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Full Length Research paper

Antimicrobial compounds from marine halophytes for silkworm disease treatment

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Forty-five crude methanolic extracts from 23 marine halophytes were screened against five bacterial and two fungal saprophytic pathogens of diseased silkworm *Bombyx mori*. Among thirty-four mangrove samples screened for antibacterial activity, the leaf extract of *Rhizophora mucronata* showed maximum inhibitory activity against *Staphylococcus aureus* (20 mm dia.). The seaweed species of *Padina tetrostomatica* showed maximum inhibitory activity against *Proteus vulgaris* (11 mm dia) and the seagrass species of *Syringodium isoetifolium* showed maximum inhibition against *P. vulgaris* (9 dia). The solar saltern cyanobacterium *Phormidium fragile* exhibits higher inhibitory activity against *P. vulgaris* (17 mm dia). The growth of fungal pathogens of *Aspergillus niger* was highly inhibited by *S. isoetifolium* (15 mm dia) and *Padina tetrostomatica* (13 mm dia) respectively compared with the other extracts. It is also noted that 16x concentration of the leaf extract of *R. mucronata* and *S. isoetifolium* showed complete reduction in the bacterial and fungal viable counts within 3 h of exposure.

Key words: Mangroves, *Bombyx mori*, seaweed, sea grass, time kill assay herbal disinfectants, cyanobacteria.

INTRODUCTION

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role as therapeutic remedies in many developing countries (Czgan, 1993; Ody, 1993). As a consequence of an increasing demand for biodiversity in the screening programmes seeking therapeutic agents from natural products, there is now a greater interest in marine orga-nisms. Marine halophytes are the specialised group of plants adopted for high saline conditions which include mangroves, seaweeds, sea grass, and blue green algae. They are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceu-tical potential (Shanmugam and Mody, 2001; Ravikumar et al., 2002; Padmakumar, 2002; Bandaranayaka, 2002; Mayer and Hamann, 2002; Sureshkumar et al., 2002; Ostenvix, 1998) . But biological control of silkworm dis-ease pathogens by herbal derived compounds has not been attempted so far.

Sericulture has been one of the main branches of agriculture in Asiatic countries for hundred of years. Silk production is only about 0.2% of the total textile fibre production in the world. Production increase is therefore slow but production falls may be sudden due to many technical and non-technical problems. Silkworm disease is considered as a one of the major technical problem. Bacterial diseases of Silkworms (“Flacherie”) are usually only secondary to virus diseases. Several bacteria cause Septicaemia and toxaemia (*Bacillus* , *Streptococcus, Staphylococcus*, *Escherichia coli*, and *Proteus* sp.). All these pathogens cause softening and putrefaction of the dead worms. Fungal (“Muscardine”) diseases can be caused by over eight fungi viz., *Beaueriana bassiana*, *Aspergillus flavus*, *A. oryzae*, *A. tanei*, *Paecilomyces farinosus*, *Sporosporella uvella*, *Metarhizium anisopliae*

.The obvious characteristic of this type of infection is mummification of the dead larvae which become hard and powdery white like sticks of chalk. Generally hygiene will reduce the incidence of these organisms. Prevention of these diseases could also be possible by spraying 1 - 2% Dithane M 45 in slaked lime or captain in kaolin, 3%

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formaline and sanjeevini dust. Tolerance to all the agents is another approach to decreasing the incidence of those diseases. Finding natural "eco-friendly" plant products that prevent or treat these diseases could be an alternative treatment process. Hence, this present communication is dedicated to find out the cheapest and eco-friendly disinfectants from marine halophytes.

MATERIALS AND METHODS

Collection of marine halophytes

Algae and the sea grass were collected from two representative stations viz., Arockiapuram and Vattakottai South west coast of India (Lat. 8^{o}N, Long 76^{o}E). Healthy and well-grown seaweeds viz., *Gracillaria edulis* (Gracilariaceae), *Colpomania sinuosa* (Phocophyceae), *Chaetomorpha linoides* (Cladophoracea), *Sargassum wightii* (sargassaceae), *Caulerpa toxifolia* (caulerpaceae), *Padina tetrostomatica* (Dictyotaceae), *Hypnea muciformis Enteromorpha intestinalis* (Caulerpaceae) and the seagrass *Syringodium isoetifolium* (Potamogentonales) were collected from rocks and shells submerged under water during low tides. They were separately cleaned on the spot with many changes of seawater in a trough to remove epiphytes, shells and other extragenous matter and were immediately transferred to separate polythene bags and placed on ice for return to the laboratory. Each alga was again cleaned in running tap water and further once with distilled water and shade dried for further use.

Twelve species of mangrove marine halophytes viz., *Acanthus illicifolius* (L.) [Acanthaeceae], *Aegiceros corniculatum* (L.) [Myrsinaceae] , *Avicennia officinalis* (L.) [Avicenniaceae], *Bruguiera cylindrica* (L.) Bl, [Rhizophoraeceae], *Ceriops decandra* (Griff) Ding hou [Rhizophoraeceae], *Exoecaria agallocha* (L.) [Euphorbiaceae], *Luminetzera racemosa* (Wild) [Combretaceae], *Rhizophora apiculata* (Blume), [Rhizophoraceae], *Rhizophora mucronata* (Lamk) [Rhozophoraeceae], *Salicornia brachiata* Roxb, [Chaenopodiaeceae], *Suaeda maritima* Dumort [Chaenopodiaeceae], and *Sesuvium portulacastrum* [Aizoaceae], and their leaf, bark, seeds, seedlings and flower were also collected from Pichavaram mangrove forest of South East coast of India (Lat. 11^{0} 27'N, Long 79^{0} 47' E). All the collected halophytes were washed once with tap water and distilled water and shade dried under room temperature (28±2ëC) for further use.

Isolation and mass cultivation of halophilic cyanobacteria

Fresh algae samples were collected from solar salt pan situated in Kanyakumari District, Tamilnadu southwest coast of India. The collected samples were packed separately in a labelled, previously unused polythene bags and brought to the laboratory in an iced chest. Further, the algae samples were inoculated into BG –11 medium (gl^{-1}) [Solution - 1 Macronutrients ($NaNO_3$ 300.0, K_2HPO_4 8.0, $MgSO_4.7H_2O$ 15.0, $CaCl_2.7H_2O$ 7.0, (COH) (COOH (CH_2 $COOH)_2.H_2O$ 1.2, Na_2 EDTA,$2H_2O$ 0.2, Na_2CO_3 4.0); Solution 2 Micronutrients (H_3BO_3 6.280, $CuSO_4.5H_2O$ 0.0179, $MnCl_2$ 1.810, $NaMoO_4.2H_2O$ 0.390, $CO(NO_3)_2.6H_2O$ 0.049 gl^{-1}). Pure culture of *Phormidium tenue* (Menegheni) Goment and *Phormidium fragile* (Menegheni) Goment were isolated by frequent subculture. Growing them in seawater for three weeks under ambient sunlight carried out mass culture of the isolated cyanobacterial species.

Isolation of silkworm pathogens

Fourth instar infected silkworm *Bombyx mori* L. (Race: Local x NB4D2) was obtained from Model Sericulture Development Centre and also from sericulture farms in and around Kanyakumari District. The collected samples were macerated in sterile distilled water and subjected for the isolation of silkworm bacteria by subsequent streaking on solidified Nutrient Agar (Peptone -0.5 g; NaCl -0.5 g; yeast extract -0.2 g; beef extract- 0.3 g; agar -1.5 g; distilled water – 100 ml; pH 7.0±0.2). Potato dextrose agar (Potato-20 g; dextrose – 5 g; agar –2 g; distilled water-100 ml; pH 5.8-6.0) was used for the isolation of fungal pathogens. Five bacteria viz., *Bacillus cereus*, *B. megaterium*, *Proteus vulgaris*, *Streptococcus lactis* and *Staphylococcus aureus* and two fungal strains *Aspergillus niger*, *M. anisopliae* were identified (Alexopolus, 1979; Holt et al.,1994) and stored in the respective agar slants at 4ëC in a refrigerator for further use. To confirm the pathogenicity, re –infection study was carried out by the supplementation of identified bacterial and fungal strains swapped on the surface of the mulberry leaf. Ten healthy silkworm 4^{th} instar larvae were chosen for each pathogenic treatment. Control was maintained without the supplementation of pathogens.

Extraction of antimicrobials

Completely dried materials of marine halophytes were weighed and ground finely in a mechanical grinder. About 50 g of powdered material was soaked with methanol in an aspirator bottle for a period of 1 to 2 weeks. Extracted plant components are separated and filtered with Whatman No. 1 filter paper to remove other suspended particles. The collected filtrate was evaporated under reduced pressure by using a rotary flash-evaporator at 40ëC (Buchi-JAPAN). The crude extract is collected and stored in a refrigerator for antimicrobial sensitivity assay against silkworm pathogens.

Antibacterial sensitivity assay

The antimicrobial activity of marine halophytic extracts was determined by disc diffusion method (Bauer et al., 1985). Disc prepared with currently used disinfectant (sanjeevini) (Karnataka State Sericulture Research and Development Institute, Suraksha Biochem, Pvt. Ltd., Bangalore. India) served as control.

Sterile Muller– Hinton agar medium (Beef infusion –30 g; casamino acid- 15 g; starch –15 g; agar - 17 g; distilled water-1000 ml; pH –7.4) was poured into sterilized standard Petri plates and allowed to solidify. Later, 0.1 ml of each inoculum in phosphate buffered saline (PBS) of 24 h cultures of bacteria grown on nutrient broth (peptone 1.5 g; sodium chloride -5 g; beef extract -1.5 g; yeast extract- 1.5 g; and distilled water- 1000 ml; pH 7.4±0.2) was overlaid on Muller Hinton agar medium. It was placed aseptically above the seeded agar and pressed a little to facilitate proper diffusion and incubated at 37±1ëC for 24 h. The diameter of inhibition was measured by using graduated scale. The activity index (Activity index = Inhibition zone of sample/Inhibition zone of standard x 100) and the percentage increase or decrease over commercial disinfectant (Treatment – Control / Treatment x 100) were calculated for each extract against each pathogen.

Antibacterial time kill assay

The potential candidate species of *R. mucronata* (mangrove) extracts carryover during the process was selected for its effect on the more susceptible bacterial pathogen *B. megaterium*. The inoculums of *B. megaterium* at the concentration (10^{8} $CFU.ml^{-1}$) starting from a 0.5 Mc Farland was prepared from fresh colonies. The extract of *R. mucronata* was prepared at a concentration of $mg.ml^{-1}$. And different strength of the extract- 1x 6.25, 4x 25 and 16 x100 mg was prepared with nutrient broth and inoculated with equal

volume of bacterial inoculam and incubated at 35ëC for 24 h. Plating was made at every 3 h interval and the colony forming unit was calculated (NCCLS,1990).

Antifungal assay

The antifungal activity of the extract was tested against isolated fungal pathogen viz., *A*. *niger* and *M*. *anisopliae*. Inocula were prepared by growing isolates on PDA slopes. As described by NCCLS slopes were flooded with phosphate buffered saline containing 0.05% Tween 80. Fungal growth was gently probed and the resulting suspension was removed and mixed thoroughly with the use of vortex mixer. After settling larger particles, suspensions were diluted as necessary to correspond to final inoculam concentration of $0.4X10^4$ – $5.0x10^4$ $CFUml^{-1}$. Sterile potato dextrose agar medium was poured into sterilized Petri plates and allowed to solidify. 0.1 ml of fungal inoculums was seeded on the prepared plates. About 5 mm of sterile disc, which contains 25 mg of halophytic extract, was placed and incubated for 48 h at 29±1ëC. After 48 h, the zone of inhibition was measured.

Antifungal time kill assay

The potential candidate species of *S*. *isoetifolium* (seagrass) extracts carryover during the process was selected for its effect on the more susceptible fungal pathogen, *A*. *niger*. Its extract was prepared in 1 ml volume at the concentration of 1x 6.25, 4x 25 and 16x100 mg in phosphate buffered saline. 1 ml of fungal inoculums $0.4\text{-}5.0x10^4$ $CFUml^{-1}$ was taken. Different concentration of halophytic extract was inoculated with 1 ml volume of fungi. Sanjeevini served as control. 100 l samples were taken at 2, 4, 6, 8 and 24 h from each test sample and plated on sabourd dextrose agar plates to find out the total number of viable counts. Triplicates were maintained for each assay.

RESULT

Effect of marine halophytic extracts against bacterial pathogens

The antibacterial activity of the crude methanolic extracts of seaweed marine halophytes showed considerable inhibitory effect (7 - 11 mm) against both gram positive and gram negative bacterial pathogens. The highest zone of inhibition was observed in *C* . *sinuosa* (11 mm) against *S*. *lactis* and *S*. *aureus* (9 mm) whereas *P*. *tetrostomatica* showed higher inhibition against *P*. *vulgaris* (11 mm) with the activity index of 122.2 and *G*. *edulis* showed maximum inhibitory activity (10 mm) against *B*. *megaterium* with the activity index of 76.9. Surprisingly no inhibition was observed against *B*. *cereus* by all the seaweed extracts (Table 1).

The mangrove leaf extracts of *E*. *agallocha* showed maximum inhibitory activity (18 mm) against *S*. *aureus* with 180 activity index and *R*. *mucronata* exhibited higher activity (20 mm) against *S*. *lactis*. The mean zone of inhibition among the bacterial pathogens reveals that the gram positive bacterial pathogens are more susceptible than the gram negative one (Table 1). Compared with the leaf extracts, bark extracts of *R*. *mucronata* and *B*. *cylindrica* showed maximum inhibitory activity (9 mm) against *B*. *megaterium* with 69.2 activity index and *S*. *lactis*, respectively. The whole plant extract, the *S*. *portulacastrum* showed higher inhibitory effect (10 mm) against *P*. *vulgaris* with the activity index of 111.1. The seedling extracts of *A*. *corniculatum* exhibited maximum inhibition (12 mm) against *B*. *cereus* with the activity index of 92.3. The flower extracts of *A*. *illicifolius* showed higher inhibition (12 mm) against *S*. *aureus* with the activity index of 12. Of the thirty four mangrove extracts, the leaf extract of *R*. *mucronata* broadly inhibits all the bacterial isolates. Interestingly, the cyanobacterial extracts of *P*. *fragile* showed remarkable inhibitory activity against *P*. *vulgaris* (17 mm) with the activity index of 188.9 compared with the *P*. *tenue* (Table The commercial disinfectant (Sanjeevini) showed inhibitory activity against all the pathogens except *S*. *lactis* and *M*. *anisopliae* (Table 2).

Time kill assay against *B*. *megaterium*

The effect of *R*. *mucronata* extract against *B*. *megaterium* was performed with different concentrations 1x, 4x and 16x. As shown in Figure 1 the viable cells of *B. megaterium* compared with control, 1 log of CFU/ml were reduced by higher concentration of *R* . *mucronata* extract 4x within 3 to 6 h, and 6 to 12 h in 16x concentration. Nearly 11 log CFU/ml was eliminated within 3 to 12 h at higher concentration.

Effect of marine halophytic methanolic extracts against fungal pathogens

Of the eight seaweed species, *C*. *linoides* showed higher inhibitory activity (14 mm) against the fungal pathogen, *A*. *niger* with the activity index of 155.5 (Table 2) and *G*. *edulis* , *C*. *linoides* and *C*. *toxifolia* showed higher inhibitory activity (9 mm) against *M*. *anisopliae*. The average inhibitory value among the seaweed extracts indicates that the *A*. *niger* (15 mm) is more susceptible than *M*. *anisopliae*. Of the eight mangrove plant leaf extracts tested, *R*. *mucronata* showed maximum inhibitory activity (7 mm) against *M*. *anisopliae* and *A*. *officinalis*, and *A*. *marina* showed maximum inhibition (7 mm) against *A*. *niger* with the activity index of 77.7. The commercial disinfectant sanjeevini showed maximum inhibition (9 mm) against *A*. *niger* but no inhibition was noticed against *M*. *anisopliae* (Table 2).

Time Kill assay against *A*. *niger*

The saprophyte, *A*. *niger* was subjected to different strength of crude extract of the seagrass *S*. *isoetifolium* which is fourfold diluted with methanol 1x concentration of extract that reduces the viable count at 3 to 12 h by>$2\log_{10}$ (Figure 2). But at 4x concentration complete

Table 1. Antibacterial activity of marine halophyte extracts against silkworm pathogens.

Name of the Halophytes	Zone of inhibition (mm)				
	Bacillus cereus	***Bacillus megaterium***	***Proteus vulgaris***	***Streptococcus lactis***	***Staphylococcus aureus***
A. Seaweed					
Gracilaria edulis	-	10 (76.9)	7 (77.8)	8	-
Chaetomorpha linoides	-	7 (53.8)	7 (77.8)	11	9 (90.0)
Colpomenia sinuosa	-	7 (53.8)	7 (77.8)	7	8 (80.0)
Padina tetrastromatica	-	-	11 (122.2)	7	8 (80.0)
Caulerpa taxifolia	-	-	8 (88.8)	8	-
Saragassum wightii	-	-	-	-	-
Enteromorpha intestinalis	-	7 (53.8)	7 (77.8)	8	8 (80.0)
Hypnea musciformis	-	-	-	-	-
B. Sea grass					
Syringodium isoetifolium	-	7 (53.8)	9 (100.0)	7	7 (70.70)
C. Mangrove					
(i) Leaf extract					
Acanthus illicifolius	-	5 (38.5)	-	-	6 (60.0)
Ceriops decandra	11 (84.6)	7 (53.8)	-	7 (100)	8 (80.0)
Rhizophora apiculata	10 (76.9)	8 (61.5)	7 (77.8)	9	-
Lumnitzera racemosa	7 (53.8)	-	-	-	11 (110.0)
Excoecaria agallocha	12 (92.3)	11 (84.6)	11 (122.2)	10	18 (180.0)
Rhizophora mucronata	16 (123.0)	15 (115.4)	7 (77.8)	20 (100)	12 (120.0)
Avicennia officinalis	-	8 (61.5)	-	15	13 (130.0)
Aegiceras corniculatum	7 (53.8)	6 (46.1)	-	6	8
Bruguiera cylindrica	11 (84.6)	8 (61.5)	-	10	-
(ii) Bark extract					
Aegiceras corniculatum	8 (61.5)	8 (61.5)	-	-	-
Avicennia officinalis	6 (46.2)	-	8 (88.8)	-	7 (70)
Bruguiera cylindrica	8 (61.5)	8 (61.5)	9 (100)	7	-
Ceriops decandra	-	7 (53.8)	6 (66.7)	6	8 (80.0)
Excoecaria agallocha	6 (46.1)	7 (53.8)	6 (66.7)	7	-
Lumnitzera racemosa	-	-	9 (100)	-	7 (70.0)
Rhizophora apiculata	8 (61.5)	8 (61.5)	7 (77.8)	8	-
Rhizophora mucronata	7 (53.8)	9 (69.2)	-	9	-
(iii) Whole plant extract					
Salicornia brachiata	7 (53.8)	7 (53.8)	9 (100)	-	10 (100)
Suaeda maritima	8 (61.5)	11 (84.6)	-	8	-
Sesuvium portulacastrum	7 (53.8)	9 (69.2)	10 (111.1)	7	8 (80.0)
(iv) Seedling extract					
Aegiceras corniculatum	12 (92.3)	11 (84.6)	9 (100)	6	8 (80.0)
Avicennia officinalis	-	8 (61.5)	7 (77.8)	6	-
Bruguiera cylindrica	8 (61.5)	10 (76.9)	-	6	7 (70.0)
Ceriops decandra	-	-	6 (66.7)	-	8 (80.0)
Rhizophora apiculata	6 (46.1)	8 (61.5)	-	6	-
Rhizophora mucronata	8 (61.5)	6 (46.6)	9 (100)	-	7 (70.0)
(v) Flower extracts					
Acanthus ilicifolius	-	-	7 (77.8)	10	12 (120.0)
Avicennia officinalis	-	-	8 (88.9)	7	-
Bruguiera cylindrica	-	-	-	-	-
Excoecaria agallocha	7 (53.8)	6 (46.2)	-	-	9 (90.0)
Lumnitzera racemosa	-	-	-	-	-

Table 1. Cont.

Rhizophora apiculata	6 (46.2)	-	-	-	-
Rhizophora mucronata	8 (61.5)	7 (53.8)	7(77.8)	-	6(60.0)
D. Cyanobacteria					
(*i*) *Phormidium fragile*	11 (84.7)	-	17(188.9)	16	15(150.0)
(*ii*) *Phormidium tenue*	7 (53.8)	-	7(77.8)	9	8(80.0)
Sanjeevini powder (standard/control)	13	13	9	-	10

Values in parenthesis indicate the activity index.

Table 2. Antifungal activity of marine halophyte extracts against silkworm pathogens.

Name of the halophytes	Zone of inhibition (mm)	
	Aspergillus niger	***Metarrhizum anisopliae***
A. Seaweed		
Gracilaria edulis	11 (122.2)	9
Colpomenia sinuosa	-	-
Chaetomorpha linoides	14 (155.5)	9
Sargassum wightii	-	-
Caulerpa taxifolia	-	9
Enteromorpha intestinalis	-	-
Hypnea musciformis	-	-
B. Seagrass		
Syringodium isoetifolium	15 (166.6)	9
C. Mangrove		
(i) Leaf extract		
Acanthus illicifolius	-	-
Ceriops decandra	-	-
Rhizohora apiculata	-	-
Lumnitzera racemosa	-	-
Excoecaria agallocha	-	-
Rhizophora mucronata	-	7
Avicennia officinalis	7 (77.7)	-
Avicennia marina	7 (77.7)	7
Sanjeevini powder (standard/control)	9	0

Values in parenthesis indicate the activity index.

reduction in fungal growth at 12 h and 0.6 log 10 value was observed at 9 h. In case of 16x concentration, there was a complete inhibition of *A*. *niger* from 6 h itself.

DISCUSSION

Molecules derived from natural products have an excellent record of providing novel chemical structure for development as new therapeutic agents. Many of the worlds most valuable and successful medicines have been derived from nature. Ten of the world's twenty-five top selling pharmaceuticals were derived from natural products and accounted for global sales of almost US$14 billion in 1995. An antimicrobial agent from marine halophytes is immediate need of ethno pharmacological science in developing novel marine pharmaceuticals. Research has been initiated by the present study to screen 45 extracts belonging to different groups of marine organisms including mangroves, seaweeds, sea grasses and cyanobacteria to explore novel antibacterial and antifungal compounds against silkworm pathogens. Among the different groups of halophytes, leaf extracts of *R*. *mucronata* showed higher inhibitory activity against the bacterial and fungal pathogens. A number of mangroves and mangrove associates proved to have antibacterial, antifungal, antifeedent, molluscisidal and

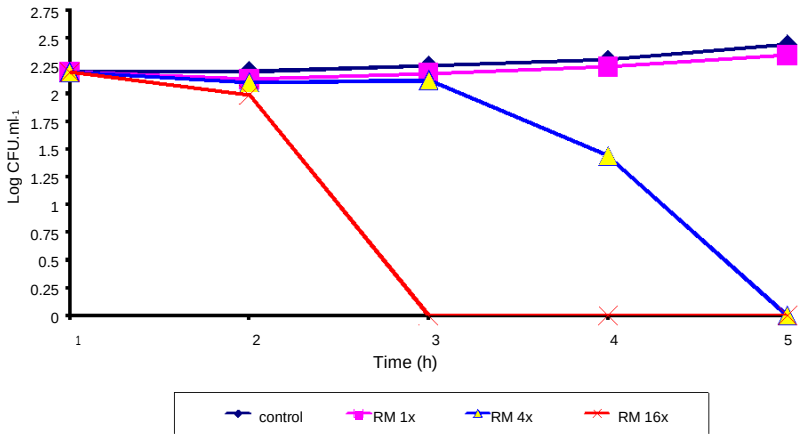

Figure 1. Concentration and time dependent assay of *Rhizophora mucronata* extract against *Bacillus megaterium*.

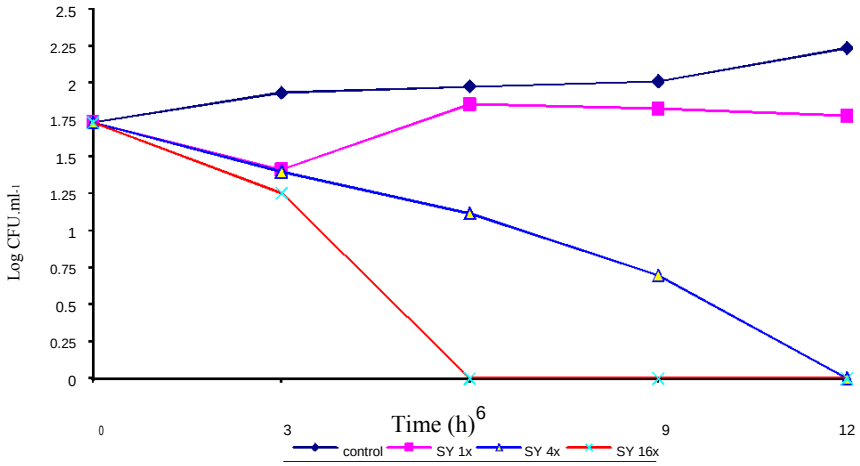

Figure 2. Concentration and time dependent assay of *Syringodium isoetifolium* extract against *Aspergillus niger*

pesticidal properties. Akalanka et al. (2002), Chou et al. (1977) and Padma kumar (1988) also reported that the leaf and bark extracts of *R*. *mucronata* showed antibacterial compounds against some human bacterial pathogens. This highest antibacterial activities are believed to be the presence of high content of phenols which includes tannins (Ravi et al., 1993), coumarines and their glycosides, anthraquinones and their glycosides, napthaquinones flavones and related flavonoides, polysaccharides (Trease and Evans, 1997); sulfated compounds which include brugierol, Isobrugierol, 4-hydroxy-1,2m dithiolane (Kokpol and Chittawong,1987) in mangrove halophytes. Phenolic substances tend to be water soluble, since they most frequently occur combined with sugar as glycosides and they are usually located in the cell vacuole (Glossary of Indian medicinal Plants, Part 1 1992). Prabha et al. (1997) reported that the fouling bacteria were particularly sensitive to the action of the crude methanolic extracts of mangove halophytes like S. *caselolaris*, S. *persica* and *A*. *marina*.

In the present study, seaweed marine halophytic species of *C*. *linoides* exhibit both antifungal and antibacterial activity against all silkworm pathogens. Seaweeds are already known for the antimicrobial activity (Naqui et al., 1980; Sreenivasarao and Parekh, 1981; Padmini et al., 1986; Padmakumar and Ayyakannu, 1986; Ravikumar et al., 2002). The antimicrobial activity is due to the presence of active principles viz., sulfated polysacccharides (Bergold and Murphy, 1976), sulfated galactose unit of the phycocolloid, 2-N-palmitoyl 1-4-5 dihydro 1,3,4,5 tetrahydroxyl spingosine (Mithlesh et al., 1988) and halogenated compounds (Hay et al., 1988; Nys et al., 1995). The methanol soluble fraction of ethanol extracts of *S*. *asperum* and *S*. *wartzii* and methanol and chloroform

soluble fraction of *S. variable* also proved to have antibacterial compounds (Jehan et al., 2000). Likewise, the seagrass marine halophytes of *S. isoetifolium* showed inhibitory activity against silkworm pathogens. Zapata and Mc Millan (1979) reported that the role of phenolic compounds present in seagrasses could also enhance the antimicrobial activity. Reichelt et al. (1983) have also detected antibacterial activity in the seagrass *P. australis* against gram Positive gram negative human bacterial pathogens. Compared with the higher marine halophytes, the unicellular photosynthetic cyanobacterium *P. fragile* also exhibited remarkable anti-bacterial and anti-fungal activity. This is due to the presence of antimicrobial substances and reflects the variety of secondary metabolites (Patterson et al., 1994).

It was also reported that, the species of *P. tenue* and *P. fragile* showed antibacterial activity against *S. aureus* and *Pseudomomas* sp. (Rao et al., 1994). Singh et al. (1999) reported that antifungal lactone tanikolide was isolated from the marine cyanobacterium *Lyngbia majuscula* that showed highest zone of inhibition (100 ug/disk) against the fungus *Candida albicans*. The methanol extract of *Anabaena variabilis*, *Gloeocapsa caldarriorum*127, *Pseudoanabaena catenata* and *Limnothrix redekei* HUB 051 inhibited the growth of *B. subtilis* SBUG 14 (Sabine et al., 2003). Decades of screening programmes have revealed that cyanobacteria are a potential source of new active substances for medicine and pharmacy (Falch, 1996, Hayashi et al., 1996; Horgen et al., 2000; Jaki et al., 1999; Kaijiyama et al., 1998; Luesh et al., 2000; Moore et al., 1989; Namikoshi and Rinerhart, 1996; Patterson et al., 1994; Papendorff et al., 1998; Singh et al., 1999). The anti-bacterial activity of cyanobacteria against methicillin resistant *Staphylococcus* strains is well known due to the presence of polyunsaturated fatty acid, linolenic acid (Mc Donald et al., 1981) and hydroxylated unsaturated fatty acids, corolic acid (Mundt Sabine et al., 2003). It is inferred from the present study that the seaweed methanolic extracts of *S. isoetifolium* and the cyanobacterial methonolic extract of *P. fragile* are recommended as alternative therapeutic agents to control Silkworm pathogens viz., *B. cereus*, *B. megaterium*, *P. vulgaris*, *S. aureus* and *A. niger*, respectively. Further attempts will be made for the development of new disinfectant to control the *M. anisopliae* and *S. lactis*.

Differences in the antimicrobial effect of the marine halophytic extracts against both gram-positive and gram-negative bacteria may be due to differences in permeability barriers. In gram-negative species outer membrane is a fairly effect barrier for the extract and also the active compounds persist in the marine halophytes. The differences in antifungal activity is due to the potential difference in the susceptibility of conidia, germinated conidia and hyphae to antifungal compound and the time duration for the expouser of the compound.

Generally the assay with comparatively long incubation times such as 24 - 48 h showed no significant differences in susceptibility, whereas studies using shorter incubation times showed differences (De lucca et al., 1997). In addition, the test agent assessed had different mechanisms of antifungal action. As reported by Cheng and Levin (1970), the thickness and density of the conidial wall may be responsible for the reduced susceptibility of conidia to antifungal agent. Based on the both inhibitory and fungicidal action the seagrass *S. isoetifolium* extract may be a useful agent for saprophytic infection of silkworms.

In the present work the antibacterial property of *R. mucronata* and the antifungal effect of *S. isoetifolium* suggest its potential in alternative disinfectant for the flacheriae and grasseriae disease of silkworm. Further studies are needed to elucidate the structure and mechanism of action of these marine halophytic extracts.

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