# SUBSTRATUM STRATEGIES ON MICROFOULING AND ANTIFOULING POTENTIALS OF MANGROVES *AVICENNIA OFFICINALIS AND RHIZOPHORA MUCRONATA*


*By*

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# INTRODUCTION

- Marine biofouling is the undesirable colonization / settlement of microbes, algae and sessile invertebrates on submerged surfaces of both natural and man made marine structures.
- Marine biofouling majorily classified into two
    1. Microfouling comprises of bacteria, fungi and diatoms
    2. Macrofouling comprises of algae, barnacles, mussels, bryozoans and polychaetes
- This is one of the serious problem that marine technology faces. It increases the fuel consumption and speed reduction in ships and both about 17% of fuel cost lost by biofouling of ship hulls.
- It not only affect the ships, but also causes serious damages to cooling systems of power stations, marine based oil industry, fishing nets, pipelines any marine infrastructures and also aquaculture systems.

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- Though the TBT, Copper, Zinc like heavy metals based antifouling paints are effective, are possess non-specific toxic threat to both marine and terrestrial environment.

- Hence, International Maritime Organization (IMO) proposed phasing out of TBT and heavy metal based antifoulants by 2003 followed by complete ban by 2008 (Lewis, 2001).

- It is the hourly need to evaluate novel eco-friendly antifouling compounds as an alternate of banned commercial products

- Eco-friendly antifouling compounds are present in both plants, animal resources and in microbes as secondary metabolites.

- Our earlier study indicated the potential antifouling property of methanolic extracts derived from ayurvedic plant materials (Immanuel *et al*., 2005)

- Consequently number of antifouling compounds have been identified from marine fauna and flora by number of workers in the past
- However little attention has been paid towards the antifouling potentials of Mangroves
- In light of the above the present work has been undertaken to explore the antifouling potentials of mangroves ***AVICENNIA OFFICINALIS*** **AND** ***RHIZOPHORA MUCRONATA***

# ECONOMICAL LOSS DUE TO BIOFOULING

- Fouling related costs of marine industries showed an increasing trend which includes prevention cost (Antifouling protection cost), increased pressure cost, replacement of fouling damaged plants and restoration after fouling caused accidents.
- Fouling related damage to US oil industry costs around 16 - 18 billion dollars per year (Arouja-Jorge et al., 1992).
- The additional fuel consumption of US Navy costs around 75 – 100 million dollars per year (Lewis, 1994).
- As little as 5% fouling on the hull of a tanker ship can increase fuel costs by 17% and a study has shown that 1 mm thick layer of slime can cause 15% loss in ship speed (Lewis, 2001).
- In India, the cost of cleaning the merchant ships has been estimated to Rs.373 million (Balaji, 1988). This estimate will be doubled when an increase of fuel consumption due to fouling is also considered.
- These are the serious problems encountered by marine industries due to marine biofouling.

# AIM

This present work has been undertaken to study the substratum strategies on microfouling and also to identify the novel eco - friendly antifouling compounds from mangroves ***AVICENNIA OFFICINALIS*** **AND** ***RHIYZOPHORA MUCRONATA***

# METHODOLOGY

## ❖ 1. Fouling study

- To study the substratum dependent both micro fouling patern, substrates such as wood (*Artocarpus* sp), fibre reinforced plastic (FRP), stainless steel (316L) and carbon steel were selected, considering their applications in construction of ship hulls, boats and other marine infrastructures.
- Substrates were made into panels of desired size (15cm length x 10cm breadth and width thickness of 2mm) for assessing the fouling pattern and was immersed in seawater at chinnamuttom fisheries harbour, Kanyakumari District, Tamilnadu, India (Lat 8° 6' 12" N and Long 7° 34'09"E) over a period of 72 h..
- During this period, the physico-chemical parameters of source water was analysed periodically by following APHA (1985)
- The biofilm samples were collected at an interval of 24 h and the samples were used for bacterial enumeration.

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## ❖ 2. Anti-fouling study

- **For antifouling study, the marine bioresource such as mangroves were collected freshly from Manakkudy estuary (8.5º23'Lat and 77.28º40.9'Long), Kanyakumari District, Tamilnadu, India.**
- **The collected mangroves were shade dried and extracted with solvents by percolation method.**
- **Antibiofilm bacterial assay was performed for both the mangrove extracts by following protocol described by Bauer et al. (1966).**
- **Mussel antifouling assay was performed for both the mangrove extracts by following the protocol described by Wilsanand et al., (1999) and Murugan and Santhanam Ramasamy (2003).**

# RESULTS

**Table 1. Daily variations in physical and chemical parameters of seawater of Study area during experimental period**

| Time (h) | Salinity (ppt) | Temperature ($^0$C) | DO (mg/l) | pH | Silicate (µmol / L) | Nitrate (µmol / L) | Nitrite (µmol / L) | Total phosphorous (µmol / L) | Phosphate (µmol / L) | Ammonia (µmol / L) |
|---|---|---|---|---|---|---|---|---|---|---|
| **24** | **36.00 ± 0.00** | **28.00 ± 0.00** | **5.10 ± 0.10** | **8.0 ± 0.50** | **0.165 ± 0.007** | **2.229 ± 0.012** | **3.323 ± 0.002** | **35.51 ± 1.10** | **24.85 ± 0.80** | **0.021 ± 0.002** |
| **48** | **35.00 ± 0.00** | **27.00 ± 0.00** | **5.30 ± 0.20** | **8.0 ± 0.00** | **0.133 ± 0.003** | **2.049 ± 0.008** | **3.527 ± 0.004** | **28.40 ± 1.50** | **21.36 ± 1.40** | **0.023 ± 0.001** |
| **72** | **36.00 ± 0.00** | **28.00 ± 0.00** | **5.20 ± 0.15** | **8.0 ± 0.00** | **0.159 ± 0.005** | **2.065 ± 0.016** | **3.139 ± 0.003** | **33.73 ± 1.20** | **23.08 ± 1.50** | **0.027± 0.003** |

**Table 2. Bacterial population ($10^{-3}$ CFU/ml) in different panels exposed at seawater of study area during different time intervals**

| Time (hours) | Panels | | | |
|---|---|---|---|---|
| | Wood | Stainless steel | FRP | Carbon steel |
| 24 | 158.60 ± 12.60 | 131.30 ± 6.98 | 85.30 ± 4.90 | 64.60 ± 5.73 |
| 48 | 219 ± 7.72 | 177 ± 4.08 | 121 ± 6.16 | 73 ± 3.40 |
| 72 | 221 ± 11.86 | 213 ± 10.62 | 165.60 ± 4.08 | 89 ± 5.35 |

**Each value is a mean of three replicates**

## Table 3. Percentage Diversity of biofilm bacteria in different panels

| Bacterial strains | % diversity in tested panels | | | |
|---|---|---|---|---|
| | Wood | Stainless steel | FRP | Carbon steel |
| *Pseudomonas aeruginosa* | 19 ± 1.63 | 23 ± 2.49 | 20 ± 1.63 | 24 ± 2.49 |
| *Vibrio cholerae* | 15 ± 1.63 | 7 ± 1.63 | 13 ± 2.49 | 12 ± 1.63 |
| *Serratia marcescens* | 17 ± 1.24 | 15 ± 1.24 | 11 ± 1.24 | 18 ± 1.24 |
| *Enterobacter aerogens* | 8 ± 0.81 | 10 ± 0.81 | 11 ± 0.81 | 10 ± 0.62 |
| *Halomonas aquamarina* | 5 ± 0.24 | 8 ± 1.24 | 10 ± 0.62 | 11 ± 0.81 |
| *Shigella flexneri* | 7 ± 0.62 | 4 ± 0.81 | 14 ± 0.47 | 4 ± 0.62 |
| *Vibrio parahaemolyticus* | 16 ± 1.24 | 19 ± 0.81 | 12 ± 1.24 | - |
| *Aeromonas hydrophila* | 4 ± 0.81 | 9 ± 0.47 | - | 9 ± 0.47 |
| *Enterobacter agglomerans* | 3 ± 0.47 | 5 ± 0.62 | 9 ± 0.47 | 6 ± 0.47 |
| *Serratia liquefaciens* | 6 ± 1.24 | - | - | 6± 0.62 |

Each value is a mean of three replicates

Table 4. Antimicrofouling activity ( Zone of inhibition – m.m) of *A. officinalis* extracts

| Bacterial strains | *A. officinalis* | | |
|---|---|---|---|
| | Methanolic extract | Chloroform extract | Hexane extract |
| *Pseudomonas aeruginosa* | 8.6 ± 0.40 | 5.9 ± 0.44 | R |
| *Halomonas aquamarina* | 10.3 ± 0.23 | 7.5 ± 0.44 | R |
| *Serratia marcescens* | 10.0 ± 0.62 | 6.5 ± 0.62 | 5.5 ± 0.62 |
| *Enterobacter aerogens* | 7.0 ± 0.40 | R | R |
| *Shigella flexneri* | 12.5 ± 0.44 | 7.3 ± 0.44 | R |
| *Aeromonas hydrophila* | 8.5 ± 0.40 | R | R |
| *Vibrio cholerae* | 8.6 ± 0.62 | R | 6.0 ± 0.44 |
| *Vibrio parahaemolyticus* | 9.0 ± 0.44 | R | R |
| *Serratia liquefaciens* | 10.6 ± 0.11 | R | 6.6 ± 0.40 |
| *Enterobacter agglomerans* | 15.1 ± 0.62 | 12.3 ± 0.40 | 6.8 ± 0.23 |

R : Resistance ; Each value is a mean of three individual estimates

Table 5. Antimicrofouling activity ( Zone of inhibition – m.m) of *R. mucronata* extracts

| Bacterial strains | *R. mucronata* | | |
|---|---|---|---|
| | Methanolic extract | Chloroform extract | Hexane extract |
| *Pseudomonas aeruginosa* | 10 ± 0.44 | R | R |
| *Halomonas aquamarina* | 10.5 ± 0.23 | R | R |
| *Serratia marcescens* | 8.6 ± 0.62 | R | R |
| *Enterobacter aerogens* | 8.5± 0.40 | R | R |
| *Shigella flexneri* | 12.0 ± 0.40 | R | R |
| *Aeromonas hydrophila* | 5.8 ± 0.62 | R | R |
| *Vibrio cholerae* | 7.3 ± 0.62 | R | R |
| *Vibrio parahaemolyticus* | 6.6 ± 0.47 | R | R |
| *Serratia liquefaciens* | 6.5 ± 0.11 | 11.5 ± 0.40 | 7.5 ± 0.40 |
| *Enterobacter agglomerans* | 11.5 ± 0.40 | R | R |

R : Resistance ; Each value is a mean of three individual estimates

## Table 6. EC50 and LC50, inhibition of byssal attachment and mortality of *P. indica* after 24h of exposure to methanolic extract of selected Mangroves

| Mangrove extracts | $EC_{50}$* (µg/ml) | $LC_{50}$* (µg/ml) |
|---|---|---|
| *A. officinalis* | 69.17 ± 5.72* | 357.33 ± 2.49* |
| *R. mucronata* | 121.54 ± 4.77* | 414.19 ± 13.14* |

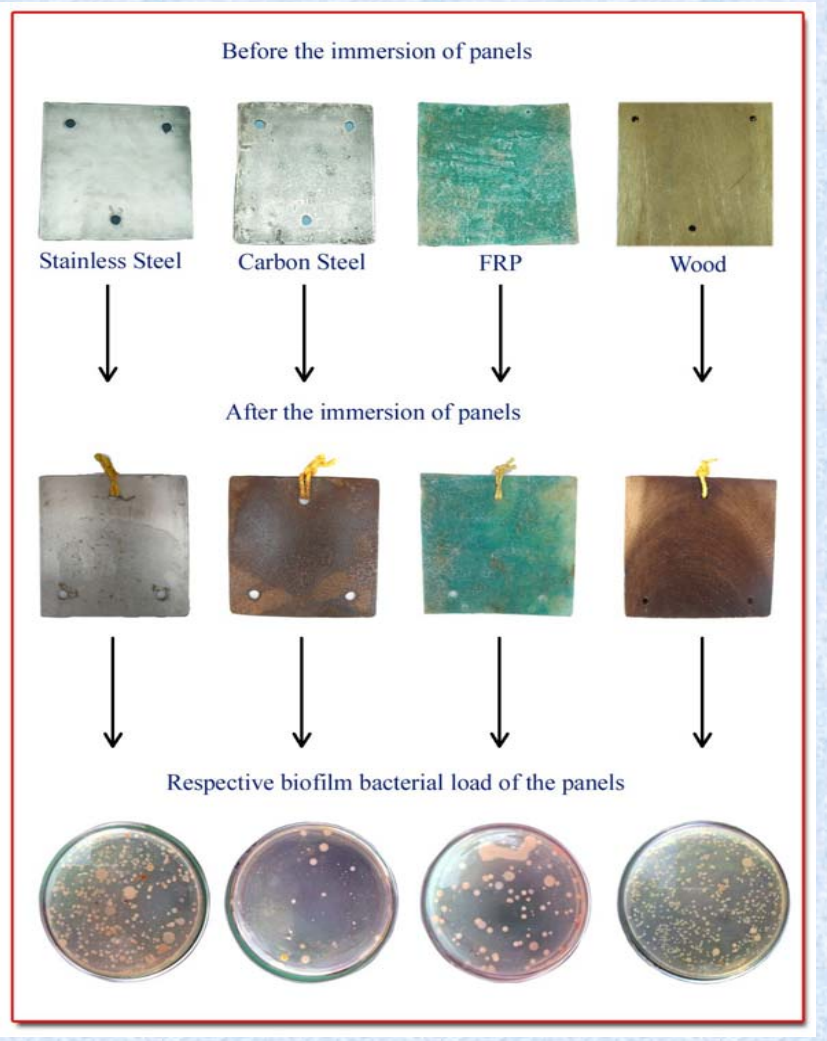
* $EC_{50}$ and $LC_{50}$ of methanolic extract of mangroves through probit analysis

# CONCLUSION

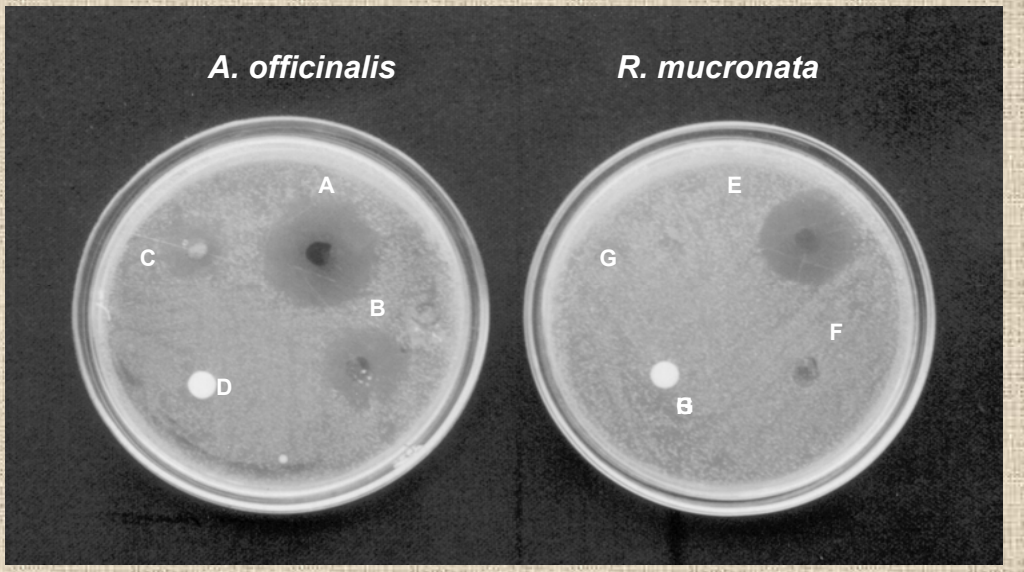
- **The present study revealed that, wood is highly vulnerable towards the biofilm bacterial attachment.**
- **In fishing harbour environment, the *P. aeroginosa* was found to be the dominant biofilm bacterial strain.**
- **The better antimicro and macrofouling activity extended by *A. officinalis extract* concluded its potential application on antifouling preparations.**

# PLATES

# Isolation of biofilm bacteria from the selected substrata



# Antimicrofouling activity of the mangrove extracts against biofilm bacterium




A : Methanol extract of *A. officinalis*
B : Chloroform extract of *A. officinalis*
C : Hexane extract of *A. officinalis*
D : Control

E : Methanol extract of *R. mucronata*
F : Chloroform extract of *R. mucronata*
G : Hexane extract of *R. mucronata*
H : Control

# Antimacrofouling activity of selected mangrove extracts by the inhibition of byssal production

***A. officinalis***


***R. mucronata***


THANK YOU