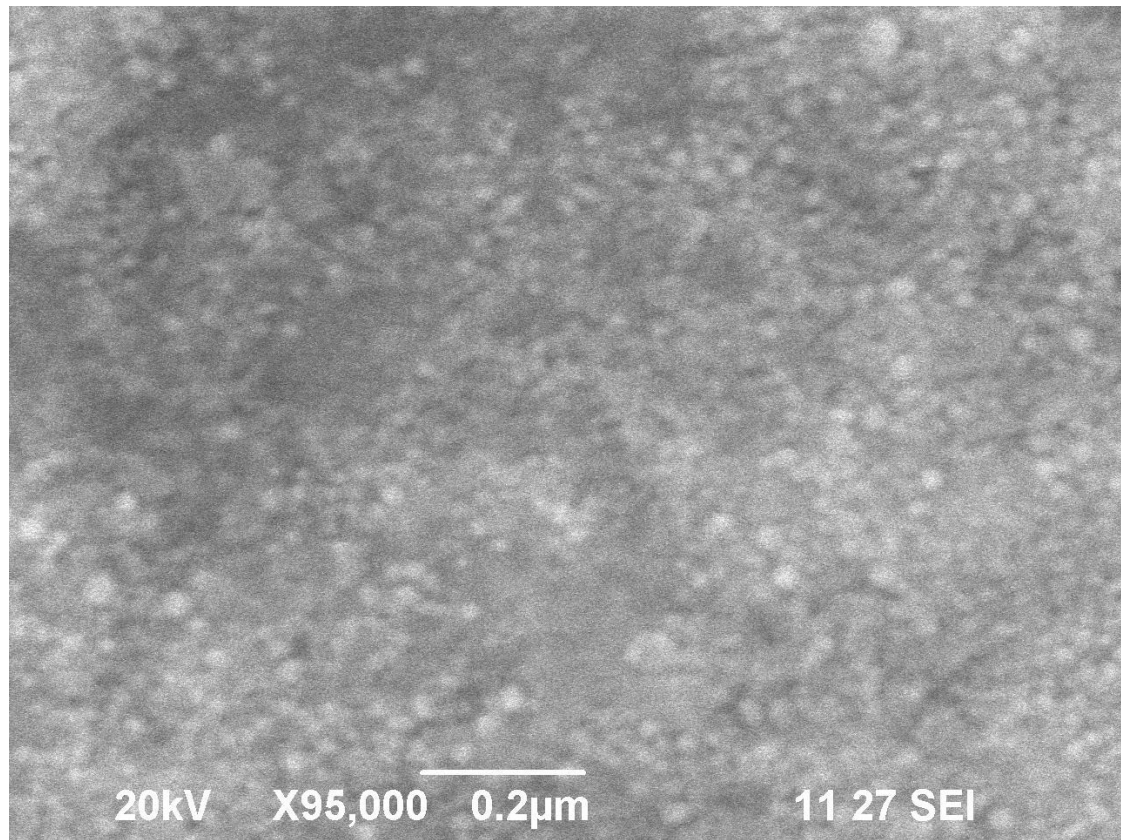
F. Control sampling site (2) - 10th day



G. Turmeric sampling site (2) - 0th day



H. Turmeric sampling site (2) - 10th day



CHAPTER 6

SUMMARY AND CONCLUSION

Due to the wide spread usage of synthetic biofouling agents against biofouling associated with various environmental sources, it is necessary to suggest an alternative, safe anti biofouling agents with this background. The present study is undertaken to evaluate antibiofouling efficacy of herbal plant extracts against biofouling organism associated with prawn culture pond water.

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