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Effect of mangrove plant (*Sesuvium portulacastrum*) extract against *Vibrio harveyi* during shrimp larviculture

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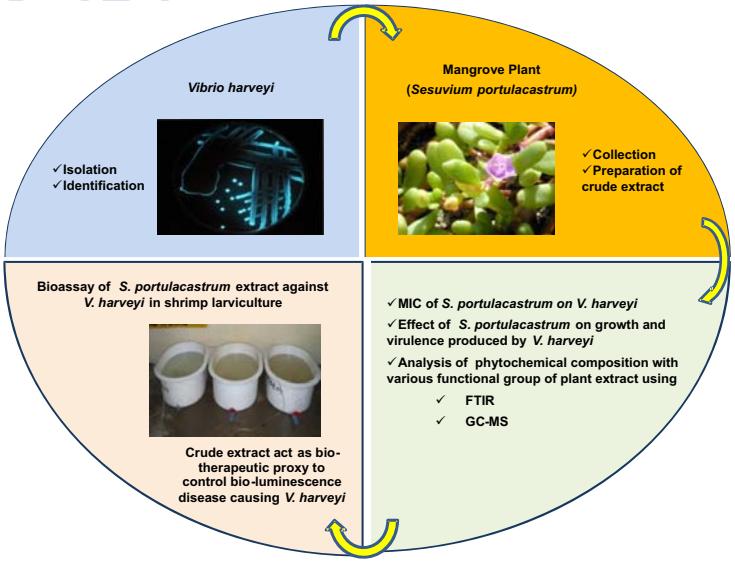
Abstract

Aim : The present study aimed at finding a potential antagonistic activity of the leaves of mangrove plant *Sesuvium portulacastrum* against pathogenic *Vibrio harveyi* during shrimp larviculture.

Methodology : Crude extract obtained from the leaves of mangrove plant *S. portulacastrum* was screened for its antagonistic activity against *V. harveyi* and the minimum inhibitory concentration was determined. Crude extract was characterized for its phytochemical composition by GC-MS and FTIR analysis. The antagonistic activity of *S. portulacastrum* crude extract was tested against *V. harveyi* during *Penaeus monodon* larviculture.

Results : MIC of *S. portulacastrum* extract was 100 µg against 1×10^{8}CFUml^{-1}of *V. harveyi*. When *V. harveyi* was cultured in Luria Bertani broth with crude extract of *S. portulacastrum* (100 µg ml^{-1}) the growth and its virulence factors became weak. The cumulative percentage on mortality of postlarvae was 54% in 30 days, whereas control tank showed 67% mortality. Differences in reduction on the cumulative percentage mortality in the treatment tank was 13% as compared to control. Using statistical analysis, significant differences ($P<0.05$) were observed between control and treatment.

Interpretation : This study endorse that crude extract of *S. portulacastrum* can be used as non-antibiotic and bio-therapeutic proxy to control bio-luminescence disease causing *V. harveyi* in the shrimp larviculture practice.



Introduction

Vibrio harveyi is one of the primary and virulent bacterial pathogen of Vibrionaceae family, which has been documented as causative agent for large-scale mortality in all the stages of *Penaeid* shrimp hatchery systems. Inappropriate application of chemicals or disinfectants for controlling *Vibrio* outbreaks could outcome in the progress of resistant among the bacteria (Vaseeharan *et al*., 2010). Dissimilar technologies have been familiarized to govern bio-luminescent bacteria in the shrimp grow-out and larviculture systems. "Green-water culture system" which is an innovative technique used in aquaculture, wherein the shrimps or fish are co-cultured with abundant microalgae such as *Chlorella*, but yet to be proved in farming trials. Probiotic bacteria have been used comprehensively for controlling various fish diseases, but high doses are indispensable and unable to maintain in many cases (Defoirdt *et al*., 2007). Therefore, the quest for alternate methods to control infection caused by antibiotic-resistant *Vibrios* are imperative challenge for sustainable development of aquaculture. Henceforth, in lieu of the chemical preservatives alternative bio-inhibitors extracted from marine plants may be used to control marine *Vibrios*.

Mangrove plants have primary and secondary metabolites such as proteins, carbohydrates, carotenoids, hydrocarbons, aliphatic alcohols, polyunsaturated fatty acids, lipids, pheromones, phorbol esters, phenolics, steroids, terpens, tannins and glycosides etc. (Bandaranayake, 2002). These metabolites were described for bioactive substances as bactericidal, fungicidal, pharmaceutical agents for animal and human beings (Suryati *et al*., 2002). Among the mangrove associated plants, *Sesuvium portulacastrum* is known as "*Sea purslane"* belongs to family *Aizoaceae* and grows in the Mediterranean coast and sub-tropical areas around the world. In traditional medicine, *Sesuvium* has been used for the treatment of conjunctivitis, leprosy, dermatitis and toothache (Chandrasekaran *et al.,* 2011). *S. portulacastrum* has a long history of use in folk medicine in countries such as Zimbabwe and South Africa. Abirami and Rameswari (2013) had described anti-*Vibrio cholera* activity against the chloroform and petroleum ether extracts obtained from *S. portulacastrum*. Oil was extracted from the fresh leaves of *S. portulacastrum* and used as antagonistic agent against bacteria and fungi (Michael *et al*., 2006). Therefore, the present study divulges that the antagonism of crude ethyl acetate extracts of *S. portulacastrum* against growth, virulence factors of *V. harveyi* and its effect on *V. harveyi* during larviculture of *Penaeus monodon*.

Materials and Methods

Isolation of microorganism : *V. harveyi* was isolated from the water samples collected from Muttukadu Experimental Station (MES) of ICAR-CIBA at Chennai. Isolates were identified by various bio-chemical tests such as arginine dihydrolase (-), lysine (+), ornithine decarboxylase (+), gelatinase (+), Vogesproskauer (-), D-glucosamine (-), etc (Abraham and Palaniappan, 2004) and compared with standard type strain *V. harveyi* ATCC 25919. The isolates were re-confirmed by streaking in *V. harveyi* Selective agar (VHSA) and stored in VHSA slants at 4°C (Harris *et al*., 1996).

Collection of *S. portulacastrum* and preparation of extract : *Sesuvium portulacastrum* was collected from MES of ICAR-CIBA, Tamil Nadu, India. The leaves were shade dried at room temperature then pulverized by a mechanical grinder and stored in an airtight container**.** Fourty g of *S. portulacastrum* powder was extracted with 200 ml of ethyl acetate (boiling point 76 -78°C) by Soxhlet apparatus.

Antagonism of *S. portulacastrum leaf* extract against *V. harveyi* by Well diffusion method : The crude extracts obtained from leaf of *S. portulacastrum* were subjected to antimicrobial assay through "Agar well diffusion assay". One hundred ml of Luria Bertani (LB) agar medium was prepared, inoculated with 100 µl of *V. harveyi* (1.8 OD), mixed thoroughly and poured into Petri-dishes in replicates. Appropriate number of circular wells each 6 mm was cut in the solidified agar medium. A drop of soft agar (0.85%) was used to seal the well. The wells were filled with extracts of various concentrations after dissolving in 30% Dimethylsulfoxide (DMSO). The inoculated plates were incubated at 28ºC for 24 h. Zone of inhibitions were measured and tabulated (Ravikumar *et al*., 2011).

Fourier transform infra-red spectroscopy (FTIR) analysis : The shade dried *S. portulacastrum* leaves were ground as a fine powder with a pestle and mortar. The FTIR spectra were recorded using BRUKER IFS 66 model FTIR spectrometer in the region 4000–400 cm^{-1} by employing standard KBr pellet technique (D'Souza *et al*., 2008).

Gas chromatography and mass spectrometry analysis (GC-MS) : Gas chromatography-mass Spectrometry (GC-MS) analysis for Ethyl acetate extract of *S. portulacastrum* was performed by using Agilent GC-MS-5975C with the Triple-Axis Detector equipped with an auto sampler. Peak identification of crude extract was performed by comparing with retention times of standards and the mass spectrum obtained was compared with those available in the NIST libraries (NIST 11- Mass Spectral Library 2011 version) with an acceptance criterion of a match above critical factor 80% (Musharraf *et al*., 2012).

Effect of *S. portulacastrum* extracts against the growth and virulence's produced by *V. harveyi* : LB broth of each 100 ml was inoculated with 1.0 ml of *V. harveyi* (1.8 OD) separately for control (without crude extract of *Sesuvium* leaves) and treatment (with crude extract of *Sesuvium*). One 100 µg of extract per ml of LB broth was added and shaker incubated at 28°C/100 rpm for 5 days. Growth and luminescence produced by *V. harveyi,* both in

the treatment as well as control was monitored for 5 days (Kannappan *et al*., 2013) using spectrophotometer (OD at 600 nm) and Luminometer (Victor TM-X3, Perkin Elmer, USA).

Determination of virulence factors produced by *V. harveyi* : Two ml of *S. portulacastrum* extract was added in LB broth was taken every day from control and treatment. Various virulence factors such as phospholipase activity, crude extracellular protein (bacteriocin), protease and luciferase produced by *V. harveyi* were determined. Cells harvested from the broth were spot-inoculated onto nutrient agar separately with 5% egg yolk for phospholipase test (Manilal *et al.,* 2010). The plates were then incubated at 37°C/24 h. The enzymatic activity of the bacteria (control and treated) was detected by observing the presence of clear zones around the spot.

Antagonism of *S. portulacastrum* against *V. harveyi*

Determination of protease using azocasein : The supernatant of *S. portulacastrum* leaves extract added in LB broth was used as crude protease enzyme and analyzed for protease activity (Olajuyigbe and Ajele, 2005). Protease enzyme activity was assessed using 500 µl of 0.5% azocasein (w/v) (Sigma, USA) in Tris HCl buffer with 100 µl enzyme solution and incubated for 60 min at 37°C. The reaction was stopped by adding 500 µl of 15% Trichloro acetic acid, incubated at 30°C/ 15 min and then centrifuged at 4°C/15 min at 3000 rpm. One ml of supernatant was added with 1.0 ml of NaOH and absorbance was measured at 440 nm (One unit of protease activity is defined to be the amount of enzyme required to produce an absorbance change of 1.0 in a 1.0 cm cuvette under the condition of the assay). For estimating crude extracellular protein (bacteriocin), the cell suspension from *S. portulacastrum* extract added in LB broth from control and treatment were centrifuged at 5000 × g for 10 min. The cells were separated out and supernatant was used for estimating the crude extracellular protein (Lowry *et al*., 1951).

Salt aggregation test : Adherence of *V. harveyi* was determined by Salt aggregation test. The cells obtained from the broth (treated and control) were washed with sterile 2.5% NaCl. The cells were suspended in sodium phosphate buffer (pH 6.8) and OD was adjusted to 1.0 at 420 nm. Fifty micro litre of cell suspension was mixed with equal volume of various molarities of ammonium sulphate ranged from 0.05 to 4.0 M (molarity) in 96 well plates, kept at room temperature and observed for agglutination. The SAT values were recorded as lowest molarity of ammonium sulfate giving visible bacterial aggregation while evaluating bacterial cell surface hydrophobicity. Strains with SAT values >4.0 M, 2.0 - 4.0 M, 1.0 - 2.0 M and 0.0 - 1.0 M were designated as no, low, moderate and high hydrophobicity, respectively (Qiao *et al*., 2012).

Bioassay of *S. portulacastrum* leaf extract against *V. harveyi* during shrimp larviculture : Plastic tanks (30 litre) were washed with 1% $KMnO_4$ solution and filled with 10 litre of 18 ± 1 PSU low saline water. Disease free postlarvae (PL 15 days) of *Penaeus monodon*, procured from private shrimp hatchery were acclimatized in 18 PSU for 3 days before starting the experiment at 28±1°C with continuous aeration. The postlarvae (PL) had an average body weight of 18-19 mg and 450 numbers of PL were stocked in each experimental tanks. Control tank was inoculated with 10 ml *V. harveyi* (1.8 OD) alone and the treatment tank was inoculated with 10 ml *V. harveyi* (1.8 OD) with crude extract of *S. portulacastrum* (100 µg ml^{-1}). The experiment was conducted for 30 days without exchanging water and PL feed given twice a day. The tanks were covered to evade air-borne contamination and the effect of *S. portulacastrum* leaf extract was tested against *V. harveyi* during shrimp larviculture. Dead PL were collected and cumulative percentage mortality was ascertained from the tanks. Water quality parameters like salinity, temperature, and pH were measured using salinometer, thermometer and pH meter respectively (Kannappan *et al*., 2012). Enumerations of total heterotrophic bacteria (THB) and *V. harveyi* counts (Harris *et al.,* 1996) were determined both in the treatment and control tanks using Trypticase soya agar (TSA) and *V. harveyi* selective agar medium (VHA) for 5 days. All the experimental values were average of three replications, expressed with standard deviation.

Statistical analysis : The replicated data obtained from the *S. portulacastrum* extract against growth and virulence factors of *V. harveyi* with cumulative percentage of mortality (CPM) during *P. monodon* larviculture were expressed with mean value ± standard deviation. CPM was calculated by Cumulative frequency / total number of observations (n) × 100. Analysis of variance following the statistical programme for the social sciences (SPSS, ver. 16.0) was used to assess the significance ($P<0.05$) between control and treatment.

Results and Discussion

The minimum inhibitory concentration (MIC) of *S. portulacastrum* extract was 100 µl (1 mg ml^{-1}) against 100 µl of $1x10^8$ CFU ml^{-1} of *V. harveyi* as determined by "Agar well diffusion assay". But *S. portulacastrum* extract showed MIC and MBC of 50 µg ml^{-1} against *Staphylococcus aureus* and *E. coli* (Al-Azzawi *et al*., 2012). Crude *S. portulacastrum* extract showed a maximum zone of inhibition, 13.9 mm (excluding the well) against *V. harveyi* at 200 µg ml^{-1} concentration, whereas no inhibition was observed when DMSO (30%) was used as positive control against *V.*

Table 1 : Antagonism of crude leaf extract of *S. portulacastrum* against *V. harveyi*

Antagonism	Concentration (µg)	Zone of inhibition (mm)
Crude extract	100	9.4 ± 0.2
Crude extract	150	11.3 ± 0.4
Crude extract	200	13.9 ± 0.6
Control (30 % DMSO)	50 (µl)	ND

Values are mean ± S.D. for three observations, ND = Not detected

Table 2 : GC-MS analysis of various compounds present in the leaf extract of *S. portulacastrum*

Peak No.	Retention time (min)	Compound name	Peak area (%)	Molecular formula	Molecular weight
1	9.375	Pyrazine carboxamide, 3,4-dihydro-3-oxo-	2.28	$C_5H_5N_3O_2$	139.112
2	12.585	Decane, 2- methyl-	2.28	$C_{11}H_{24}$	156.308
3	13.456	2 (3H) – Benzoxazolone	50.09	$C_7H_5NO_2$	135.12
4	13.573	2 (4H) - Benzofuranone, 5,6,7,7a-tetra hydro - 4,4,7a-trimethyl-	7.51	$C_{11}H_{16}O_2$	180.243
5	14.313	Tetradecane	1.76	$C_{14}H_{30}$	198.388
6	15.461	Eicosane, 10 - methyl-	3.01	$C_{21}H_{44}$	296.574
7	16.55	Hentriacontane	1.12	$C_{31}H_{64}$	436.839
8	16.942	Bicyclo [4.1.0] heptane, 2-methyl-	6.08	C_8H_{14}	110.109
9	17.000	2-Pentadecanone, 6,10,14-trimethyl	6.75	$C_{18}H_{36}O$	268.477
10	17.378	3,7,11,15 - Tetramethyl-2-hexadecen- 1-ol	2.43	$C_{20}H_{40}O$	296.531
11	18.162	n-Hexadecanoic acid	7.18	$C_{16}H_{32}O_2$	256.424
12	18.598	Ethylamine, 2-(adamantan-1-yl)-1-methyl-	1.74	$C_{13}H_{23}N$	193.183
13	19.542	3,3 - Dimethyl - 4- methylamino-butan-2-one	1.89	$C_7H_{15}NO$	129.115
14	19.861	4,6- dimethyl - 2-propyl -1,3,5-dithiazinane	3.65	$C_8H_{17}NS_2$	191.08
15	19.905	L-Alanine-4-nitroanilide	2.24	$C_9H_{11}N_3O_3$	209.201

Table 3 : Various functional groups of compounds detected in leaf extract of *S. portulacastrum* by FTIR

Frequency (cm^{-1})	Bond	Functional group
2921.8	C–H stretch, O–H stretch	Alkanes, carboxylic acids
1639.1	C–C stretch (in–ring)	Aromatics
1446.2	C–O stretch, C–N stretch	alcohols, carboxylic acids,
1046.2	C–Cl stretch,C–Br stretch	esters, ethers
596.2		aliphatic amines, alkyl halides

Table 4 : Effect of crude *S. portulacastrum* leaf extract against growth and virulence factors of *V. harveyi*

Day	Growth of *V. harveyi* (600 nm)		Luciferase activity (CPS)		Phospholipase activity		Salt aggregation test (M - Molarity)		Bacteriocin (660 nm)		Protease (440 nm)	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treatment	Control	Treatment	Control
1	0.41 ± 0.05	0.47 ± 0.01	170	254	ND	+++	1.00	0.75	1.36±0.01	1.79±0.03	0.22±0.04	0.30±0.01
2	0.38 ± 0.02	1.01 ± 0.09	154	216	ND	+++	1.25	0.50	1.21±0.01	1.86±0.07	0.19±0.07	0.33±0.08
3	0.36 ± 0.02	0.98 ± 0.02	104	224	+	++++	2.00	0.75	1.18±0.02	1.90±0.20	0.14±0.09	0.38±0.06
4	0.34 ± 0.00	1.03 ± 0.01	115	138	+	++++	1.75	1.00	1.16±0.02	1.53±0.10	0.118±0.01	0.44±0.03
5	0.28 ± 0.01	1.11 ± 0.01	143	198	++	++++	2.75	0.75	0.83±0.01	2.04±0.11	0.081±0.06	0.48±0.09

Control : *V. harveyi* untreated with crude extract and treated: treatment of crude extract of *S. portulacastrum*; Here : + = low or weak, ++ = moderate, +++ = high, ++++ = very high, ND= not detected

harveyi. Paulpriya *et al.* (2013) reported that *S. portulacastrum* extract had phenolic and flavanoid compounds, which were responsible for bacterial inhibition. Various concentrations of *S. portulacastrum* extract showed antagonism against *V. harveyi* (Table 1).

Chandrasekaran *et al*. (2011) described antimicrobial activity of *S. portulacastrum* extract against human pathogenic bacteria. The essential oil extracted from *S. portulacastrum* exhibited antagonism against *Acetobacter calcoacetica*, *B.subtillis*, *Clostridium sporogenes*, *C. perfringens*, *E.coli*, *Salmonella typhii*, *Staphylococcus aureus* and *Yersinia enterocolitica* (Magwa *et al*., 2006). The antimicrobial activity of leaf extract obtained from *S. portulacastrum* shown antagonism against *S. aureus*, *E. coli, Klebsiella pneumonia, V. cholerae* and *Pseudomonas aeruginosa* (Abirami and Rameshwari, 2013). Chandrasekaran *et al*. (2011) reported higher amount of saturated and unsaturated fatty acids from *S. portulacastrum*, which might be responsible for antagonism. The GC-MS profile of ethyl acetate extract obtained from *S. portulacastrum* leaf shown several compounds such as 2(3H)-Benzoxazolone (50.09%),2(4H)-Benzofuranone, 5,6,7,7a-tetra-hydro-4,4,7a-trimethyl (7.51%) and n-Hexadecanoic acid (7.18%) (Table 2).

Table 5 : Bioassay of crude extract from *S. portulacastrum* leaf extract against *V. harveyi* with cumulative percentage mortality reduction on *P. monodon* larviculture

Day	Cumulative percentage mortality reduction				Treatment tank (CFU ml^{-1})		Control tanks (CFU ml^{-1})		Average body weight of postlarvae (mg)		Changes on water quality parameters-treatment and control tubs			
	Control tank with *V. harveyi*	Treatment with *Sesuvium* extract with *V. harveyi*	Treatment with *Sesuvium* extract and PL alone	Treatment with PL alone	Total plate count	*V. harveyi* count	Total Plate count	*V. harveyi* count	Treatment tubs	Control tubs	Temp. (ºC)	Salinity (PSU)	pH in control tubs	pH in treatment tubs
0	0	0	0	0	2.10×10^{7}	4.50×10^{7}	2.40×10^{7}	4.40×10^{7}	18.9 ± 2	17.3 ± 3	30 ± 1.0	18 ± 1.0	8.3 ± 0.2	8.2 ± 0.2
5th	9.5 ± 0.3	4.3 ± 0.2	3.9 ± 0.1	3.1 ± 0.1	1.60×10^{8}	1.47×10^{6}	2.42×10^{8}	1.97×10^{6}	60.5 ± 5	55.6 ± 9	31 ± 1.0	19 ± 0.5	8.3 ± 0.2	8.3 ± 0.2
10th	12.2 ± 0.9	9.5 ± 0.3	6.8 ± 0.5	6.3 ± 0.2	1.18×10^{8}	1.00×10^{6}	1.70×10^{8}	1.57×10^{6}	134.3 ± 8	128.3 ± 7	30 ± 1.0	20 ± 0.5	8.3 ± 0.2	8.1 ± 0.2
15th	21.4 ± 1.2	18.3 ± 1.5	11.7 ± 0.4	11.6 ± 0.5	7.20×10^{5}	5.20×10^{5}	8.40×10^{5}	9.10×10^{5}	168.6 ± 10	163.8 ± 13	31 ± 1.0	20 ± 0.5	8.2 ± 0.2	8.3 ± 0.2
20th	46.3 ± 1.6	34.2 ± 1.4	17.1 ± 0.6	17.9 ± 0.5	1.80×10^{5}	1.20×10^{5}	5.10×10^{5}	2.70×10^{5}	216.9 ± 11	212.9 ± 12	31 ± 1.0	21 ± 0.5	8.3 ± 0.2	8.1 ± 0.2
25th	58.6 ± 2.1	46.5 ± 1.7	21.6 ± 0.6	21.4 ± 1.0	1.50×10^{5}	8.00×10^{4}	3.70×10^{5}	2.40×10^{5}	264.3 ± 14	260.2 ± 16	30 ± 1.0	21 ± 0.5	8.3 ± 0.2	8.3 ± 0.2
30th	66.8 ± 2.3	53.8 ± 2.5	26.6 ± 1.1	27.2 ± 1.0	5.60×10^{4}	3.73×10^{3}	2.90×10^{5}	2.40×10^{5}	289.6 ± 23	287.9 ± 21	31 ± 1.0	21 ± 0.5	8.2 ± 0.2	8.3 ± 0.2

Values are average of three determinations with standard deviation

FTIR analysis, (Table 3) of the crude extract of *S. portulacastrum* showed various functional compounds such as alkanes, aromatics, alcohols, carboxylic acids, esters, ethers, aliphatic amines, alkyl halides, etc. Sheela and Udayakumari (2013) reported about 1-Monolinoleoyl-glycerol trimethylsilyl-esther (19.29%) and Vitamin E (44.79%) in the leaf extract and Benzoic acid, 4-ethoxy-ethyl ester (23.03%), Oleic acid (15.99%) as chief phyto-constituents in the stem of *S. portulacastrum*. However, *S. portulacastrum* extract prepared from the leaves were reported to have 2 (3H)–Benzoxazolone at high level (50.09%) followed by Benzofuranone (7.51%) and Hexadecanoic acid respectively. In the present study, the extracts of *S. portulacastrum* using different solvents, showed various phytochemical compounds based on their solubility. The antagonistic effect of *S. portulacastrum* might be due to presence of phytochemicals in the extract.

The extract of *S. portulacastrum* was suppressed the growth of *V. harveyi* from 0.41 OD to 0.28 OD for first 5 days in treatment whereas growth increased from 0.47 to 1.11 OD in control. Similarly, virulence activities such as luciferase, bio-luminescence (Table 4), phospholipase became weak. But in control these activities were strong. Correspondingly, in treatment crude bacteriocin was reduced from 1.36 to 0.83 OD as compared to control (1.79 to 2.04 OD) and the level of protease production also decreased from 0.22 to 0.08 OD. These values were statistically interpreted and significant differences ($P<0.05$) between the leaf extract of *S. portulacastrum* treated against *V. harveyi* and control.

Hydrophobic microbes are capable of adhering to the oil/water interface and utilizing oil components as a source of energy for growth and metabolism. This hydrophobic nature would also help the bacteria to adhere on the external surfaces and tissues of a host which is in initial step in the infection of a host and subsequent occurrence of disease. It was broadly accepted that hydrophobic and biofilm productions were the major factors in the adhesive process and survival of pathogens in cells (Zoueki *et al.,* 2010). But, *S. portulacastrum* extract treated *V. harveyi,* cells showed SAT values in between 1.00 - 2.75 M (Table 4) which was indicates hydrophobic nature of bacteria. Qiao *et al.* (2012) also reported that, *V. harveyi* cells were more hydrophobic in nature. Under larviculture experiment, the cumulative percentage of mortality value increased in control due to *V. harveyi* (Sivakumar *et al.,* 2014) from 9 to 67% in 5th to 30th days. But in treatment, the CPM was reduced from 4 to 54% till 30th day. The difference among the CPM in the treatment tank was 13% as compared to control (Table 5). The essential oil of *S. portulacastrum* exhibited antibacterial activity against *Acetobacter calcoacetica*, *B. subtillis*, *C. porogenes*, *C. perfringens*, *E.coli*, *S. typhii*, *S.aureus* and *Yersinia enterocolitica* (Michael *et al.,* 2006). Crude aqueous extract of *S. portulacastrum,* even show significant enhancement on growth and metabolism in *Labeo rohita* fish (Johnson and Banerji, 2007).

The water quality parameters were observed as quiet normal and much dissimilarity was not observed due to treatment. The average initial load of *V. harveyi* was 4.45×10^7 CFU ml^{-1} during the first day of treatment and control tanks. The growth was then reduced to 1.47×10^6 to 3.73×10^3 CFU ml^{-1} respectively in the treatment tank as compared to control tank 1.97×10^6 to2.40×10^5CFU ml^{-1}. The inhibitory activity may be due to the antioxidant, phenolic and flavonoids compounds present in the bark and leaf extracts of *S. portulacastrum* (Singh *et al.,* 2013). It has been proved that mangrove plant extract controls the infection caused by *V. alginolyticus* in Clown fish (Dhayanithi *et al.,* 2012). Recent study showed that the essential oil extracted from the leaves of *Sesuvium* showed significant antifungal and antioxidant activity (Magawa *et al.,* 2006).

The results of the present study revealed that the concentration of 100 µg ml^{-1} of *S. portulacastrum* extract was able to antagonize 10^7 to 10^3 CFU ml^{-1} of *V. harveyi* in the treatment tank. Also the THB count of treatment tank decreased from 2.10×10^7 CFU ml^{-1} to 5.60×10^4 CFU ml^{-1} during the entire experiment period. The values observed from the bioassay of *S. portulacastrum* extract against *V. harveyi* during the *P. monodon* larviculture, proved significant differences ($P<0.05$) between the *S. portulacastrum* extract treated with *V. harveyi* and control.

It can be concluded that the crude extract of *S. portulacastrum* was reduced even the mortality on *P. monodon* during larviculture caused by *V. harveyi*. The cost of production of crude *S. portulacastrum* extract was compared with molecular grade chemicals in the animal experiments. The decreased amount of cost was worked out up to 20%.

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