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Full Length Research Article

Physicochemical Analysis Isolation and Identification of some Endophytic Fungi from Marine Environs

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Abstract

The present investigation suggested that the endophytic fungi that are residing asymptotically in internal tissues of all plants and promising sources of biologically novel active compounds were recognized. The marine soil physiochemical parameters of Mallipattinam and Kollukadu of Pattukkottai taluk were analyzed. The Kollukadu soil has rich physio chemical properties when compared with Mallippattinam soil sample obviously water sample in Mallipattinam was excellent parameters found to be recorded when compared with Kollukadu water sample recorded respectively. Isolation and identification of endophytic fungi like, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. ochraceous*, *Chaetomiun sp*., *Fusarium sp*., *F. moniliforme*, *F. oxysporum*, *Pencillium chrysogenum*, *P. citrinum*, *P. janthinellum*, *P. purpurrescens*, *R. stolonifer*, *Trichoderma harzianum* from marine associated medicinal plants such as *Acanthus ilicifolius*, *Aegiceras corniculatum*, *Avicennia marina*, *Ceriops decandra*, *Excoecaria agallocha*, *Rhizophora mucronata* were determined respectively. Among the six-marine associated medicinal plants the *Acanthus ilicifolius* showed maximum fungal colonies (67) were recorded and minimum fungal colonies (48) in the *Rhizophora mucronata* were recognized respectively. The statistical analysis of Shannon and Simpson index of endophytic fungi were performed. The fungi that make up charge of different biological characteristics that are used in industrial important fungi and environmentally beneficial methods of living.

***Key words*:** Endophytic fungi, Physico-chemical analysis, Host plant, Novel natural agents

Human activities are responsible for a major decline of the world's biological diversity and the problem is critical that combined human impacts could have accelerated present extinction rates to 1000–10,000 times the natural rate [1]. Water is an essential resource for industries, agriculture, manufacturing and other activities of human beings [2]. Water quality is described in term of its physical, chemical and biological factors [3]. Soil is a vital component, medium of unconsolidated nutrients and materials forms the life layer of plants [4]. Soil is made up of broken rock particles that have been altered by chemical and environmental conditions such as weathering and erosion [5]. Soil is a mixture of mineral and organic constituents that are in solid, gaseous and aqueous States [6]. The soil quality analysis includes an analysis of parameters and processes which effects on soil to operate efficiently as a component of a sound ecosystem [7]. Soil physic-chemical properties influence the behavior of soil and hence knowledge of soil property is important [8].

Endophytic fungi are mitosporic and meiosporic ascomycetes that asymptomatically reside in the internal tissues of plants beneath the epidermal cell layer, whereas fungi colonize healthy and living tissue via quiescent infections [9]. Endophyte is defined as all microorganisms residing in their host plant tissue either rhizome, stems, leaves and flowers without causing any disease to their host [10]. The relationship between the plant and endophytes is symbiotic [11], the fungi receive shelter and nutrients from the plant while plant is protected from inhibition pathogens and herbivores [12-13] as well as increased resistance to factors that are responsible for abiotic stress and toxicity to high concentrations of heavy metals [14]. The endophyte may survive as a latent pathogen causing or quiescent infections for a long period and symptoms only when physiological or ecological conditions favors of virulence [15-16]. The present study is focused on the Physico-chemical analysis and isolation of endophytic fungi from marine environs.

MATERIALS AND METHODS

Study site

Mallipattinam and Kollukadu is a coastal village in the Pattukkottai taluk of Thanjavur District, Tamil Nadu, India. It

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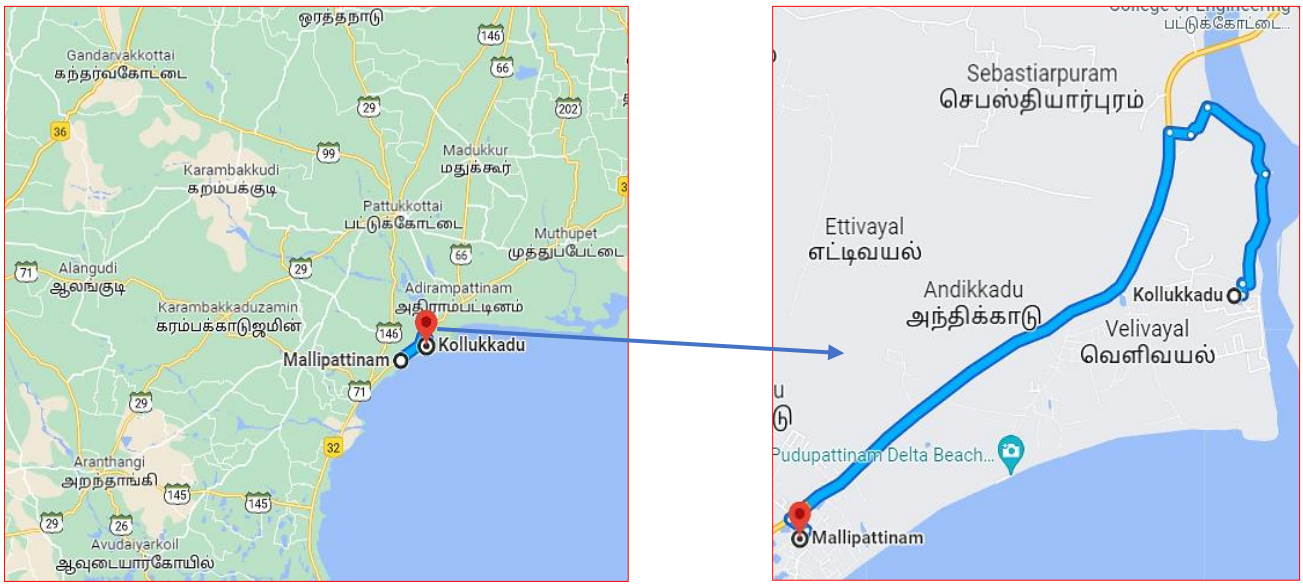
is located between Thiruthuraipoondi and Pattukkottai around 360 km away from Chennai Tamil Nadu.

Collection of samples

The soil and water samples were collected from Mallipattinam and Kollukadu. The soil site at depth of 10cm by using spatula and sterilized every time with 70% alcohol. The seawater sample were taken at a depth of 0-50cm by fighting ocean currents [17]. At each station 2 samples were collected randomly and were pooled together. The samples were kept in sterilized polythene bags sealed and transported to the laboratory.



Sample collection site and location of Mallipattinam and Kollukadu area of pattukkottai talik

Fig 1 Sample location

Collection of plant samples

The following medicinal *Acanthus ilicifolius, Aegiceras corniculatum, Avicennia marina, Ceriops decandra, Excoecaria agallocha* and *Rhizophora mucronata* were collected from respective site. At each station 5 to 7 leaves were collected randomly and were pooled together. The samples were kept in sterilized polythene bags sealed and transported to the laboratory.

Physico chemical analysis of soil

The physico-chemical parameters of soil and water samples were analyzed by standard methods. The physico-chemical parameters such as colour, temperature, pH, salinity (%), organic carbon (%), organic matter (%), available nitrogen (mg/kg), available phosphorus (mg/kg), available potassium(mg/kg), available zinc (ppm), available copper (ppm), available iron (ppm), available manganese (ppm), calcium (mg/kg), sodium (mg/kg) potassium (mg/kg), oil and grease mg/ml, chromium mg/ml, dissolved oxygen mg/ml and biochemical oxygen demand (mg/ml) of the soil and water samples were analyzed by using standed method [18], [20-21].

Isolation of endophytic fungi from marine medicinal plant samples

Endophytes were isolated from plant leaf [21] method. Surface sterilization of roots [22]. Leaf segments (5mm) were washed thoroughly in running tap water, sterilized with sodium hypochlorite (NaCl, 5%) for 3min, rinsed three times in sterile distilled water and then dried on a sterile filter paper. Segments were placed in a 90mm diameter Petri dish containing a mixture of potato dextrose agar (PDA) medium and amoxiallin (15mL/l), and incubated at 25 °C with darkness. After 7 days culture were transferred to fresh PDA.

Identification

The identification of fungi taxa using standard Manual of soil fungi such as A Manual of Penicillia [23], A, Manual of soil fungi [24], Manual of AspergilIi [25], Hyphomycetes [26], Dematiaceous Hyphomycetes [27].

Shannon index of diversity

The values of diversity has been calculated as follows: $H= - \sum Pi \, Ln \, Pi$

Simpson index of diversity

Simpson index is calculated using the following equation: $D =1-\sum(Pi)^2$

RESULTS AND DISCUSSION

In the present study that the physicochemical parameters including soil texture the maximum parameters in Kollukadu such as Colour, Temperature (°C), pH, organic carbon, organic matter, available nitrogen, phosphorus, potassium, sodium, calcium, magnesium, zinc, copper, iron, manganese, potassium and salinity exchange capacity was (Brown), (16°C), (7.9), (0.77), (0.96), (256.02 mg/kg), (31.0 mg/kg), (269.03 mg/kg), (1.58 mg/kg), (1.97 mg/kg), (1.69 mg/kg), (1.02 ppm), (0.56ppm), (5.67ppm), (2.89 mg/kg), (0.79 mg/kg), (23) found reported, while the minimum in (15°C), (7.8), (0.68), (0.87), (220.20 mg/kg), (29.3 mg/kg), (254.03 mg/kg), (1.29 mg/kg), (1.79 mg/kg), (1.64 mg/kg), (0.96 ppm), (0.46 ppm), (5.45 ppm), (2.48 mg/kg), (0.69 mg/kg) and (24) exhibited from soil sample in Mallipattinam respectively.

The maximum physico chemicals such as, pH, dissolved oxygen, biochemical oxygen demand, available nitrogen, potassium, calcium, zinc, iron, potassium, salinity, oil and grease and chromium was (8.0), (0.5 ml/l), (10 ml/l), (36.1 g/l), (11.3 g/l), (18.3 g/l), (0.78 g/l), (3.53 g/l), (11.0 g/l), (27), (5.0 g/l), (0.73 g/l) from water sample of mallipatinam respectively whereas minimum in (7.9), (0.5 ml/l),(12 ml/l), (29 g/l), (9.47 g/l), (14.1 g/l), (0.71 g/l), (0.71 g/l), (10.2g/l), (24), (3 g/l), (0.69 g/l) exhibited from water samples in Kollukadu respectively (Table 1-2).

Table 1 Analysis of physicochemical parameters of different soil samples

Physicochemical parameters	Mallipattinam	Kollukadu
Colour	Brown	Brown
Temperature (°C)	15°C	16°C
pH	7.8	7.9
Organic carbon (mg/kg)	0.68	0.77
Organic matter (mg/kg)	0.87	0.96
Available nitrogen (mg/kg)	220.20	256.02
Available phosphorus (mg/kg)	29.3	31.0
Available potassium (mg/kg)	254.03	269.03
Sodium (mg/kg)	1.29	1.58
Calcium (mg/kg)	1.79	1.97
Magnesium (mg/kg)	1.64	1.69
Available Zinc (ppm)	0.96	1.02
Available Copper (ppm)	0.46	0.56
Available Iron (ppm)	5.45	5.67
Available Manganese (mg/kg)	2.48	2.89
Potassium (mg/kg)	0.69	0.79
Salinity (PPT)	24	23

Mean ± standard deviation

The fungal colonies were isolated using PDA agar plate by incubated at room temperature (27°C) for five days. Totally (15) endophytic fungi were isolated in different marine associated medicinal plants. The isolation of endophytic fungi using marine medicinal plants like *Acanthus ilicifolius*, *Aegiceras corniculatum*, *Avicennia marina*, *Ceriops decandra*, *Excoecaria agallocha* and *Rhizophora mucronata*. The endophytic fungi such as *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. ochraceous*, *Chaetomiun sp.*, *Fusarium sp.*, *F. moniliforme*, *F. oxysporum*, *Pencillium chrysogenum*, *P. citrinum*, *P. janthinellum*, *P. purpurrescens*, *R. stolonifer* and *Trichoderma harzianum*. The maximum number of fungal colonies (67) were presented in *Acanthus ilicifolius* plant and minimum number of fungal colonies (48) were recorded in *Rhizophora mucronata* leaves respectively (Table 3).

Table 2 Analysis of physicochemical parameters of different marine water samples

Physicochemical parameters	Mallipatinam	Kollukadu
pH	8.0	7.9
Dissolved Oxygen (ml/l)	0.5	0.5
Biochemical Oxygen demand (ml/l)	10	12
Available nitrogen (g/l)	36.1	29
Available potassium (g/l)	11.3	9.47
Calcium (g/l)	18.3	14.1
Available Zinc (g/l)	0.78	0.71
Available Iron (g/l)	3.53	3.21
Potassium (g/l)	11.0	10.2
Salinity (PPT)	27	24
Oil and grease (g/l)	5.0	3
Chromium (g/l)	0.73	0.69

Mean ± standard deviation

Table 3 Isolation and identification of endophytic fungi from marine associated medicinal plants

S. No.	Name of the endophytic fungi	Different medicinal plants					
		Acanthus ilicifolius	*Aegiceras corniculatum*	*Avicennia marina*	*Ceriops decandra*	*Excoecaria agallocha*	*Rhizophora mucronata*
1.	*Aspergillus flavus*	5	2	3	6	6	-
2.	*A. fumigatus*	3	5	2	5	4	2
3.	*A. niger*	2	3	-	3	6	5
4.	*A. terreus*	5	4	3	-	8	6
5.	*A. ochraceous*	6	9	6	8	3	4
6.	*Chaetomiun sp.*	4	5	6	8	-	2
7.	*F. moniliforme*	6	3	-	2	5	6
8.	*F. oxysporum*	4	3	-	4	6	-
9.	*Fusarium sp.*	2	4	5	6	3	3
10.	*Pencillium chrysogenum*	8	5	4	5	4	3
11.	*P. citrinum*	6	3	3	6	3	-
12.	*P. janthinellum*	4	5	2	-	-	2
13.	*P. purpurrescens*	5	-	5	3	5	6
14.	*R. stolonifer*	4	4	6	5	2	4
15.	*T. harzianum*	3	2	4	4	-	5
Total number of colonies		67	57	49	65	55	48

Table 4 Shannon and Simpson index of endophytic fungi from marine associated medicinal plants

Name of the medicinal plants	Inference	
	Shannon index (H)	Simpson index (D)
Acanthus ilicifolius	2.6439	0.9456
Aegiceras corniculatum	2.5596	0.9354
Vicennia marina	2.4205	0.9336
Ceriops decandra	2.0809	0.9389
Excoecaria agallocha	2.4192	0.9259
Rhizophora mucronata	2.4090	0.9193

The present study shannon index and simpson index with highest value is H=2.6439 and D= 0.9456 respectively in the *Acanthus ilicifolius* plant (Table 4).

The physicochemical analysis of the soil samples included pH, organic matter content, moisture content, temperature, water holding capacity, and texture. The changes are pH, water holding capacity, organic matter content and moisture content [28]. The phisico chemical characterization of seawater at ship dismantling area. Generally, the smell of seawater was as similarly with oil smell [29]. The value of pH showed lie in the alkaline side, pH of these soil is greater than 7. Alkalinity is measure of saline or salt effected soil [30]. The parameters like organic matter percentage, total chloride, calcium, nitrogen, magnesium was higher in test soil [29]. Sampling and analysis for physicochemical parameter on seawater at surrounding the area of ship dismantling activities, some parameters such as TSS, BOD, phosphate, nitrate, oil and grease, and some heavy metals (Pb, Cd, Zn) showed higher value than the seawater quality standard [31]. A strategic

marine environmental quality management plan has been proposed [32]. In the present investigation suggested that the maximum physico chemical parameters at Mallippattinam water sample whereas Kallukadu soil sample was excellent physico chemical parameters found to be recorded respectively.

According to microscopic characteristics and ITS-rDNA sequences, 17 isolates of fungal endophytes from *A. macrosperma* were obtained. Among the identified fungi, 11 isolates belonged to 5 different taxa (*Acremonium furcatum, Cylindrocarpon pauciseptatum, Trichoderma citrinoviride, Paecilomyces marquandii* and *Chaetomium globosum*) [33]. The diversity of endophytic fungi at older stage of leaves was abundant with high potential source of antimicrobial properties [34]. A total of 142 endophytic fungi were successfully isolated from young, mature, old and senescent leaves of *C. mangga* using six different agar medium viz., PDA, PDA supplemented with host plant powder, PDA with host plant extract, MEA, MEA supplemented with host plant powder and MEA with host plant extract [35]. There are factors determined the presence of endophytic fungi in plants, such as environment, species, and isolation technique [36]. The species identified as endophytic fungi from another citrus plant, *C. nobilis* var. microcarpa [37]. Colletotrichum species can be found as pathogens, endophytes, and as *Citrus sp*., *C. medica* var. sarcodactylis, and *C. nobilis* var. microcarpa [37-39]. Saprobes in a variety of plant hosts [40]. The endophytic fungi are also found in other citrus plants in the present study isolation of endophytic fungi from *Acanthus ilicifolius, Aegiceras corniculatum, Avicennia marina, Ceriops decandra, Excoecaria agallocha, Rhizophora mucronata* plants were such as *Aspergillus flavus, A. fumigatus, A. niger, A. terreus, A. ochraceous, Chaetomiun sp., Fusarium sp., F. moniliforme, F. oxysporum, Pencillium chrysogenum, P. citrinum, P. janthinellum, P. purpurrescens, R. stolonifer* and *Trichoderma harzianum* isolated respectively.

CONCLUSION

Conclusively from study area Mallipattinam and Kollukadu of Pattukkottai taluk of Thanjavur District, Tamil Nadu, India. The highest results from the physicochemical assessment of soil and water samples in the two areas can be found in Kollukadu soil and Mallipattinam for water. The Kollukadu soil quality can be carried out by different parameters. Most of the parameters are quite higher or lower than acceptable limits. Conclusively, In this study the water quality properties in terms of its physico-chemical parameters maximum results were showed in Mallipattinam and some parameters showed higher reading than standard, indicated it needed some efforts to remediate those location so that the environmental quality can be improved. The isolation of endophytic fungi from *Acanthus ilicifolius, Aegiceras corniculatum, Avicennia marina, Ceriops decandra* and *Excoecaria agallocha* plants respectively. *Acanthus ilicifolius* leaves showed that 15 fungi were observed the endophytic fungal colonies were excellent candidature for conservation of environment and mankind. The minimum number of fungi were isolated in Rhizophora mucronat plant.

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