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(Under Section 3 of UGC Act 1956)

DEPARTMENT OF ELECTRICAL AND ELECTRONICS ENGINEERING

LIST OF ADVANCED LEARNERS

Program: B.E – EEE-M | **Batch / Group: 2015 – 2019 / I**

S.No	Roll No	Name of the Student	Selection Criteria*	Remarks
1	EE269	ANANDHAN UNNI	a,b,c	Good, Have to improve
2	EE275	JAYA KRISHNAN	a,b,c	Very Good
3	EE279	MANIKANDAN	a,b,c	Good, Have to improve
4	EE282	OSWIN SANCHO	a,b,c	Very Good
5	EE284	SIVARAMAN	b,c	Good, Have to improve
6	EE288	SURYA KUMAR	a,b,c	Good
7	EE289	VIGNESH	a,b,c	Good, Have to improve
8	EE294L	BAJARE SHARDUL JAYATHA	a,b,c	Very good

Selection Criteria*

a) Based on student previous academic performance (above 6.5 CGPA)
b) Students with good problem solving and creative skills
c) Students with good critical thinking and leadership quality

Faculty In-Charge

Dean/ EEE

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CERTIFICATE OF PARTICIPATION

This is to certify that Mr./Ms. JAYA KRISHNAN of AMET University, Chennai has participated in the One day National seminar on "Current Research Trends in Power Engineering" organized by the School of Electrical Engineering (SELECT), VIT Chennai on 25th June, 2016.

S. Krithiga

Dr. Krithiga S. / Prof. S. Angalaeswari
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DEPARTMENT OF MARINE BIOTECHNOLOGY

PROFORMA OF LIST OF ACTIVITIES PARTICIPATED BY ADVANCED LEARNERS

Course name: M.Sc., Marine Biotechnology **Academic Year:** 2015-2016

S.No	Reg.No	Name of the student	Students participation						
			NPTEL	AMET Seed money project	Mini Project	Paper presentation in Intl/National conference	Innovation programmes	Other professional Events (symposium, workshop, short term training courses, Internship, etc.,)	Documents Attached (Detailed Report, certificate, etc.,)
1	AMBT1402	Gokulalakshmi E						✔	Research publication
2	AMBT15001	Anoop Raj			✔				Internship Certificate

Virgibacillus marismortui strain AMET F001 16S ribosomal RNA gene, partial sequence

GenBank: KT072095.1

FASTA Graphics

Go to:

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LOCUS       KT072095            1590 bp    DNA     linear   BCT 28-JUN-2015
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REFERENCE   1  (bases 1 to 1590)
  AUTHORS   Vanitha,C.M., Sri Kumaran,N., Gokulalakshmi,K. and Ramalingam,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-JUN-2015) Department of Marine Biotechnology,
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            India
COMMENT     Sequences were screened for chimeras by the submitter using
            Pintail.
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            Sequencing Technology :: Seq Scape
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      121 cctgtggaac gagcggcgga cgggtgagta acacgtgggc aacctgcctg taagactggg
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      601 gaattattgg gcgtaaagcg cgcgcaggcg gtcctttaag tctgatgtga aagcccacgg
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Extraction and Characterization of Chitosan Obtained from Scales of *Clarias gariepinus* (Catfish)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Fish waste is considered to be one of the major bio-pollutants which are generally discarded in coastal regions through local markets and fish processing industries. Nowadays, it is being eyed as a newer bioresource. Fish scales are good source of chitin and chitosan. Very little information is available related to extraction of chitosan from fish scales. In this study fish scale waste from markets around Chennai was used for extraction of chitosan. Chitosan is extracted from the fish scales and the physicochemical properties of the extracted chitosan were characterized by FTIR, UV-Visible spectroscopy, and Scanning Electron Microscopy. The antibacterial activity of chitosan against different sea food pathogens pathogens like *Vibrio parahaemolyticus, Vibrio cholerae, Staphylococcus aureus, Salmonella typhii*, *Escherichia coli* and *Shigella dysenteriae* was evaluated by calculation of minimum inhibitory concentration (MIC). Antioxidant activity for chitosan was

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performed with various concentration of 20 µm-100 µm and the scavenging activity of the sample is calculated as 15%. The physiochemical properties and FTIR and XRD studies confirm the production of chitosan, because of its high antibacterial activity against pathogens, hence chitosan can be widely used in food preservation, manufacture of wound dressing and in antimicrobial finished textiles.

Keywords: Fish scales; chitosan; antimicrobial activity; scanning electron microscope; fourier transform infrared spectra; antioxidant activity.

1. INTRODUCTION

The sea food industries waste poses environmental hazard due to easy deterioration, even though the wastes generated are biodegradable, it degrades slowly. About 130 million tons of fish waste is generated each year in the world [1]. Fish waste is nothing but pile of the leftover parts of dead fish - heads, tails, internal organs etc. So quick and effective method to utilize these wastes could be to produce biological sustainable materials is the current challenge in research. Chitin is a major structural component of all the crustaceans. Chitosan can be produced by partial deacetylation of chitin with alkali solutions at elevated temperature [2-4]. It is a Bio-polymer composed of β-(1-4)-linked D-Glucosamine. Chitosan has many industrial application based on the modification of reactive functional group. These modifications can be carried out using chemical or biological method [5,6]. Chitosan and its derivatives are inhibiting the growth of many bacteria, fungi and pathogens [7,8]. This important property of acting as antimicrobial agent is useful for food and pharmaceutical industry. Some of the important applications of chitosan are as biomaterial in tissue engineering, food preservatives, drug delivery. Numerous research studies have been undertaken to extract chitosan from prawn shell and lobster. The commercial production of chitosan from shrimp and crab shells requires high production cost and multiple chemical processes such as demineralization, deproteinization, and decolourization [9]. Therefore fish scale waste is another low cost raw material for chitin production. “Fish scales” are good source of chitin and chitosan. Generally, fish scales consist of protein (type I collagen and ichthylepidin) and apatite (calcium phosphate, magnesium carbonate and calcium carbonate) [10]. Research on extraction of chitosan from fish scale is scares and the present investigation focuses on its extract from *Clarias gariepinus* sps and determination of it physico-chemical properties along with antimicrobial and antioxidant properties [11].

2. MATERIALS AND METHODS

2.1 Collection of Fish Scales

Fish scale from *Clarias gariepinus*, was collected from the fish market in Chennai, India. The scales and meat portions were separated and cleaned. The scales were dried at 50°C in an oven for 24 hours. The dried material was homogenized in a laboratory mixer. The moisture content was determined by weighing before and after being dried. The scales were then crushed and preserved in air tight containers.

2.2 Extraction of Chitin and Chitosan

The extraction of chitin and chitosan involved sequence washing of crushed fish scales. These were placed in 1000 ml beakers and soaked in boiling sodium hydroxide (2 and 4% w/v) for one hour in order to dissolve the proteins and sugars thus isolating the crude chitin. 4% NaOH is used for chitin preparation [12]. After the samples were boiled in the sodium hydroxide, the beakers containing the fish scales samples were allowed to cool for 30 minutes at room temperature.

2.2.1 Demineralization

The grounded scales were demineralized using 1% HCl with four times its quantity. The samples were allowed to soak for 24 h to remove the minerals (mainly calcium carbonate) [13]. The remaining chitin was washed with deionized water, which is then drained off. The chitin was further converted into chitosan by the process of deacetylation.

2.2.2 Deacetylation

The deacetylation process was carried out by adding 50% NaOH and then boiled at 100°C for 2 h on a hot plate. The samples were then

placed under the hood and cooled for 30 min at room temperature. Then the samples are washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan. The samples were then left uncovered and oven dried at 110°C for 6 h. The chitosan obtained was in a creamy-white form [9].

2.2.3 Purification of chitosan

The processed chitosan has to be purified to make it suitable for further Industrial applications. The purification process was designed in three steps (a) Removal of insoluble with filtration. (b) Reprecipitation of chitosan with 1N NaOH (c) Demineralization with hydrochloric acid and NaOH.

2.3 Characterization of Prepared Chitosan

2.3.1 pH

The pH measurements of the chitosan solutions was carried out using a microprocessor pH meter.

2.3.2 Ash value

To determine the ash value of chitosan, 2.0 g of chitosan sample were placed into previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 65°C for 4 hr. The crucibles were allowed to cool in the furnace to less than 200°C and then placed into desiccators with a vented top. Percentage of ash value is calculated using the following [14].

$$\%Ash = weight(g)/sampleweight(g)\times 100$$

2.3.3 Loss on drying

Loss on drying of the prepared chitosan was determined by the gravimetric method. The water loss has been determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass (or weight) will be the difference between the weights of the wet and oven dry samples [15].

$$\%\ Loss\ on\ drying = Wet\ weight\ (g) - Dry\ weight(g)/\ Dry\ weight(g)\times 100$$

2.3.4 Fourier transform infrared spectra (FTIR) studies of chitosan

The samples of Chitin and Chitosan produced were characterized in KBr pellets by infrared spectrophotometer in the range of 400 to 4000 cm^{-1} (Brucker Equinox 55).

2.3.5 Scanning electronic microscopy (SEM)

Scanning electron microscope (model 2360, Leo Oxford, England) was used to evaluate the surface and shape characteristics of the particles.

2.3.6 UV-Vis absorption

Each sample was analyzed by UV-visible spectrophotometer (Optima 3200, Tokyo, Japan) in the range 200-750 nm and the wavelength corresponding to maximum absorption (μ max) was recorded. 0.1% (w/v) and 0.5% (w/v) chitosan in 1% (v/v) acetic acid solutions irradiated at the same dose (20±2 kG) were used as blank samples.

2.4 Screening for Antimicrobial Activity

The Chitosan was tested for their antimicrobial activity by the agar well diffusion method and the Minimum inhibitory Concentration was calculated by the lowest concentration of chitosan that inhibits the growth of bacteria was considered as the minimum inhibitory concentration or MIC. The microbial strains *Vibrio parahaemolyticus, Vibrio cholerae, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Shigella dysenteriae* were used for this analysis. This culture suspension was seeded in agar plates by the pour plate technique. Three cavities were made using a cork borer (10 mm diameter) at equal distance and were filled with the chitosan solution and then incubated at room temperature for 24 hrs. The formation of a clear zone (restricted microbial growth) around the cavity is an indication of antimicrobial activity.

2.5 Scavenging Ability on 1, 1-diphenyl 1-2-picryl Hydroxyl Radicals (DPPH)

The scavenging effect of chitosan on DPPH radical was examined using the modified method described earlier [16,17]. Each chitosan sample (0.1-10 mg/ml) in 0.2% acetic acid solution was mixed with 1 ml of methanolic solution containing DPPH radicals, results/resulted in a final concentration of 10 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark and the absorbance was then measured at 517 nm against a blank [18]. Ascorbic acid was used as standard. The scavenging ability was calculated as follows:

Scavenging ability (%) = [ΔA517 of control-ΔA517 of sample) /ΔA517 of control] X100.

3. RESULTS AND DISCUSSION

Chitosan was extracted from the fish scales which are discarded daily as waste materials from fish markets of Kanathur. Extraction of chitosan from fish scales was done using chemical treatments. The results of physiochemical properties of chitosan are given in (Table 1). The prepared chitosan was confirmed as reported by Sunithakumari et al. (2014).

Table 1. Physiochemical parameters of chitosan

Physiological parameters	Chitosan
Yield	45.56 %
Moisture content	5%
Ash value	1.26%
Loss on drying	3%
pH	7
Solubility	Acetic acid

The absorption bands of FTIR of chitosan were observed in the range of 3643 -3938 cm^{-1} related to associated in N-H bond primary amines, 2920 cm^{-1} – 3440 cm^{-1} was associated with C═O of carboxylic acid, 1787-2522 cm^{-1} was associated with C ═ N, C ≡ N of aliphatic amine and 433 cm^{-1} - 860 cm^{-1} C ─ N Aromatic (Bending) (Fig. 1). The band at 1567 cm^{-1} has a larger intensity than at 1655 cm^{-1}, which suggests effective deacetylation. When chitin deacetylation occurs, the band observed at 1656 cm^{-1} decreases, while a growth at 1597 cm^{-1} occurs, indicating the prevalence of NH2 groups [19,20].

Fig. 2 show the SEM photographs of prepared chitosan from fish. The sample exhibited rough and thick surface morphology under electron microscopic examination at 50X magnification. This was in accordance to the previous data [21].

The antibacterial activity of the extracted chitosan samples against gram negative and gram positive bacteria are shown in Fig. 3. The plates were checked for anti-bacterial activity after 24 hours of incubation. Data indicated that, chitosan markedly inhibited the growth of most of gram negative bacteria tested; however, the inhibitory effects differed depending on the types of chitosan and the tested bacteria. It could be reported that, good antibacterial activity was observed in the plates containing test strains *Vibrio parahaemolyticus* (20 mm), *Vibrio cholera* (17 mm), *Staphylococcus aureus* (18 mm), *Salmonella typhii* (17 mm), *Escherichia coli* (15 mm), *Shigella dysenteriae* (16 mm). The bacterial pathogen *V. parahaemolyticus* for chitosan showed 20 mm inhibition zone whereas the Chitosan showed little inhibition for test strain *E. coli* compared to others (Table 2). Chitosan from fish scales show efficient antibacterial property due to their extremely large surface area, which provides better contact with microorganisms [22,23].

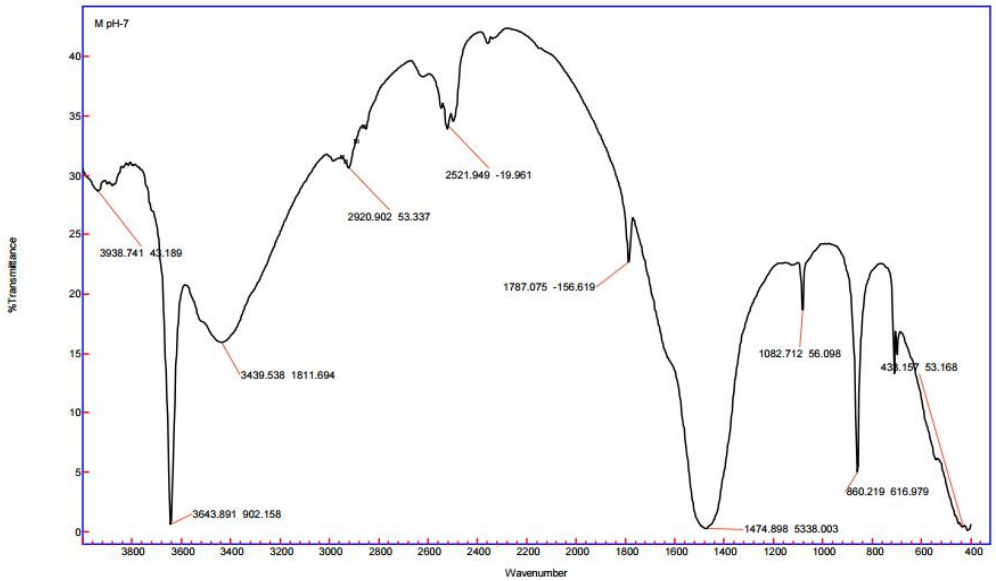

Fig. 1. FTIR of chitosan

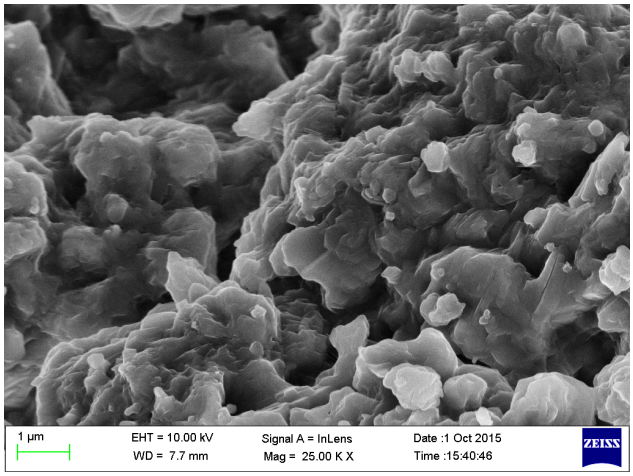

Fig. 2. SEM image of chitosan

Table 2. Antimicrobial activity of chitosan derived from fish scales

Test organism	Concentration of chitosan (µg/mL)	Concentration of acetic acid (µL/mL)
	0.001	20
	Zone of inhibition(mm)	
V. parahaemolyticus	20	
Vibrio cholera	17	
S. aureus	18	
S. typhii	17	
E. coli	15	
Shigella dysenteriae	16	

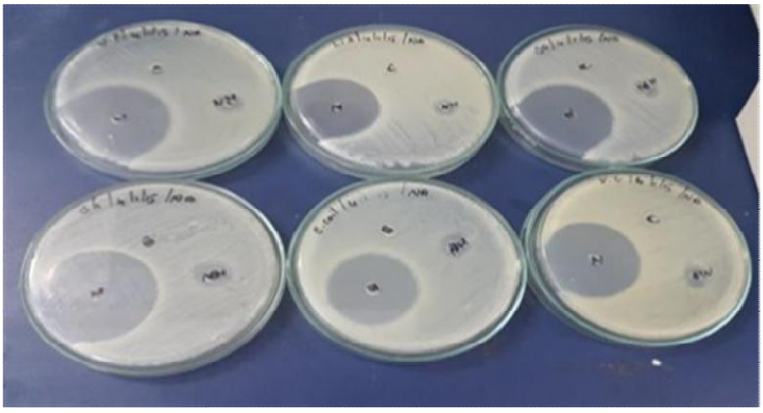

Fig. 3. Zone of inhibition of chitosan derived from fish scales against various pathogens

Antioxidant activity of chitosan at different concentration was shown in Fig. 4. Yen et al. [18] reported that the scavenging activity of the extracted chitosan sample on (DPPH) radicals was increased with their concentration increased. The DPPH radical scavenging activity of the chitosan was significantly lower than that of Ascorbic acid at the same concentration. The activity varied from 2.5 to 15%, which corresponds to 0.2 to 1.0 mg of the chitosan per mL. Chitosan scavenges various free radicals through the action of nitrogen on the C-2 position of the chitosan [24,25].

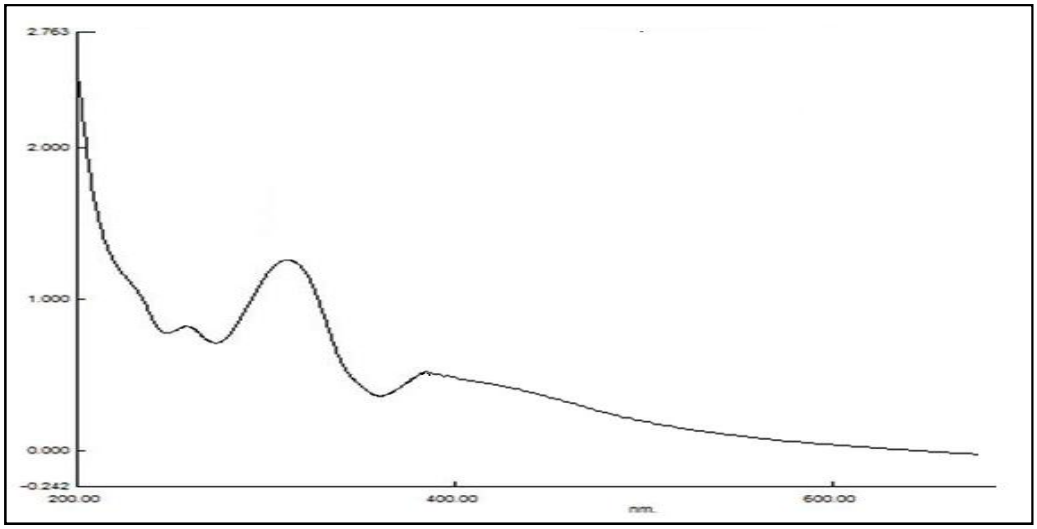

Fig. 4. UV visible spectroscopy of chitosan scales (x-axis wavelength in nm, y-axis absorbance in nm)

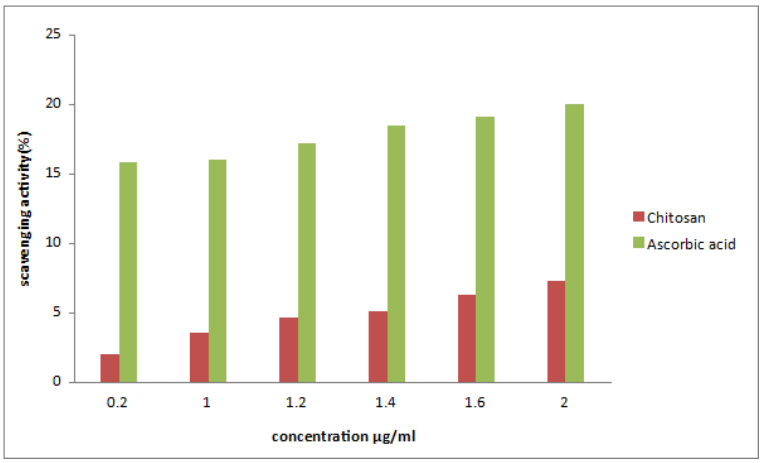

Fig. 5. Antioxidant activity of chitosan scales from *Clarias gariepinus* (X axis = concentration of DPPH µg/mL, Y axis = % of scavenging activity)

4. CONCLUSION

In this study, waste fish scale collected from markets were used for extraction of chitosan. Chitosan was synthesized from the fish scales and the physicochemical properties of the extracted chitosan were characterized by FTIR, UV-Visible spectroscopy, and Scanning Electron Microscopy. The antibacterial activity of chitosan against different sea food pathogens pathogens was evaluated by calculation of minimum inhibitory concentration (MIC). Antioxidant activity for chitosan was performed with various concentrations and the scavenging activity of the sample is calculated. This study shows the production of chitosan from fish scales, would successfully reduce the environmental pollution. The physiochemical properties and the FTIR and XRD studies confirm the production of chitosan. Because of its high antibacterial activity against sea food pathogens chitosan can be widely used in food preservation, manufacture of wound dressing and in antimicrobial finished textiles. Further plan of work would be on exploiting the use of chitosan in the field of environmental studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Antibacterial activity of crab-chitosan against *Staphylococcus aureus* and *Escherichia coli.* J Adv Scient Res. 2011; 2:63-66.

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Research Article

BENEFICIAL MICROBES AS PROBIOTICS ON AQUACULTURE TO BRING SUSTAINABILITY IN BLUE REVOLUTION

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ABSTRACT

The use of probiotics or beneficial bacteria, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment, which cause attendant problems like drug residue in tissues, export rejection etc. The present study was aimed to determine the probiotic effectiveness of Bacillus sp, *Lactobacillus sp* and *Arthrobacter sp* (isolated from marine samples) on Litopenaeus vannamei culture for the prevalence of white spot syndrome virus (WSSV) under laboratory scale conditions. All the three selected isolates were included in the diet of juvenile shrimp at different combination and concentration. Two bioassays were conducted with treatments by triplicate. Based on the, Initial mean weight, Mean weight gain, FCR, DWG, Yield, Survival rate and Vibrio load, the test group T-5, where the shrimps fed with all the three different probiotic strains (Bacillus sp, *Lactobacillus sp* and *Arthrobacter sp*) incorporated feed (in the range of 5×106 cfu mL) showed significant changes in regard to the mentioned biometric parameters than other groups. In bioassay II, after 21 days of culture, the maximum shrimp survival (46%) was observed in treatment II comparatively than control (28%). The consortium of three potential beneficial probiotic bacterial consortiums didn't eliminate the WSSV in cultured shrimps, but increase the survival rate and decrease the vibrio load in the culture systems and water.

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INTRODUCTION

Intensive development of these shrimp industries and extensive culture of these aqua farms has created various ecological, economical and social problems. During the last few years white spot syndrome virus (WSSV) disease has spread worldwide and caused large scale mortalities and economic loss in shrimp culture particularly in Asia (Danya and Jagadish, 2014). Due to the continuous outbreak of this WSSV disease in *Penaeus monodon* culture leading to loss of shrimp culture in India the farmers are seriously looking for alternative shrimp species for culture (Karthik *et al*., 2014). In 2008, the Coastal Aquaculture Authority of India (CAA) introduced a new shrimp species *Litopenaeus vannamei* as an alternative to Penaeid species in India to culture and export. Since the *Litopenaeus vannamei* exhibits fast growth rate and its culture period is significantly shorter compared to *Penaeus monodon*. Several maritime countries have switched over to *Litopenaeus vannamei* culture instead of *Penaeus monodon* as a prospective species in terms of economical gain and standing top production in short periods (Karthik *et al*., 2015 a&b; Karuppasamy, *et al*., 2013).

The newest attempt to improve water quality in aquaculture is the application of probiotics and/or enzymes to ponds. This approach of biotechnology is also known as bioremediation, which involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and get rid of undesirable waste compounds. The concept of biological disease control, particularly using microbiological modulator for disease prevention has received widespread attention (Karthik *et al*., 2016). A bacterial supplement of a single or mixed culture of selected non-pathogenic bacterial strains is termed as probiotics. Thus this chapter of the study, focused to evaluate the effectiveness of potentially selected and/or

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developed suitable microalgae and a mixture of beneficial and/or probiotic bacterial consortium along with nitrifying and denitrifying bacteria on prevalence of WSSV on (*Litopenaeus vannamei*) culture under laboratory scale experimental conditions.

MATERIALS AND METHODS

Mass culture of potential bacterial strains and preparation of probiotic feed

The bacterial strains namely Bacillus sp, *Lactobacillus sp* and *Arthrobacter sp* were separately grown in nutrient broth in a shaking incubator at 30°C for 24 hours. After the incubation period, the cells were harvested by centrifuging at at 4000 rpm for 15 min at 4ºC. The obtained pellet washed twice with phosphate-buffered saline (pH 7.2) and re-suspended in the same buffer and the number bacteria (5×10^{13} CFU/ml) was standardized by dilute plating method. Spraying method was used to prepare the diet according to Gildberg and Mikkelsen (1998). For feed preparation, according to Avakh (2006), 1 kg of the commercial pellet feed containing 38% crude protein was sprayed (Individually or in a combine) by 3 ml of bacteria solution followed Gildberg and Mikkelsen (1998). Then the feed was oven-dried at 35ºC for 1 - 2 hours. The amount of bacterial load in the feed was determined by standard plate count method using respective media (Ajitha *et al*., 2004).

Mass culture of Nitrosomonas sp and Nitrobacter sp

The potential *Nitrosomonas* sp and *Nitrobacter* sp strains were mass cultured in a 2L fermentor by using sodium nitrite (0.25mg NaNO2/l Winogradsky broth) and ammonium sulphate (5.0mg (NH4)2SO4/l Winogradsky broth) as medium for *Nitrobacter* sp and *Nitrosomonas* sp respectively with proper pH (8), temperature (28°C), agitation (200 rpm) and aeration (at the rate of 0.6 L min"1). The fermentor was covered with a black cloth to protect the culture from light inactivation. When the rate of substrate uptake and product formation declined (indicating the attainment of stationary phase) the culture was harvested by centrifuging at 8000 rpm for 20 minutes at 4°C. The obtained culture filtrate was washed with fresh medium and re suspended in corresponding medium (containing 10 µg mL^{-1} substrate) and it was stored in air tight container at 4°C. Then, the absorbance at 600 nm was adjusted to 0.25 ± 0.05 in order to standardize the number of bacteria (5×10^2 CFU/ml) it was subjected to dilute plating method.

Experimental animal

Litopenaeus vannamei (nauplii 24 h) seeds were obtained from a commercial shrimp hatchery located in Marakanam, Kanchipuram District, Tamil Nadu, India. They were kept in seawater with aeration for a period of 6 h in order to avoid any stress to the animals and then used for further experiments.

Feeding schedule from zoea to post larvae

The Zoea of *Litopenaeus vannamei* kept in the experimental tanks was fed with *Cheatoceros calcitrans*. On 1st day of the Zoea (Z) of I- III stages were fed thrice with 30×10^4 cells/mL of algal cells. On the 2nd day of the Mysis (M) (I- III/Postlarve 1) of *Litopenaeus vannamei* were fed thrice with 40×10^4 cells/mL of algal cells. From 3rd day up to 20th day they were fed thrice with 3-8 No/mL of *Artemia salina* nauplii enriched with *Cheatoceros calcitrans*. The 24 h old *Artemia salina* nauplii enriched with *Cheatoceros calcitrans* for a period of 24 h was fed to *Litopenaeus vannamei*. This experiment was conducted up to when the larvae reach PL15 stage. Then from PL15 to PL25 animals were fed with *Artemia salina* and commercial pellet feed at daily rate of 8% body weight, three times daily.

Shrimp acclimation for experimental conditions

A total of six hundred and fifty shrimps were individually weight and placed into thirteen batches (each containing 50 shrimps) and acclimated to culture and/or experimental conditions for five days. During the first five days of experimental condition, animals were fed with commercial feed. Two bioassays were conducted to evaluate the effect of feed supplemented with different probiotic bacteria to evaluate in terms of growth performance, survival and prevalence of WSSV.

Experimental design for Bioassay I

After acclimation of five days, shrimps in the control tank were fed only with commercial feed and the shrimps in the experimental tank 1 to 12 were fed with probiotic bacterial isolates mixture of two or three probiotic bacteria) supplemented feed (Table). The experiment carried for 60 days, during the culture period shrimp in all groups were fed twice daily at 9am and 5pm. Shrimps were fed twice daily and half of the water was exchanged at day three and the uneaten food and waste matter were removed daily before feeding.

Table 1 Experimental setup for probiotics administration (Bioassay I)

Treatments	Feed incorporated with mixture of multi probiotic isolates @ ratio 1:1	Experiments	Dosage	Water Probiotics in the range of 5×10^2 CFU/ml
T – 1	Commercial feed	Control	-	-
		E – 1	10^2 CFU mL^{-1}	Once in a week
T – 2	Bacillus sp + *Lactobacillus sp*	E – 2	10^4 CFU mL^{-1}	Once in a week
		E – 3	10^6 CFU mL^{-1}	Once in a week
		E – 4	10^2 CFU mL^{-1}	Once in a week
T – 3	Bacillus sp + *Arthrobacter sp*	E -5	10^4 CFU mL^{-1}	Once in a week
		E – 6	10^6 CFU mL^{-1}	Once in a week
		E – 7	10^2 CFU mL^{-1}	Once in a week
T – 4	*Lactobacillus sp* + *Arthrobacter sp*	E – 8	10^4 CFU mL^{-1}	Once in a week
		E – 9	10^6 CFU mL^{-1}	Once in a week
		E -10	10^2 CFU mL^{-1}	Once in a week
T – 5	Bacillus sp + *Lactobacillus sp* + *Arthrobacter sp*	E – 11	10^4 CFU mL^{-1}	Once in a week
		E – 12	10^6 CFU mL^{-1}	Once in a week

During the culture period, both the potentially selected nitrifying and denitrifying bacterial strains such as, *Nitrosomonas* sp and *Nitrobacter* sp (each in the range of 5 × 10^2 CFU/ml) were added (through water) in all the experimental tanks (7, 14, 21, 28, 35, 42, 49 and 56th day). The growth parameters were calculated according to Robertson *et al*. (2000), Felix and Sudharsan (2004) and Venkat *et al*. (2004).

1. Weight gain (g/shrimp) = Final weight (g) – Initial weight (g)
2. Weight gain (%) = Final weight (g) – initial weight (g) X 100
3. Initial weight (g)
4. Food conversion ratio (FCR) = Total feed given (g)
5. Wet weight gain (g)
6. Daily weight gain (DWG; g/days) = Final weight (g) x Initial weigh t (g)
7. Days
8. Yield of shrimps (g) = Mean body weigth (g) x Total viable shrimps at harvest
9. Survival rate (%) =Total number of larvae survived Initial number of larvae stocked X 100

Experimental design for Bioassay II

Total of one experimental with one control trial were conducted in aerated 120-L indoor plastic tanks containing 80 L of filtered (20 µm) sea water (34 to 35 g/l) and constant aeration in groups of 50 animals per tank. The second bioassay experiment was conducted for 21 days. After acclimation of five days (Similarly to Bioassay I), shrimps in the control tank were fed only with commercial feed and the shrimps in the experimental tank were fed with probiotic bacterial isolates mixture of two or three probiotic bacteria) supplemented feed (Table) for the first seven days . Shrimp were fed twice daily and half of the water was exchanged at day three and the uneaten food and waste matter were removed daily before feeding. Meanwhile, WSSV infected shrimps were obtained from a disease spread out pond and confirmed for positive by RT-PCR. One gram of homogenised WSSV positive shrimp paste was fed to the animals in both the treatments (control and experiment) on day eight. Then from day nine to 21 animals were fed as the first seven days. During the culture, both the potentially selected nitrifying and denitrifying bacterial strains such as, *Nitrosomonas* sp and *Nitrobacter* sp (each in the range of 10^5 ml^{-1}) were added (through water) in the experimental tank (7, 14and 21st day). At the end of 21st day, shrimp survival in the control and experimental groups were determined and shrimp samples were taken for WSSV analysis.

Table 2 Experimental setup for probiotics administration (Bioassay II)

Treatments	Feed incorporated with mixture of multi probiotic isolates @ ratio 1:1	Experiments	Dosage	Water Probiotics in the range of 5×10^2 CFU/ml
T – 1	Commercial feed	Control	-	-
T – 2	Bacillus sp + *Lactobacillus sp* + *Arthrobacter sp*	E -10	10^2 CFU mL^{-1}	Once in a week

Water quality analysis

Water quality analysis has done using following standard methods. pH pen (Scan – 2- Eutech cybernetics PTE Ltd, Singapore) used to measure the water pH and handy refractometer (Atago, Japan) for estimating salinity. Dissolved oxygen and temperature together were measured with the help of handy D.O meter (YSI 55 model). Ammonium and nitrite was estimated by an ammonia meter respectively. The shrimp culture systems were cleaned daily by siphoning out the wastes and uneaten feed. Water exchanging was done 30% daily. All the control and experimental tanks were supported by well-built aeration.

Microbiological analysis

Shrimps and the water samples were taken from all the control and experimental tanks during the first bioassay at every 15 days time intervals to enumerate and check the *Vibrio* sp on TCBS agar medium (Sivakumar *et al*., 2012; Akponah *et al*., 2014).

RESULTS AND DISCUSSION

The farming of whiteleg shrimp (Litopenaeus vannamei) is an important economic activity in India. However, in the last decade, this industry has been threatened by viral diseases that have affected its production performance. While white spot syndrome virus (WSSV) can cause cumulative mortalities of up to 100% within 3–10 days (Chou *et al*., 1995; Wang *et al*., 1995). One of the strategies involved in aquaculture to prevent losses caused by diseases are the basic practices of good management, chemotherapy and vaccination (Subasinghe and Barg, 1998). However, in the last years, biological control has become a useful technique in aquaculture. It consists in the use of probiotic bacteria capable of stimulating growth and improving animal health (Farzanfar, 2006). In aquaculture, the term probiotic is defined as a microbial supplement formed by a single or a mixed culture of selected microorganisms that are added to a culture system in order to manipulate the microbial communities present in the pond (Balcázar, 2002). Probiotics have multiple mechanisms of action to inhibit pathogens which include competitive exclusion, production of substances that inhibit growth of opportunistic pathogens (antagonism), stimulation of the immune response, antiviral effects, increase of digestive function through production of enzymes, improved nutrition by providing essential nutrients, and improved water quality (Balcázar, 2002; Balcázar *et al*., 2006; Farzanfar, 2006). In the two last decades, many studies reported promising results using a single beneficial bacterial strain in the culture of many finfish species (Avella *et al*., 2010a). The application of probiotics
against viruses in shrimp cultivation is a novel and safe approach. Looking for novel approach, Hence, this chapter of the study, focused to evaluate the effectiveness of potentially selected and/or developed suitable microalgae and a mixture of beneficial and/or probiotic bacterial consortium (at different combination and concentration) along with nitrifying and denitrifying bacteria on prevalence of WSSV on *Litopenaeus vannamei* culture under laboratory scale experimental conditions.

Water quality parameters

During the experimental period, the temperature (26 – 28°C), salinity (25 – 28%), total ammonium (0 – 0.1 mgl−1), nitrite (0 – 0.05 mgl−1) and pH (7.0 – 7.6) were maintained in the suitable range and found to be stable in all the control and experimental tanks. Shan and Obbard (2003) reported that reduction of TAN in aquaculture system can be facilitated by providing and maintaining an optimum environment condition for nitrifying bacteria. Nitrification is the aerobic oxidation of ammonia to nitrite followed by the aerobic oxidation of nitrite to nitrate. Nitrification is a two step process in which ammonia is oxidized to nitrite by ammonia oxidizing bacteria (AOB) or ammonia oxidizing archaea (AOA) and nitrite is then oxidized to nitrate by nitrite oxidizing bacteria (NOB). Hence, in this study during the culture, both the potentially selected nitrifying and denitrifying bacterial strains *Nitrobacter* sp and *Nitrosomonas* sp were added (through water) in all the experimental tanks on weekly intervals. While, checking the Ammonia (NH_4^+), Nitrite (NO_2) and Nitrate (NO_3) in all the tank water on day the ammonia and nitrite concentration decreased and the nitrate concentration increased in the both *P.monodon* and *L.vannamei* culture experimental tanks.

Effectiveness of bacterial mixtures on Bioassay I

The first bioassay was carried out for 60 days. In this, study all the three potentially selected probiotic bacterial strains viz., Bacillus sp, *Lactobacillus sp* and *Arthrobacter sp* were incorporated in to feed at different combination and concentration and fed to the shrimps. At the beginning of study there were no significant differences for initial mean weight of shrimps calculated between all the control and experimental groups. After 60 days of culture, there were significance differences for survival rate between and the other treated and control groups and also the mean yield. There were significance differences in final mean weight (10.2±0.20 g), Mean weight gain (6.17±17 g), Mean weight gain (153.10±18%), FCR (2.65±0.07), DWG (0.68±0.05 g/days), Yield (265.31±15.11 g) and Survival rate (86.1±1.18) in Treatment T-5, where the shrimps fed with all the three different probiotic strains such as, Bacillus sp, *Lactobacillus sp* and *Arthrobacter sp* incorporated feed (1×10^6 CFU mL^{-1}) followed by T-3, T-4, T-2 treatments and control groups.

The first bioassay results indicates that, incorporating a mixture (consortium) of Bacillus sp, *Lactobacillus sp* and *Arthrobacter sp* as feed probiotics in the concentration of 1×10^6 CFU mL^{-1} along with *Nitrobacter* sp and *Nitrosomonas* sp each in the concentration of 5×10^2 CFU/ml through water (Weekly once) will absolutely provide additional support against the unfavorable conditions or pathogen attacks during the culture and increased significant survival and yield. Other studies previously demonstrated enhanced protection with multi-species probiotics (Timmerman *et al*., 2007; Zoppi *et al*., 2001), based on the theory that multiple species-specific benefits possessed broaden spectrum of probiotic effect. Indeed, three LAB probiotics were effective against *Vibrio harveyi*, *V. parahaemolyticus* and *Pseudoalteromonas piscicida* in an *in vitro* assay (Talpur *et al*., 2012). The higher survival of shrimp fed with probiotic supplemented feed might be related to an immune reactive effect of probiotics on the host immune system, by producing extracellular compounds to stimulate the non specific immune response in vertebrates (Marteau *et al*., 2002; Gill, 2001).

Effectiveness of bacterial mixtures on control of Vibrio load

While checking the vibrio load in the culture water and shrimp (in both control and experimental groups) on 7th, 14th and 21st day, the higher Vibrio load was observed in shrimp intestine and culture water where the shrimps fed with control diet, and it was decreased in treatment (T- II), where the shrimps were fed with all the three different probiotic strains such as, Bacillus sp, *Lactobacillus sp* and *Arthrobacter sp* incorporated feed (10^6 CFU mL^{-1}). Far *et al*., 2009 as also suggested that the use of Bacillus sp as a probiotic in shrimp culture will colonize both the culture water and the shrimp digestive tract and also replace *Vibrio* spp. in the gut of the shrimp, thereby increasing shrimp survival. Abrashev *et al.* (1998), have reported that, some *Arthrobacter sp*ecies have the ability to produce a number of valuable substances like amino acids, vitamins, enzymes, specific growth factors, and polysaccharides and its possess many advantageous properties and nutritional benefits to be probiotics in aquaculture (Li *et al*., 2006). Recently, Amnah, (2013) have reported that the *Arthrobacter sp* can be regarded as a probiotic bacterium for the culture of shrimp while *β*-1,3 glucan and, *Moringa oleifera* leaf were considered as immunostimulants for cultured of shrimp *Penaeus indicus* Juvenile against pathogenic vibrios Juvenile. Natesan *et al*., 2012 also observed the maximum zone of inhibition (16mm) against *V. alginolyticus* using their strain *L. acidophilus* 04. The previous authors also described that, the antibacterial activity of *Lactobacillus sp* against the pathogenic microbes may be due to the production of its metabolites such as, organic acids (lactic and acetic acid), hydrogen peroxide, diacetyl and bacteriocins (Valenzuela *et al*., 2010).

Effectiveness of bacterial mixtures on Bioassay II

WSSV is a large dsDNA virus infecting crustaceans and is the most important viral pathogen of cultured penaeid shrimp worldwide. In cultured shrimp, WSSV causes a cumulative mortality of up to 100% within 3-10 days. Due to its rapid spread and high associated mortality rates, WSSV is an extremely virulent pathogen in shrimp culture (James *et al*., 2010). Hence, in this study, the second bioassay was conducted for 21 days with shrimp weighing (12.8 ± 1.8 g). After acclimation of seven days with probiotic diet, animals in the control and experimental treatments were fed with only 1 g per tank of muscle shrimp paste positive for WSSV and confirmed positive for the White Spot Syndrome Virus. During the experimental period, the temperature (26 – 28°C), salinity (25 – 28%), total ammonium (0 – 0.1 mgl−1), nitrite (0 – 0.05 mgl−1) and pH (7.0 – 7.6) were maintained in the suitable range and found to be stable in all the control and experimental tanks. After 21 days of culture, the maximum shrimp survival (46%) was observed in treatment II comparatively than control (28%), and the shrimps from both control and experiments were confirmed with WSSV positive. As the same beneficial microbes also play a vital role in aqua ponds therefore using commercially available water and soil probiotic products such as, Grobac, Good Earth, Prorich and Nature - 365 (Guybro Chemical Pvt Ltd, Mumbai) which enhances the crop

production in aquaculture sector. Farmers feedback and trial reports of these products also have proven that the product works effectively *viz.*, oxidize organic matter into enzymes and available nutrients, produce antimicrobial compounds and eliminates pathogens, removes bad odor, sludge and black soil from the pond bottom, biological oxidation of ammonia and ammonium to nitrite, biological reduction of nitrite into useful nitrate and improves the growth of beneficial microbes and plankton under different regions and farming situations in India and as well as in other countries.

From the results, it has been suggested that the, the consortium of these potential and/or beneficial probiotic bacterial consortium have not eliminated the WSSV infection in cultured shrimps, but increased the survival rate and decreased the vibrio load in the culture systems and water.

A perusal of literature revealed thus far no reports on the effect of incorporating a mixture (consortium) of Bacillus sp, *Lactobacillus sp* and *Arthrobacter sp* as probiotics in shrimp feed and WSSV prevalence in *L. vannamei* or other penaeid shrimp. Similarly, Partida Arangure *et al*., 2012, reported that, inulin and probiotic bacteria increased the shrimp survival of 100% and a decrease in the prevalence of WSSV (22.2%) in shrimp fed inulin (8.0 g/kg feed) and bacteria (1 x 105 CFU/g feed) compared with control (44.4 and 51.8%).

However, study of the antiviral activity of the probiotics is an upcoming research which needs a deep insight. Further research works should be needed to adapt the WSSV to the BHK cell lines, to study their antiviral efficacy and cytopathic effect (CPE) in the BHK cell lines for the qualitative assessment of antiviral efficacy of the probiotic bacteria strains used in this study.

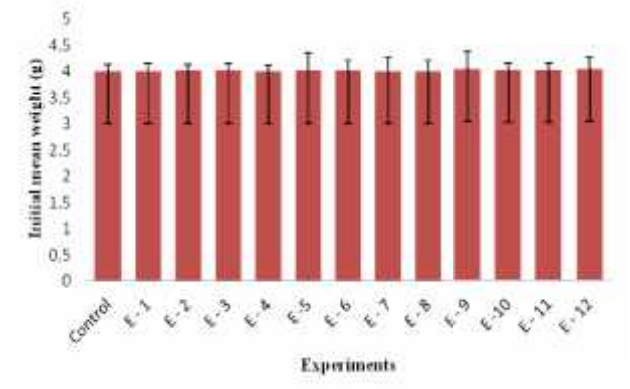

Fig 1 Initial mean weight (g) of *L. Vannamei*

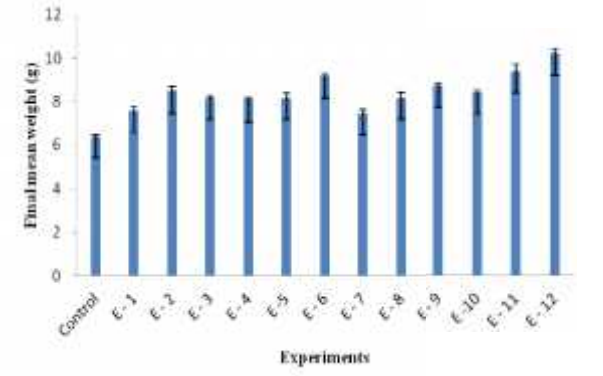

Fig 2 Final mean weight (g) of *L. vannamei*

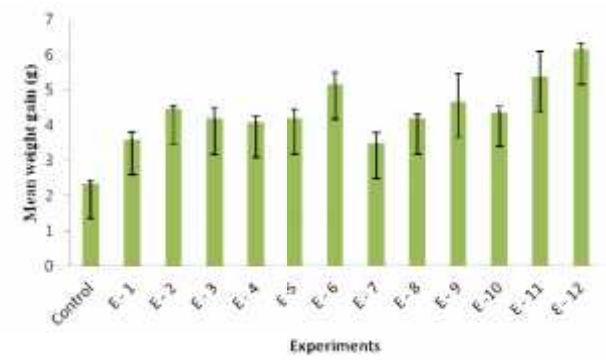

Fig 3 Mean weight gain (g) of *L. vannamei*

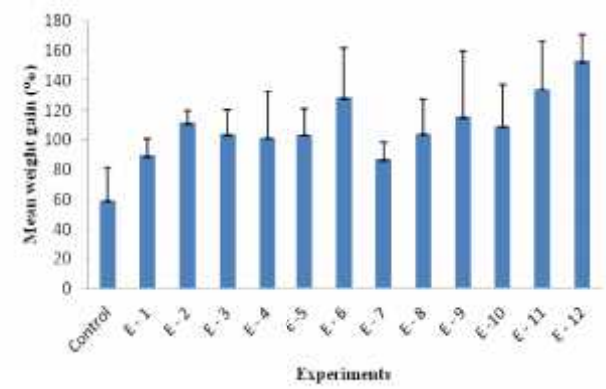

Fig 4 Mean weight gain (%) of *L. vannamei*

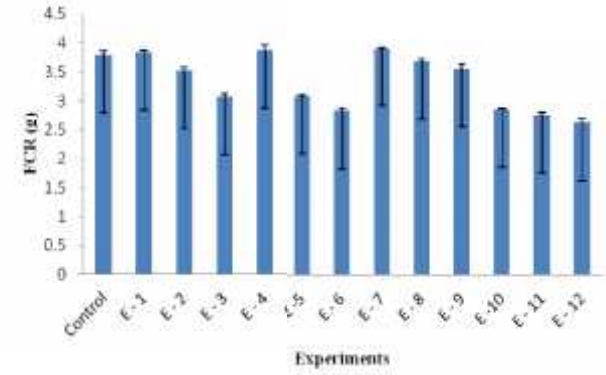

Fig 5 FCR (g) of *L. vannamei*

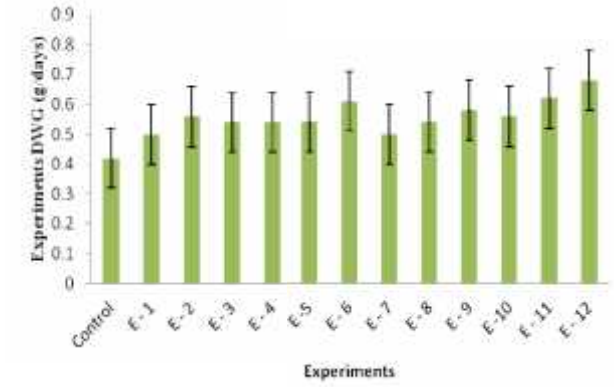

Fig 6 DWG (g/days) of *L. vannamei*

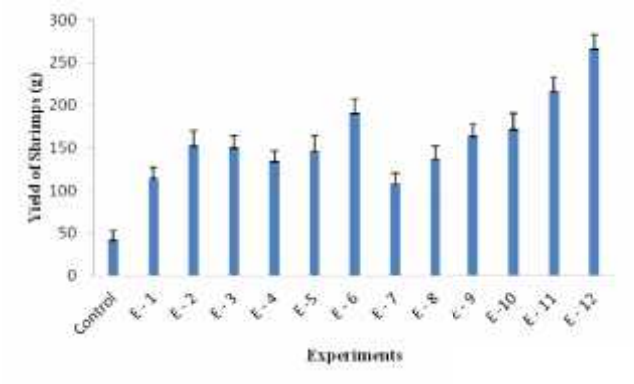

Fig 7 Yield of Shrimps (g) of *L. vannamei*

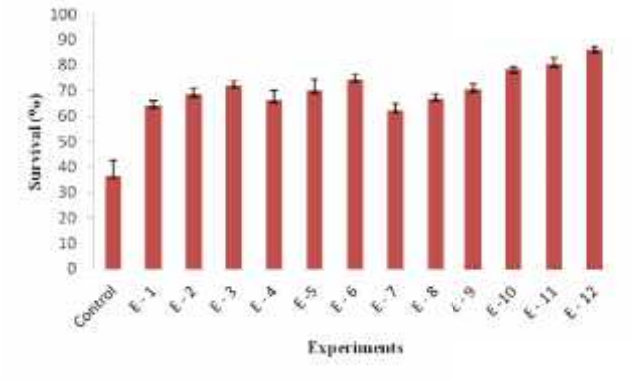

Fig 8 Survival (%) of *L. vannamei*

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Citation : GOOD PERFORMANCE IN CAT

P.N. Mauthake

HOD

Department of Petroleum Engineering

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Citation : ACTIVE IN CLASS & GOOD ATTENDANCE Y.

HOD
Department of Petroleum Engineering

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Citation : ACTIVE & GOOD COMMUNICATION SKILL

Prof. A. Kawitkar

HOD

Department of Petroleum Engineering

BEST STUDENT

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DEPARTMENT OF MECHANICAL ENGINEERING

PROFORMA OF LIST OF ADVANCED LEARNERS

Academic Year: 2014-18 (Batch 1)

S. No	Reg. No	Name of the Student	Selection Criteria*	Remarks
1.	BME14145	RANJITH MENON.D	a, b, d	Good academic performance, better modern tool usage & Good communication skills
2.	BME14159	VENKATSRINIVASAN.S	a, c, d	Good academic performance, better modern tool usage and Leadership quality and team work
3.	BME14138	NARAYANA SWAMY.S.B	a, c	Students with better modern tool usage and good academic performance
4.	BME14161	ASISH THOMAS	a, b, c	Students with good problem solving and creative skills and good academic performance
5.	BME14114	AKHIL GEEVARGHESE	a, d	Good academic performance ,Team work and good communication skills
6.	BME14111	ABDUR RAHMAN. KM	b ,c	Student with good creative skills and better modern tool usage
7.	BME14153	SANTHOSH. S	a, b	Good academic performance, good problem solving and creative skills
8.	BME14120	AVINASH.M	c, d	Students with better modern tool usage and good communication skills
9.	BME14149	RUBAN KUMAR S	b ,c	Student with good creative skills Team work
10.	BME14162	PIYUSH KUMAR	b, c	Team work & Good problem solving skill
11.	BME14129	GOPALA KRISHNAN.K	b, c	Student with good Creativity and Team work skill
12.	BME14158	TAMIZH THENDRAL. U	b, d	Leadership quality and team work
13.	BME14116	ALEX DEEPAN. P	d	Team work and good communication skills

Selection Criteria*

a) Based on student previous academic performance

b) Students with good problem solving and creative skills

c) Students with better modern tool usage

d) Team work and good communication skills

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DEPARTMENT OF MECHANICAL ENGINEERING

PROFORMA OF LIST OF ADVANCED LEARNERS

Academic Year: 2015-19 (Batch 2)

S. No	Reg. No	Name of the Student	Selection Criteria*	Remarks
1.	BME15009	GAUTHAM. MAHESH	a, c, d	Good academic performance, better modern tool usage and good communication skill
2.	BME15036	VINOTH. E	a, b	Students with better modern tool usage and academic performance
3.	BME15011	GIRIDHARAN.S	a, c	Leadership quality and team work
4.	BME15031	SURENDRA PAL	a, b , c	Students with good problem solving and creative skills and better modern tool usage
5.	BME15024	SATHISH KUMAR B	a, c ,d	Good academic performance, better modern tool usage and teamwork
6.	BME15042	GANESH. A	a , c	Student with better modern tool usage and Good academic performance
7.	BME15025	SEBASTIAN ANJOS	a , d	Good academic performance and teamwork
8.	BME15023	ROHIT SINGH	a , b	Good problem solving & Creativity skill with good academic performance
9.	BME15016	MOHIT MOHANDAS	b , c	Student with better modern tool usage and good problem solving skills
10.	BME15002	ABHAY PRATAP SINGH	a, d	Student with good communication skill with good academic performance
11.	BME15029	SUMANTHRAJ.R	a , c	Student with better modern tool usage and academic performance

12.	BME15020	PRATHAMESH SANJAY PATIL	a, c , d	Student with better modern tool usage and good communication skills and better academic perfomance
13.	BME15037	VIVEKANANDAN.D	a , b	Student with better modern tool usage and good creativity skill
14.	BME15018	NIKHIL FERNANDO.L	d	Team work and good communication skills
15.	BME15012	GOPINATH M	a , c	Student with better modern tool usage and better academic performance
16.	BME15033	TAMIL SELVAN.M	c , d	Student with better modern tool usage & Team work skill
17.	BME15005	ARAVINDHAN A	b	Good problem solving and creativity skills
18.	BME15043L	SAHIL OBEROI	c , d	Student with better modern tool usage, Leadership quality and team work

Selection Criteria*

a) Based on student previous academic performance

b) Students with good problem solving and creative skills

c) Students with better modern tool usage

d) Team work and good communication skills

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