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An investigation of the mycoflora in the sand dune soils of Tamilnadu coast, India

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ABSTRACT

Sand dune samples numbering 40 were collected from 8 different stations along the entire Tamil Nadu coast and examined by dilution plating method on MEA medium to assess the fungal diversity, their population density. Physico-chemical characteristics of the soil were also analyzed to find out their impact on fungal population. A total number of 24 species representing 12 genera were recorded from all the sand dune samples. Physiochemical analysis revealed the moisture content in the range from 3.6 to 34 per cent, water holding capacity from 17.4 to 21.5 %, pH from 6.65 to 8.80, EC from 0.06 to 3.1 organic carbon from 4 to 20 mg/g and available nitrogen from 0.014 to 0.046 mg/g in different stations. Correlation analysis made between fungal population density and physico– chemical factors of the soil revealed no factor as responsible for the population density changes in different stations.

Keywords: Fungal diversity, Population density, Physico – chemical character, Sand dune soils.

INTRODUCTION

Soil microorganisms act as agents of nutrient elements transformation because of their extracellular enzyme production to decompose / re-mineralize the organic matter/ insoluble elements present in the environment and store carbon and mineral nutrients with a fast turnover. Thus the quantity and activity of the soil microorganisms are the determining factors of the productivity of any kind of soil. These considerations are more important for the unfertilized soils, such as sand dune soils, forest soils etc. They even accumulate or absorb the toxic materials in the environment and thereby take part in environmental clean up process.

Fungi are one of the important microbial components of the soil. Since 1860's, research have been carried out on the fungi of different soil types, such as soils of forest,[4,2,12] driftwood,[9] grasslands,[19,6,11] polar region,[3] desert,[5] marine and mangrove habitats[22,13,10] and coastal sand belt24 from various parts of the world. All these studies revealed that the fungi might reside permanently, temporarily for a period in the soil. Their number and species composition in the soil habitat differs from place to place depending upon the physical, chemical and biological factors of the particular habitat.

Sand dune soils along the coastline are one of the specialized habitats characterized with sparse vegetation and subjected to have some influence of the sea. Thus the sand dunes provide an interesting niche for the study of microbes, especially fungi.

MATERIALS AND METHODS

Collection of Samples

Sand dune soil samples were collected from 8 stations (Kovalam, Marakkanam, Cuddalore, Vedaranyam, Manora, Dhanushkodi, Tuticorin and Tiruchendur) along the entire Tamil Nadu coast during October 2009. The soil samples were collected from 3 inches below the surface of the sand dunes, using a metal spatula. The spatula was sterilized every time with 70% alcohol. At each station 5 samples were collected randomly from different sand dunes within the radius of 1 km. The samples were kept in new polythene bags, sealed and transported to the laboratory. For the analysis of soil nutrients, one kg of soil was separately collected in polythene bags.

Analysis of physico – chemical characteristics of soil

Moisture content content was estimated by finding the weight difference of known quantity of soil before and after keeping in an oven at 60˚C for 6 hours .pH of the soil was tested using pH meter (Systronics, India) by suspending the soil in distilled water.

EC value was tested using Electrical Conductivity meter. Nutrients such as Organic Carbon and available nitrogen were estimated by adopting the conventional methods described[7] by respectively at Soil Testing Laboratory (TNAU), Kudimiyanmalai.

Analysis of soil mycoflora

Dilution plating technique was described[27] by used to isolate the fungi from soils. Soil sample weighing 1 gm was diluted in 10 ml of distilled water. One ml of the diluted sample was plated on sterilized 2% Malt Extract Agar medium (Malt extract – 20 g; Agar – 18 g; Tap water – 1000 ml) supplemented with 1% Streptopenicillin antibiotic solution (14 ml/litre).

Preparation of antibiotic solution

One gram of streptopenicillin was mixed thoroughly in 100 ml of sterilized distilled water, aseptically and kept in a refrigerator until its use. The plates were incubated in a dust free Chamber at the room temperature (24±2˚C) for 7 days.

Observations

The colonies growing on MEA plates, with different morphology were counted separately. A portion of the growing edge of the colony was picked up with the help of a pair of needless and

mounted (Hi – media). The slide was gently heated in a sprit lamp so as to remove air bubbles. The excess stain was wiped of with the help of tissue paper and then the cover slide was sealed with transparent nail polish. The slide was observed under microscope.

Identification

Colony colour and morphology were noted besides hyphal structure, spore size, shape and spore bearing structures. They were compared with the standard works[18,26,1,17,8] others for identification of the species. Microphotography of the individual fungal species were also taken using Nikon phase contrast microscope.

Presentation of data

The term diversity was used to refer the number of species. Population density was referred as Colony Forming Unit (CFU) / g . Frequency occurrence was calculated as follows in order to identify their existence in the soils collected from different areas.

$$\% \text{ frequency} = \frac{\text{Number of samples in which a particular fungus occurred}}{\text{Total number of samples examined}} \times 100$$

Based on the frequency occurrence the fungi were grouped as rare (0.25% frequency), occasional (26 – 50 % frequently), frequent (51 – 75% frequency) and common (76 – 100% frequency) species.

RESULTS

Fungal diversity in sand dune soils

Altogether forty sand dune soil samples from 8 different station representing the entire Tamil Nadu Coast were examined for fungal diversity. The study resulted the presence of 24 species of fungi in all. Of them, 1 species belonging to one genera was assignable to *Zygomycetes*, 2 species belonging to 2 genera were *Ascomycetes* and the remaining 21 species belonging to 12 genera were assignable to *Deuteromycetes* (Table 1).

Station - wise occurrence

In different stations, the fungal diversity showed variations. The minimum number of 9 species was recorded in the sand dune soils collected from Vedaranyam and Dhanushkodi. The highest number of 18 species was recorded in the samples collected from Marakkanam. Species numbering 16 were recorded from the samples collected from Cuddalore and Tuticorin. All other stations recorded 11 species each (Fig .1)

Species Composition

Among the 12 genera recorded, the genus *Aspergillus* was constituted by more number of (9) species followed by *Penicillium* (3 species) *Fusarium* and *Monodictys* (2 species each). All other genera were represented by one species each (Table 1).

Percentage frequency

All the fungi recorded in the present investigation did not show their occurrence in all the stations. Therefore the percentage frequency was calculated according to their existence in

different stations. The highest percentage frequency was recorded by *Aspergillus niger* (100%) followed by *Drechslera sp.,Humicola sp* and *A.terreus* (87.5% each); *Rhizopus stolonifer* (75%), *Fusarium oxysporum, Monodictys putredinits, A.flavus* and *A. versicolor* (62.5% each), *Penicillium citrinum, Periconia sp., A.fumigatus, A.nidulans, A.ochraceus A.restrictus* (50% each), *Emericella nidulans, Cladosporium cladosporioides, Chuppia sp., F.tabacinum* and *M.levis* (37.5% each), *Eurotium sp., Penicillium chrysogenum* and *A.sydowi (25% each)* and *Penicillium sp*. (12.5%) (Table 1).

Frequency class

Based on the frequency of fungi, *Drechslera sp.,Humicola sp, Aspergillus niger, A.terreus,* were classified as common; *Rhizopus stolonifer, Fusarium oxysporum, Monodictys putredinits, A.flavus and A.versicolor* as frequent; *Emericella nidulans, Cladosporium cladosporioide, Chuppia sp., F.tabacinum, M.levis, Penicillium citrinum, Periconia sp., A.fumigatus, A.nidulans, A.ochraceus* and *A.restrictus* as occasional and *Eurotium sp., Penicillium chrysogenum, Penicillium sp*. and *A.sydowi* as rare species in coastal sand dune habitats of Tamil Nadu (Table 1).

Population density

As that of species diversity, population density also showed variations in different stations. Mean fungal population density recorded the minimum of 8.8 X 10^{-1} CFU/g and the maximum of 20.6 X 10^{1} CFU/g in the sand dune samples collected from Manora and Tuticorin respectively. The samples collected from Kovalam, Marakkanam, Vedaranyam, Tiruchendur, Cuddalore and Dhanushkodi recorded 17.2, 17.0, 16.2, 15.0, 13.6 and 10.0 X 10^{1} CFU/g of fungal population, respectively (Fig.2).

Physico-chemical properties of sand dune soil

Physico-chemical properties of the soil showed variations in different stations. Moisture content was ranged from 3.6 to 34.0 percent, while the water holding capacity was from 17.4 to 21.5 percent in soils collected from different stations. pH was alkaline in all the sand dune soil samples except the one collected from Cuddalore, which exhibited the acidic pH of 6.65. The maximum pH was 8.8, among the different stations. Electrical conductivity showed variations from 0.06 to 2.4 in the samples collected from Dhanushkodi and Kovalam respectively. The organic carbon content was as low as 4 mg/g in the soils collected from Kovalam and Tuticorin. The maximum of 20 mg/g was recorded the soils collected from Vedaranyam. Available nitrogen content of the soil was meager and showed lesser variations. The minimum of 0.014 mg/g was recorded in the soils collected from Marakkanam, Vedaranyam and Tiruchendur while the maximum of 0.046 was recorded in Kovalam (Table 2).

Relationship between physicochemical parameters and fungal population

Correlation analysis made between physico – chemical parameters and fungal population revealed no significant relationship with moisture content, pH, organic carbon and available nitrogen contents of the soil. However water holding capacity ($r = 0.640$; $P < 0.2$) and electrical conductivity ($r = 0.338$; $P < 0.1$) showed positive correlation (Table 3).

DISCUSSION

The present investigation on soil mycoflora of sand dunes of Tamil Nadu yielded some basic information on the species diversity, population density of fungi and its associated physico – chemical characteristics.

Table 1: Occurrence, Percentage frequency class of different fungal species in sand dune soils of Tamil Nadu coast

S. No	Fungal species	Kovalam	Marakkanamm	Cuddalore	vedaranyam	Manora	Dhanuskodi	Tuticorin	Tiruchendur	% frequency	Frequency Class
	ZYGOMYCOTINA										
1.	*Rhizopus stolonifer*	+	+	+	+	+	+	-	-	75	F
	ASCOMYCOTINA										
2.	*Emericella nidulans*	-	+	+	-	-	-	+	-	37.5	O
3.	*Eurotium sp.*	-	+	+	-	-	-	-	-	25	R
	DUETEROMYCOTINA										
4.	*Aspergillus flavus*	-	+	+	-	-	+	+	+	62.5	F
5.	*A.fumigatus*	+	-	-	-	+	+	+	-	50	O
6.	*A.nidulans*	+	+	+	-	-	-	+	-	50	O
7.	*A.niger*	+	+	+	+	+	+	+	+	100	C
8.	*A.ochraceus*	-	+	+	-	+	-	+	-	50	O
9.	*A.restrictus*	+	-	+	-	+	-	-	+	50	O
10.	*A.sydowi*	-	+	-	-	+	-	-	-	25	R
11.	*A.terreus*	+	+	-	+	+	+	+	+	87.5	C
12.	*A.versicolor*	+	+	-	-	+	-	+	+	62.5	F
13.	*Cladosporium cladosporioides*	+	+	-	-	-	-	-	+	37.5	O
14.	*Chuppia sp.*	-	+	+	-	-	-	+	-	37.5	O
15.	*Drechslera sp.*	+	+	-	+	+	+	+	+	87.5	C
16.	*Fusarium oxysporum*	-	-	+	+	-	+	+	+	62.5	F
17.	*F.tabacinum*	+	+	+	-	-	-	-	-	37.5	O
18.	*Humicola sp.*	+	+	-	+	+	+	+	+	87.5	C
19.	*Monodictys levis*	-	+	+	-	-	-	+	-	37.5	O
20.	*M.putreinis*	-	+	+	+	-	-	+	+	62.5	F
21.	*Penicillium chrysogenum*	-	+	+	-	-	-	-	-	25	R
22.	*Penicillium citrinum*	-	-	+	+	-	-	+	+	50	O
23.	*Penicillium sp.*	-	-	-	+	-	-	-	-	12.5	R
24.	*Periconia sp.*	-	-	+	-	+	+	+	-	50	O

+ denotes presence of species, - denotes absence of species; C – Common; F – Frequent; O – Occasional; R – Rare.

Among the 24 species recorded in the present study the genus *Aspergillus* was represented by 9 species. This indicate that *Aspergillus* was the dominant fungal genus of sand dune soils. Dominant occurrence of *Aspergillus* was reported[16,15] from various marine soils. They have explained their suitability to grow in higher saline concentration for their dominant distribution in coastal marine habitats. A great majority of the fungi recorded in the present study were previously reported from various marine habitats such as sediment and water[23,16] sand dunes and sand belt,[24] decomposing biomass,[21,20] mangrove rhizosphere,[25] mangrove soils,[14] except, Emericella nidulans, Eurotium sp., Chuppia sp., Monodicts levis, Fusarium tabacianum and M.putredinis. Hence, these are distributional records to the coastal sand dune habitats of

Tamil Nadu. Grouping made based on the frequency occurrence of 5 species, occasional occurrence of 11 species and rare occurrence of 4 species of fungi. Among the 6 species of fungi reported as new distributional records in sand dune soils *Eurotium sp*. was rare, *Chuppia sp*., *F.tabacianum* and *M.levis* were occasional and *M.putredinis* was frequent.

All the soil samples collected were alkaline in nature, except the one collected from Cuddalore. Alkaline condition was explained as the characteristic feature of marine / marine influenced habitats are well adopted to grow in alkaline pH.[15] Therefore the fungal population did not seem to influence by the pH. EC is the indirect measure of salinity, which showed range from 0.06 to 2.4. This was comparatively lower than the marine and brackish water sediments.[23] The nutrients levels were very less than all the marine habitats. Hence, the correlation analysis did not bring any relationship with the fungal population, in contrary to the statement,[15] who revealed a significant positive correlation between OC and fungal population in the coral reef sediments.

Thus, the present study brought out the fungal diversity, population density and community structure of sand dunes and its associated physico – chemical conditions, though a definite reason for the changes in density and diversity pattern of fungi in different stations could not be arrived.

Table 2. Physico – chemical properties of sand dune soils

Station	Moisture content	Water Holding capacity	pH	EC	Organic carbon (mg/g)	Available nitrogen (mg/g)
Kovalam	3.9	20.1	7.1	2.4	4	0.046
Marakkanam	4.5	20.0	6.8	2.3	15	0.014
Cuddalore	21.1	19.3	6.6	2.0	13	0.021
Vedaranyam	34	21.5	7.9	2.1	20	0.032
Manora	11.1	18.9	8.0	0.07	7	0.018
Dhanushkodi	7.2	17.4	8.8	0.06	5	0.033
Tuticorin	20.2	17.9	8.8	0.07	4	0.023
Tiruchendur	3.6	17.8	8.8	0.08	8	0.038

Table 3. Correlation coefficient (r) values for physicochemical characteristics of the soil and fungal population

	Moisture content (%)	Water holding capacity (%)	pH	Electrical conductivity (EC)	Organic carbon (mg/g)	Available nitrogen (mg/g)	Total fungal population (CFU/g)
Moisture content (%)	1						
Water holding capacity (%)	0.405	1					
pH	-0.305	0.325	1				
Electrical conductivity (EC)	0.527	0.586	0.092	1			
Organic carbon (mg/g)	0.683	0.233	-0.637	0.381	1		
Available nitrogen (mg/g)	-0.37	-0.138	0.293	0.330	-0.59	1	
Total fungal population (CFU/g)	0.139	0.640**	0.013	0.338*	0.091	0.029	1

***P <0.2 level; *P<0.1 level*

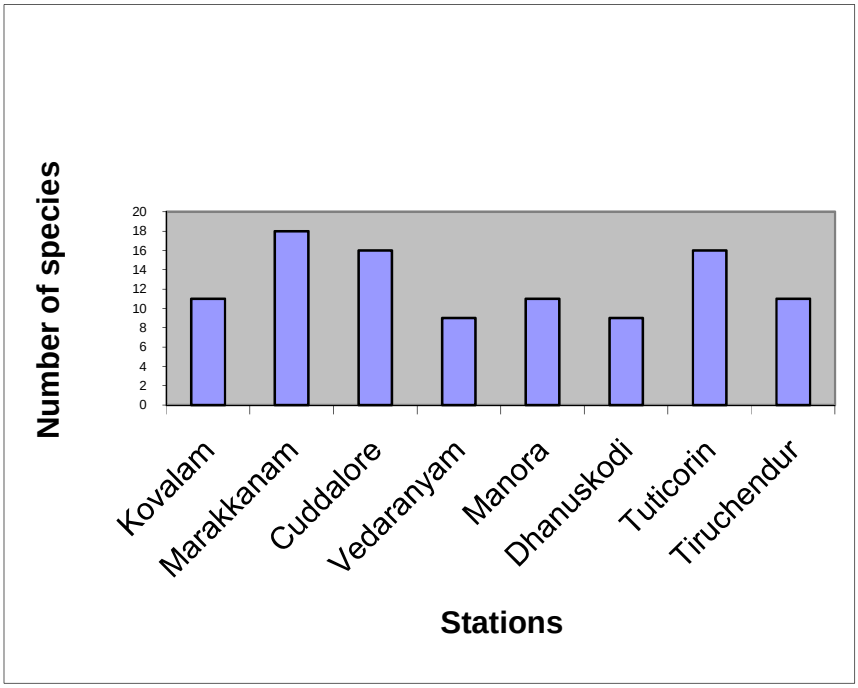

Fig 1. Fungal diversity in different sampling stations

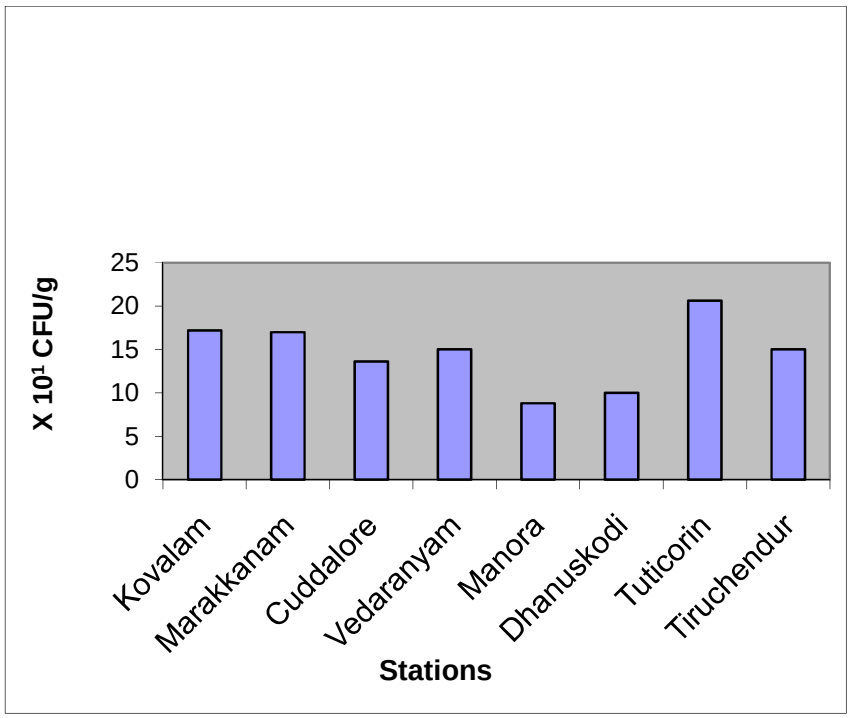

Fig 2 Mean Population density of fungi in different sampling stations

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