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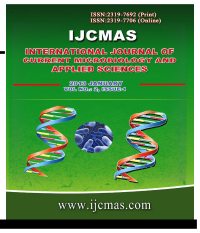

Original Research Article

Isolation, identification and Characterization of *Fusarium* species from mangrove habitat of Pichavaram, Tamil Nadu, India

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Keywords

Mangrove soil; *Fusarium* sp; Growth characteristics; Effect of physical and chemical parameters.

A B S T R A C T

The present study was confined to the Pichavaram mangrove ecosystem in Cuddalore District, Tamil Nadu. Sediment (Soil) samples of mangrove were collected to isolate the fungi. All the collected samples were plated, incubated and the fungal colonies were identified. Colony growth rate of the fungi was studied on seven different types of solid media (PDA, SDA, CMA, CZA, MA, RBA, and OMA). Effect of different ecological parameters such as pH (5-9), temperature (20-60ºC), salinity (5-40%), metals (FeSO4 and ZnSO4), carbon (CMC, starch and mannitol) and nitrogen sources (ammonium nitrate and calcium nitrate) on the growth of fungi was also determined. A total of 21 fungal species of Fusarium were isolated and enumerated by plating, techniques. Maximum growth rate of *F. oxysporum* was observed in PDA, SDA, CMA and RBA than other media. Maximum fungal growth was observed in pH 8, 30ºC (temperature), 40% (salinity), $FeSO_4$ (metal), carboxy methyl cellulose (carbon source) and ammonium nitrate (nitrogen source) after 8 days of growth in liquid medium.

Introduction

Mangrove forests are located at the interface between land and sea, a unique and extreme environment. The soils in mangrove communities are muddy or sandy with loose sediment. They contain submerged mangrove roots, trunks and branches. These conditions attract rich communities of fungi and bacteria. Biodiversity of fungi is an important aspect to be dealt with utmost scientific accuracy and accountability. One third of fungal diversity of the globe exists in India. Out of 1.5 million of fungi, only 50% are identified and remaining 50% need to be identified. Unfortunately around 5 –10% of these fungi alone can be cultured artificially. The variety and galaxy of fungi and their natural beauty occupy prime place in the biological world and India has been the cradle for such fungi. Only a fraction of total fungal wealth has been subjected for scientific scrutiny and mycologists have to explore

the unexplored and hidden wealth (Manoharachary *et al*., 2005). Mangroves are open systems with respect to both energy and matter and can be considered "interface" ecosystems coupling upland terrestrial and coastal estuarine ecosystems. (Lugo and Snedaker, 1974). While terrestrial fungi and lichens occupy the aerial parts of mangrove plants, the marine fungi occur at lower parts where their trunks and roots are permanently or intermittently submerged in water. At the high tide mark there will be an interface and an overlap of marine and terrestrial fungi that occur (Kohlmeyer, 1969; Kohlmeyer and Kohlmeyer, 1979).

Mangrove forests generate considerable amount of detritus such as leaf litter, woody debris and infloresence (Wafar *et al*., 1997) and hence constitute an ideal habitat for many detritus – dependant fauna and microbes. Chandralata (1999) and Raghukumar and Raghukumar (1998) reported adaptation and activity of terrestrial fungi under marine/ mangrove ecosystem as facultatives or indwellers or residents. Terrestrial fungi are common in mangrove water and mud (Chowdhery *et al* 1982; Garg, 1983), mangrove leaves (Raghukumar *et al*., 1995), wood (Aleem, 1980), standing senescent stems (Sadaba *et al*., 1995), decomposing mangrove palm (*Nypa fruticans*) (Hyde and Alias, 2000). Terrestrial fungi in deep - sea region of Arabian Sea were recovered (Raghukumar and Raghukumar, 1998).

Seawater, sea foam and beach soil of Arabian Gulf Coast, Saudi Arabia yielded terrestrial fungi, typical marine and freshwater fungi (Bokhary *et al*., 1992). Sampling of the leaf litter from the Nethravathi mangroves, India revealed the occurrence of many freshwater Hyphomycetes (Sridhar and Kaveriappa, 1988). Based on the necessary basic information obtained on marine fungi and mangrove ecosystem, the present study has been undertaken in the proposed study area in Pichavaram mangroves, a coastal deltaic habitat along the East coast of Palk Strait, in Bay of Bengal in Cuddalore District, Tamil Nadu.

Materials and Methods

Sample collection

Soil sample were collected from an area of mangrove forest in Pichavaram, Tamil Nadu, India. The soil sample were taken from a depth of 5-10 cm and then kept in plastic bags until drying was performed immediately after sampling in the laboratory.

Sample processing

The soil samples were air dried at room temperature (27±1°C) for seven days and then ground using a mortar and pestle. Ground soil samples were sieved with a 0.5mm sieve to remove larger particle such as stone and plant debris in order to obtain a consistent soil particle size for isolation using the soil dilution technique. Silver soils and debris were then store separately in paper bags and kept at 4°C.

Isolation and Identification of *F. oxysporum*

The method were used for isolation of *Fusarium* isolates from the mangrove soil samples namely, soil dilution, debris isolation and direct isolation techniques. After sampling, within 24 hrs the water samples from each station were subjected to appropriate dilutions (10^{-2} to10^{-5}) and 0.1 ml of sample was aseptically transferred into the plates containing Potato dextrose agar/ Czapek dox

agar/Corn meal agar/Rose Bengal agar with addition of mixture antibiotics, Tetracycline and Penicillin (Spread plate method) The plates were incubated at room temperature (28°C) for 4-5 days (Plate. 2a). Control plates were also maintained. Sterilization of glasswares and preparations of media were carried out as per the method described by Booth (1971).

Presentation of data

The semi permanent slides of the isolated fungi were prepared using Lactophenol Cotton Blue Staining method (Dring, 1976) and sealed with DPX mountant. The fungal species were photographed using photo micrographic instrument (Nikon AFX II Microscope fitted with Nikon FX-35 camera, Tokyo, Japan).

Identification of fungi

The identification of fungal taxa was based on illustrated Genera of imperfect fungi (Barnett, 1965), Hyphomycetes (Subramanian, 1971), Dematiaceous Hyphomycetes and More Dematiaceous Hyphomycetes (Ellis, 1971, 1976), Marine Mycology (Kohlmeyer and Kohlmeyer, 1979), Micro fungi on land plants (Ellis and Ellis, 1985) Micro fungi on Miscellaneous substrate (Ellis and Ellis, 1988), Illustrated key to the filamentous higher marine fungi (Kohlmeyer and Volkman - Kohlmeyer, 1991) and Manual of soil fungi (Gilman, 1957, 1998).

Maintenance of fungal cultures

Cultures are maintained by separated sub culturing on appropriate medium. Fungi cultures were inoculated into the PDA plates. The plates were maintained at room temperature for week.

Growth and Morphological characteristics of fungi on various media

In this study the *Fusarium* species (21 sp.) of fungi were selected. All the fungi were inoculated (agar block containing fungi) in center of seven fungal media such as PDA, SDA, CMA, CZA, MA, RBA and OMA. The inoculated plates were incubated at room temperature (28ºC) for 6 days. After incubation period, the radial growth (diameter in mm) of each fungus was measured (Palacios – Cabrera *et al*., 2005).

Effect of physical and chemical parameters on fungal growth

In this study, the most *Fusarium* species (21 sp.) were selected and studied for biomass, effect of various parameters such as pH, temperature, salinity and carbon and nitrogen sources (Booth, 1971; Boyd and Kohlmeyer, 1982; Aneja, 2001).

Effect of Fungal Biomass

All the fungi were inoculated into Potato Dextrose broth (PD) and incubated at room temperature. After incubation for 8 days, the optical density was measured at 600 nm. The fungal fresh and dry weights were also determined.

Effect of pH on fungal growth

All the fungi were inoculated into Potato Dextrose broth (PD) broth containing different pH ranges (5, 6, 7, 8 and 9) and incubated at room temperature. After incubation for 8 days, the optical density was measured at 600 nm. The fungal fresh and dry weights were also determined.

Effect of Temperature on fungal growth

All the fungi were inoculated into Potato Dextrose broth (PD) broth and the tubes were incubated at different temperature range (20, 30, 40, 50 and 60°C) and incubated at room temperature. After incubation for 8 days, the optical density was measured at 600 nm. The fungal fresh and dry weights were also recorded.

Effect of Salinity on fungal growth

All the fungi were inoculated into Potato Dextrose broth (PD) containing different salinity ranges such as 5, 10, 20, 30 and 40 % and incubated at room temperature. After incubation for 8 days, the optical density was measured at 600 nm. The fungal fresh and dry weights were also recorded.

Effect of Carbon and Nitrogen Sources on fungal growth

All the fungi were inoculated into Potato Dextrose broth (PD) broth containing different carbon sources (Carboxy Methyl Cellulose, Starch, Mannitol) and nitrogen Source (Ammonium nitrate and Calcium nitrate) and incubated at room temperature. After incubation for 8 days, the Optical density was measured at 600 nm. The fungal fresh and dry weights were also determined.

Effect of Heavy Metals on fungal growth

All the fungi were inoculated into Potato Dextrose broth (PD) broth containing different heavy metals (Ferric sulphate and Zinc sulphate) and incubated at room temperature. After incubation for 8 days, the Optical density was measured at 610 nm. The fungal fresh and dry weights were also measured.

Results and Discussion

Isolation of fungi from the sediment samples

In this study, 21 species of *Fusarium* species were recovered from sediment samples collecte4d mangrove habitat of Pichavaram, Tamil Nadu, India. Among the Hyphomycetes, *Fusarium* was the common genus represented by 21 species. in sediments samples also the genus *Fuarium* was also found to be dominant, includes, *F.oxysporum, F.citri, F.culmorum, F.verticillioides, F.proliferatum, F.equiseti, F,roseum, F. napiforme, F. sambucium and F.fusarioides*. This well agreed with the findings of Garg (1982), Rai and Chowdhery (1978), Raper and Fennell (1965) and Roth *et al*. (1964). According to their findings Aspergilli dominated over Mucorales and Penicillia in the mud of mangrove swamps of Sunderband. Nicot (1958) recorded the dominance of Aspergilli and Penicillia in the coastal soils of France. Furthermore, Raper and Fennell (1965) have also suggested that certain non-osmophilic species of *Aspergillus* may grow luxuriantly under halophytic conditions. Although terrestrial fungi are found in coastal environments frequently as part of the spore population, only species adapted to saline environments appear to be able complete their life cycles fully in coastal and marine environments (Jennings 1986). Sparrow (1934, 1936) reported that the presence of *Aspergillus, Penicillium* and *Fusarium* species in the marine sediments. Satio (1952) investigated the mycoflora of a salt marsh and observed that the species of *Penicillium, Fusarium* and *Trichoderma*

vignorum were the common forms encounted in the surface mud. This well correlates with the findings made by Garg (1983) in which, he came come cross highest number of fungi from surface layer of the Sunderban mangrove mud. Ito *et al.* (2001) have reported the mycobiota of mangrove forest soils from the rhizosphere of eight mangrove species collected at the Ranong Research Center (Kasetsart University) and Phang-Nga. Two methods were used to isolate the fungi: incubation at 45ºC and the standard dilution plate method. Forty-two fungal strains were documented from soil samples, all typical soil taxa, with *Penicillium* sp., *Trichoderma harzianum* and an unidentified strain were the most commonly isolated strains. Further, mangrove soil fungi have been reported by Wongthong (2001), Kongamol (2001) and Sriswadskulmee (2002).

Isolation of *Fusarium* species in greater number and frequency is due to the high nutrient level in the mangrove eco-system. These species prefer a medium with high osmotic concentration and therefore, compete more easily with other forms in the mangrove eco-system.

Growth and morphological characteristics of fungi on various media

In this study, Maximum growth rate was observed in *Fusarium oxysporum* with 85 in PDA , 83 mm in diam (CMA),84 in RBA than other media used. The minimum growth rate of *F. oxysporum* (60 mm in diam) was observed in CMA and MA agar plates. This study was well correlated with earlier findings by Palacios - Cabrera *et al*. (2005). They studied that the influence of three culture media with different water activity, time of incubation and temperature on the growth of *Aspergillus ochraceus*, *A. niger* and *A. carbonarius* on GYA, DG18, Malt agar with 40% glucose agar (Table.1).

Growth characteristics of fungi on various parameters

Fungal biomass study

In this study, *F.oxysporum* showed maximum optical density with 1.991 followed by *F.moniliforme* and other species of *Fusarium* species. Fresh weight of the fungi were also maximum in 7.81mg/g(Table.2). Minimum of fresh weight was observed in *F.solani* (1.66 mg/g). Dry weights of the fungi were also maximum in 2.75mg/g. and Minimum of dry weight was observed in *F.moniliforme* (0.20 mg/g)**.** Ecological studies were carried out by various physico-chemical parameters. Among these, pH (8), temperature (30°C), salinity (5%), metals (FeSO4), carbon source (CMC) and nitrogen source (ammonium nitrate) influenced the maximum growth of fungi in liquid media on 8 days of incubation at room temperature. Fresh and dry weights of the fungi were maximum in above conditions (Table.2).

Effect of pH on the growth of fungi

In this study, the maximum growth was observed in pH 8 after 8 days of incubation. In this pH, *F.oxysporum* showed maximum growth with 2.301 (optical density. Minimum growth rate was observed in *F.verticilliodes* (0.635) (Table.3). Fresh weight of the fungi were also maximum in *F.oxysporum* with 2.97 mg/g and Dry weights of the fungi were also maximum with 0.68 mg/g (Table.4&5). The effect of temperature, pH, salinity and salinity- temperature interaction for thermophilic and thermotolernt fungi from Sundarban

Table. 1 Growth and morphological characteristics of *Fusarium oxysporum* on various media
(The values are represented in mm in diameter).

S.No	Name of the fungi	PDA	SDA	CMA	CZA	MA	RBA	OMA
1	*Fusarium oxysporum*	85	80	83	67	60	84	60
2	*F.moniliforme*	73	24	52	36	82	39	47
3	*F.graminearum*	63	20	51	31	33	45	35
4	*F.culmorum*	72	63	15	19	59	79	28
5	*F.solani*	72	39	40	33	35	83	59
6	*F.semitectum*	49	80	68	73	53	78	83
7	*Fusarium sp.1*	51	23	19	19	20	31	26
8	*Fusarium sp.2*	25	32	12	15	31	10	16
9	*Fusarium citri*	16	18	15	25	14	13	19
10	*F.subulatum*	34	38	40	26	23	18	23
11	*Fusarium sp.3*	38	29	41	36	34	33	41
12	*Fusarium sp.4*	46	35	45	24	31	38	18
13	*F.verticillioides*	18	26	31	21	21	15	16
14	*Fusarium proliferatum*	25	22	30	32	39	14	18
15	*Fusarium equiseti*	16	17	30	23	31	14	18
16	*F.roseum*	39	33	34	26	72	31	61
17	*F.napiforme*	17	13	11	11	13	10	12
18	*F.sambucinum*	40	51	29	35	35	18	21
19	*Fusarium sp.5*	43	65	29	21	30	31	55
20	*Fusarium sp.6*	54	21	42	45	29	28	32
21	*F.fusarioides*	15	42	19	22	19	13	11

PDA- Potato Dextrose Agar, SDA- Sabouraud's Dextrose Agar,CMA-Corn Meal Agar, CZA-Czapek's Dox Agar, MA- Malt Agar, RBA- Rose Bengal Agar, OMA-Oat Meal Agar.

Table. 2 Effect of biomass of *Fusarium oxysporum*
(The values are represented in OD at 600 nm)

S.No	Name of the fungi	8days	Fresh & Dry weights in mg/g (After 8 days)	
			Fresh	Dry
1	*Fusarium oxysporum*	1.991	7.81	2.75
2	*F.moniliforme*	1.859	2.82	0.20
3	*F.graminearum*	1.524	5.47	0.28
4	*F.culmorum*	1.755	2.04	0.41
5	*F.solani*	0.410	1.66	0.56
6	*F.semitectum*	0.763	6.47	1.38
7	*Fusarium sp.1*	1.299	5.61	0.78
8	*Fusarium sp.2*	0.389	4.83	1.03
9	*Fusarium citri*	1.203	4.91	0.59
10	*F.subulatum*	1.852	5.01	2.41
11	*Fusarium sp.3*	1.873	7.80	2.41
12	*Fusarium sp.4*	0.821	4.54	0.83
13	*F.verticillioides*	1.269	5.95	1.23
14	*Fusarium proliferatum*	1.704	5.16	0.42
15	*Fusarium_equiseti*	0.421	6.98	2.72
16	*F.roseum*	1.493	2.0	0.88
17	*F.napiforme*	1.883	4.99	2.09
18	*F.sambucinum*	1.929	7.68	2.17
19	*Fusarium sp.5*	1.955	9.28	3.02
20	*Fusarium sp.6*	1.159	6.09	1.26
21	*F.fusarioides*	1.159	4.16	0.36

Table. 3 Effect of pH on *Fusarium oxysporum* growth (The values are represented in OD at 600 nm)

S.No	Name of the fungi	5	6	7	8	9
1	*Fusarium oxysporum*	1.449	1.452	1.447	2.301	1.885
2	*F.moniliforme*	0.823	0.931	0.971	0.998	0.451
3	*F.graminearum*	0.761	0.942	0.725	1.424	0.540
4	*F.culmorum*	0.368	0.763	0.802	1.657	0.648
5	*F.solani*	1.564	1.646	1.236	1.993	1.127
6	*F.semitectum*	0.695	1.273	1.158	1.315	0.602
7	*Fusarium sp.1*	0.929	1.217	0.658	2.041	1.153
8	*Fusarium sp.2*	1.063	1.177	1.009	1.518	1.174
9	*Fusarium citri*	0.668	0.641	0.607	0.782	0.697
10	*F.subulatum*	1.237	1.200	1.402	1.480	1.231
11	*Fusarium sp.3*	0.899	1.215	0.877	1.272	1.174
12	*Fusarium sp.4*	1.022	0.944	1.297	1.581	1.330
13	*F.verticillioides*	0.543	0.592	0.588	0.635	0.597
14	*Fusarium proliferatum*	0.624	1.083	0.516	1.863	0.644
15	*Fusarium equiseti*	2.148	1.876	1.587	2.876	2.174
16	*F.roseum*	0.807	0.537	0.367	0.933	0.852
17	*F.napiforme*	1.922	2.157	0.594	2.223	2.004
18	*F.sambucinum*	1.106	0.784	1.465	1.717	1.510
19	*Fusarium sp.5*	0.952	0.320	1.228	1.265	1.104
20	*Fusarium sp.6*	1.241	1.340	1.235	1.519	1.294
21	*F.fusarioides*	1.394	0.973	1.274	1.815	0.501

Table. 4 Effect of pH on fresh weight of *Fusarium oxysporum* (The values are represented in OD at 600 nm)

S.No	Name of the fungi	5	6	7	8	9
1	*Fusarium oxysporum*	*1.333*	0.62	0.12	2.99	*1.333*
2	*F.moniliforme*	1.06	1.01	0.65	1.19	1.19
3	*F.graminearum*	1.15	1.30	0.96	1.72	1.07
4	*F.culmorum*	0.79	0.80	0.78	1.25	0.90
5	*F.solani*	1.10	0.96	0.88	1.26	1.20
6	*F.semitectum*	0.84	1.13	0.50	1.15	0.91
7	*Fusarium sp.1*	1.01	0.97	1.56	1.78	1.02
8	*Fusarium sp.2*	1.10	0.70	0.28	2.18	2.43
9	*Fusarium citri*	1.05	1.18	1.10	1.27	1.17
10	*F.subulatum*	1.92	1.80	1.72	2.11	1.76
11	*Fusarium sp.3*	2.08	2.79	2.35	2.97	2.66
12	*Fusarium sp.4*	1.83	2.01	2.65	2.97	2.21
13	*F.verticillioides*	1.24	1.42	1.59	2.86	1.40
14	*Fusarium proliferatum*	1.60	1.54	2.02	2.59	2.23
15	*Fusarium equiseti*	1.74	1.52	1.85	1.96	1.79
16	*F.roseum*	1.88	2.16	2.15	2.31	1.6
17	*F.napiforme*	1.45	2.16	2.04	2.75	2.04
18	*F.sambucinum*	1.98	2.20	2.43	2.85	1.63
19	*Fusarium sp.5*	1.82	1.92	1.97	2.09	1.74
20	*Fusarium sp.6*	2.4	1.31	1.92	2.75	1.88
21	*F.fusarioides*	1.89	2.06	1.88	2.14	0.89

Table.5 Effect of pH on dry weight of *Fusarium oxysporum* (The values are represented in mg/ g)

S.No	Name of the fungi	5	6	7	8	9
1	*Fusarium oxysporum*	0.04	0.11	0.06	0.68	0.09
2	*F.moniliforme*	0.07	0.10	0.07	0.16	0.14
3	*F.graminearum*	0.47	0.80	0.08	0.90	0.13
4	*F.culmorum*	0.08	0.09	0.09	0.29	0.12
5	*F.solani*	0.06	0.10	0.07	0.11	0.08
6	*F.semitectum*	0.03	0.01	0.03	0.04	0.03
7	*Fusarium sp.1*	0.09	0.02	0.05	0.14	0.04
8	*Fusarium sp.2*	0.01	0.05	0.04	0.18	0.01
9	*Fusarium citri*	0.08	0.18	0.07	0.24	0.02
10	*F.subulatum*	0.10	0.17	0.05	0.24	0.04
11	*Fusarium sp.3*	0.02	0.12	0.25	0.28	0.08
12	*Fusarium sp.4*	0.01	0.04	0.02	0.08	0.06
13	*F.verticillioides*	0.01	0.02	0.11	0.21	0.01
14	*Fusarium proliferatum*	0.02	0.05	0.08	0.08	0.08
15	*Fusarium equiseti*	0.04	0.01	0.03	0.21	0.01
16	*F.roseum*	0.04	0.09	0.06	0.14	0.09
17	*F.napiforme*	0.06	0.06	0.07	0.15	0.06
18	*F.sambucinum*	0.02	0.01	0.02	0.20	0.03
19	*Fusarium sp.5*	0.06	0.04	0.05	0.13	0.02
20	*Fusarium sp.6*	0.02	0.02	0.03	0.16	0.01
21	*F.fusarioides*	0.01	0.05	0.06	0.14	0.02

Table.6 Effect of temperature on *Fusarium oxysporum* (The values are represented in OD at 600 nm)

S.No	Name of the fungi	20°C	30°C	40°C	50°C	60°C
1	*Fusarium oxysporum*	0.806	2.567	1.007	0.407	0.744
2	*F.moniliforme*	1.146	1.481	1.162	1.313	0.694
3	*F.graminearum*	1.307	1.722	1.287	0.177	0.608
4	*F.culmorum*	0.504	1.291	1.025	0.238	0.381
5	*F.solani*	0.968	2.546	0.717	2.092	1.791
6	*F.semitectum*	1.655	1.738	1.093	0.855	1.041
7	*Fusarium sp.1*	1.676	1.717	1.301	0.955	0.516
8	*Fusarium sp.2*	1.866	1.904	1.611	1.600	1.262
9	*Fusarium citri*	1.213	1.314	1.046	1.311	1.173
10	*F.subulatum*	1.432	1.835	1.526	1.041	1.340
11	*Fusarium sp.3*	1.272	2.645	1.920	2.176	1.454
12	*Fusarium sp.4*	2.012	2.136	1.640	1.331	2.00
13	*F.verticillioides*	1.173	2.448	1.454	0.968	0.869
14	*Fusarium proliferatum*	1.147	1.968	1.764	1.353	1.193
15	*Fusarium equiseti*	1.317	1.733	1.529	0.632	0.700
16	*F.roseum*	1.455	1.528	1.331	1.390	0.908
17	*F.napiforme*	1.170	2.109	1.770	1.221	1.031
18	*F.sambucinum*	1.473	1.620	1.249	0.653	0.281
19	*Fusarium sp.5*	1.676	1.987	1.246	1.954	1.813
20	*Fusarium sp.6*	1.568	1.574	0.882	0.403	0.437
21	*F.fusarioides*	1.470	1.637	1.611	1.153	0.327

mangrove swamp have been investigated by several investigators (Jaitly, 1982, 1983; Jaitly and Rai, 1982). They have observed that forms like *A. fumigatus, Humicola* and *Thermomyces* have a wide range of temperature tolerance.

Effect of temperature on the growth of fungi

In this study, the maximum growth was observed in temperature range of 30ºC after 8 days of incubation (Table.6). In this temperature study, *F.oxysporum* showed maximum growth with 2.576 (optical density). Fresh weights of the fungi were also maximum with 1.48 mg/g. Dry weight of the fungi was also maximum *F.oxysporum* (0.84 mg/g) (Table.7&8). This result was discussed with earlier studies by Ritchie (1957,1959). They found that water, temperature and salinity have a combined effect on the growth rate of certain fungi. Studies of some fungi isolated from mangrove swamps and marine habitats clearly indicate that the incubation temperature increases, the salinity optima also increase until the temperature becomes a limiting factor (Chowdhery, 1975; Jaitly, 1983; Ritchie, 1957, 1959).

The effect of temperature, pH, salinity and salinity- temperature interaction for thermophilic and thermotolernt fungi from Sundarban mangrove swamp have been investigated by several investigators (Jaitly, 1983; Jaitly and Rai, 1982). They have observed that forms like *A. fumigatus, Humicola* and *Thermomyces* have a wide range of temperature tolerance. Boyd and Kohlmeyer (1982) studied that the influence of temperature on the seasonal and geographic distribution of three marine fungi and dry weight of fungi analysed. The effect of temperature on the growth and sporulation of aquatic hyphomycetes has been studied by Koske and Puncan (1974), Suberkropp (1984) and Webster *et al*. (1976).

Effect of salinity on the growth of fungi

In this study, the maximum growth (optical density) was observed in salinity 40% after 8 days of incubation ((Table.9). In this salinity study, *F.oxysporum* showed maximum growth (2.781). Fresh weight of the fungi were also maximum in *F.oxysporum* (2.98 mg/g)**.** Dry weights of the fungi were also maximum *F.oxysporum* (1.08 mg/g) (Table.10&11). The above parameters were discussed with the studies carried out by Hohnk (1952, 1953, 1955, 1956) on the physiology, ecology and distribution of marine fungi in relation to salinity. Chowdhery (1975) reported that mangrove isolates have higher osmotic optima as compared to their fertile soil counterparts. In mangrove swamps, the microbial life has to withstrand high salinity and fungi found in this habitat show a high degree of osmotic tolerance and increased salinity optima. Jaitly, (1983), Jaitly and Rai, (1982) investigated the effect of temperature, pH, salinity and salinity- temperature interaction for thermophilic and thermotolernt fungi from sundarban mangrove swamp.

It is interesting therefore that in considering the physiological response of terrestrial and marine fungi to increasing salinities, it can be seen that there is good correlation with the observed distribution of these fungi under natural conditions. Typically marine fungi exhibit a broad tolerance to salinity while the terrestrial fungi are inhibited by higher salinities, especially their reproduction and spore germination. Thus, the statement of Jones

Table. 7 Effect of temperature on fresh weight of *Fusarium oxysporum* (The values are represented in mg/ g)

S.No	Name of the fungi	20°C	30°C	40°C	50°C	60°C
1	*Fusarium oxysporum*	0.87	1.48	0.71	1.0	0.62
2	*F.moniliforme*	0.89	0.94	0.65	0.63	0.77
3	*F.graminearum*	0.70	0.97	0.54	0.49	0.77
4	*F.culmorum*	0.60	0.82	0.57	0.76	0.64
5	*F.solani*	0.72	0.80	0.79	0.78	0.71
6	*F.semitectum*	0.71	0.84	0.76	0.79	0.77
7	*Fusarium sp.1*	0.98	1.27	1.11	0.61	0.94
8	*Fusarium sp.2*	0.28	0.67	0.57	0.59	0.50
9	*Fusarium citri*	0.36	1.71	0.85	0.83	1.03
10	*F.subulatum*	0.19	0.47	0.41	0.12	0.42
11	*Fusarium sp.3*	0.97	1.27	1.17	0.95	0.99
12	*Fusarium sp.4*	0.70	1.53	1.08	1.03	0.46
13	*F.verticillioides*	0.09	0.32	0.53	0.53	0.42
14	*Fusarium proliferatum*	0.98	1.09	0.92	1.16	1.07
15	*Fusarium equiseti*	0.40	0.74	0.19	0.44	0.40
16	*F.roseum*	0.93	1.01	1.00	0.97	1.02
17	*F.napiforme*	0.32	1.27	1.21	0.34	0.56
18	*F.sambucinum*	0.28	0.60	0.51	0.53	0.37
19	*Fusarium sp.5*	0.59	0.94	0.69	0.41	0.78
20	*Fusarium sp.6*	0.34	0.78	0.76	0.34	0.53
21	*F.fusarioides*	0.69	0.93	0.60	0.84	0.76

Table. 8 Effect of temperature on Dry weight of *Fusarium oxysporum* (The values are represented in mg/ g)

S.No	Name of the fungi	20°C	30°C	40°C	50°C	60°C
1	*Fusarium oxysporum*	0.09	0.84	0.09	0.10	0.08
2	*F.moniliforme*	0.11	0.19	0.10	0.10	0.08
3	*F.graminearum*	0.10	0.19	0.09	0.09	0.08
4	*F.culmorum*	0.08	0.17	0.09	0.10	0.08
5	*F.solani*	0.09	0.16	0.08	0.10	0.10
6	*F.semitectum*	0.22	0.31	0.22	0.07	0.02
7	*Fusarium sp.1*	0.23	0.32	0.28	0.09	0.04
8	*Fusarium sp.2*	0.11	0.22	0.16	0.19	0.13
9	*Fusarium citri*	0.11	0.11	0.03	0.05	0.11
10	*F.subulatum*	0.13	0.25	0.32	0.21	0.23
11	*Fusarium sp.3*	0.21	0.41	0.24	0.21	0.05
12	*Fusarium sp.4*	0.21	0.32	0.13	0.06	0.04
13	*F.verticillioides*	0.04	0.22	0.20	0.21	0.12
14	*Fusarium proliferatum*	0.11	0.22	0.21	0.09	0.04
15	*Fusarium equiseti*	0.10	0.11	0.03	0.09	0.03
16	*F.roseum*	0.21	0.41	0.23	0.21	0.06
17	*F.napiforme*	0.11	0.51	0.24	0.22	0.03
18	*F.sambucinum*	0.11	0.26	0.13	0.21	0.11
19	*Fusarium sp.5*	0.22	0.34	0.21	0.32	0.21
20	*Fusarium sp.6*	0.11	0.22	0.07	0.21	0.12
21	*F.fusarioides*	0.03	0.45	0.33	0.38	0.30

Table. 9 Effect of salinity on *Fusarium oxysporum* (The values are represented in OD at 600 nm)

S.No	Name of the fungi	5%	10%	20%	30%	40%
1	*Fusarium oxysporum*	0.947	0.674	0.475	0.457	2.781
2	*F.moniliforme*	1.405	0.695	0.347	0.474	0.411
3	*F.graminearum*	1.154	0.620	0.381	0.423	0.376
4	*F.culmorum*	0.593	0.508	0.354	0.427	0.402
5	*F.solani*	0.497	0.429	0.404	0.493	0.421
6	*F.semitectum*	0.675	0.661	0.420	0.290	0.275
7	*Fusarium sp.1*	1.788	1.058	0.516	0.345	0.180
8	*Fusarium sp.2*	0.987	0.909	0.892	0.449	0.404
9	*Fusarium citri*	0.765	0.693	0.475	0.390	0.220
10	*F.subulatum*	1.292	1.082	0.835	0.806	0.117
11	*Fusarium sp.3*	1.986	1.205	1.106	0.255	0.220
12	*Fusarium sp.4*	0.974	0.835	0.336	0.244	0.159
13	*F.verticillioides*	1.092	0.858	0.560	0.320	0.250
14	*Fusarium proliferatum*	0.983	0.975	0.720	0.635	0.520
15	*Fusarium equiseti*	0.826	0.353	0.556	0.187	0.167
16	*F.roseum*	1.007	1.001	0.523	0.288	0.132
17	*F.napiforme*	2.773	1.761	0.440	0.255	0.210
18	*F.sambucinum*	1.136	1.072	0.713	0.457	0.320
19	*Fusarium sp.5*	1.662	1.415	1.568	0.533	0.172
20	*Fusarium sp.6*	0.795	0.648	0.520	0.289	1.551
21	*F.fusarioides*	1.375	1.650	1.116	0.280	0.109

Table. 10 Effect of salinity on fresh weight of *Fusarium oxysporum* (The values are represented in mg/ g)

S.No	Name of the fungi	5%	10%	20%	30%	40%
1	*Fusarium oxysporum*	1.96	1.90	1.05	0.96	2.98
2	*F.moniliforme*	1.72	1.03	1.07	1.25	1.11
3	*F.graminearum*	1.61	0.87	1.03	1.57	1.29
4	*F.culmorum*	1.76	0.88	0.77	1.17	1.15
5	*F.solani*	1.37	0.75	0.70	1.27	1.04
6	*F.semitectum*	2.21	2.08	1.82	1.68	0.93
7	*Fusarium sp.1*	2.03	1.99	1.96	1.90	1.80
8	*Fusarium sp.2*	1.40	1.84	1.79	1.68	1.04
9	*Fusarium citri*	2.06	1.70	1.59	1.57	0.66
10	*F.subulatum*	2.18	2.10	1.92	1.92	1.01
11	*Fusarium sp.3*	1.90	1.86	1.78	1.57	1.10
12	*Fusarium sp.4*	1.85	1.49	1.41	1.09	1.08
13	*F.verticillioides*	1.96	1.80	1.14	0.61	0.80
14	*Fusarium proliferatum*	2.38	2.08	2.08	1.77	1.01
15	*Fusarium equiseti*	2.96	2.86	2.16	2.08	1.96
16	*F.roseum*	1.86	1.67	1.67	1.48	1.06
17	*F.napiforme*	1.81	1.40	1.09	1.07	0.88
18	*F.sambucinum*	2.01	1.98	1.69	1.90	2.04
19	*Fusarium sp.5*	2.31	1.58	1.58	2.10	2.23
20	*Fusarium sp.6*	2.71	1.21	1.33	1.66	2.09
21	*F.fusarioides*	1.71	1.62	1.59	1.40	1.14

Table. 11 Effect of salinity on dry weight of *Fusarium oxysporum* (The values are represented in mg/ g)

S.No	Name of the fungi	5%	10%	20%	30%	40%
1	*Fusarium oxysporum*	0.45	0.38	0.34	0.21	1.08
2	*F.moniliforme*	0.47	0.44	0.28	0.25	0.13
3	*F.graminearum*	0.53	0.25	0.19	0.16	0.09
4	*F.culmorum*	0.43	0.40	0.29	0.27	0.12
5	*F.solani*	0.45	0.40	0.29	0.18	0.12
6	*F.semitectum*	0.88	0.79	0.22	0.21	0.20
7	*Fusarium sp.1*	0.64	0.33	0.32	0.31	0.27
8	*Fusarium sp.2*	0.46	0.44	0.39	0.38	0.34
9	*Fusarium citri*	0.79	0.47	0.43	0.24	0.12
10	*F.subulatum*	0.65	0.64	0.52	0.52	0.47
11	*Fusarium sp.3*	0.43	0.32	0.28	0.25	0.18
12	*Fusarium sp.4*	0.44	0.28	0.27	0.22	0.22
13	*F.verticillioides*	0.68	0.60	0.15	0.09	0.05
14	*Fusarium proliferatum*	0.79	0.77	0.73	0.21	0.15
15	*Fusarium equiseti*	0.47	0.43	0.39	0.39	0.36
16	*F.roseum*	0.36	0.32	0.29	0.18	0.11
17	*F.napiforme*	0.50	0.24	0.21	0.13	0.12
18	*F.sambucinum*	0.37	0.29	0.27	0.26	0.22
19	*Fusarium sp.5*	0.45	0.34	0.34	0.31	0.28
20	*Fusarium sp.6*	0.62	0.21	0.17	0.10	0.09
21	*F.fusarioides*	0.50	0.36	0.34	0.29	0.18

Table 12. Effect of carbon and nitrogen sources on *Fusarium oxysporum* (The values are represented in OD at 600 nm)

S.No	Name of the fungi	CMC (1%)	Starch (1%)	Mannitol (1%)	Amm. Nitrate (1%)	Cal. Nitrate (1%)
1	*Fusarium oxysporum*	2.931	1.405	1.012	2.180	0.641
2	*F.moniliforme*	1.908	1.843	0.802	2.030	0.808
3	*F.graminearum*	1.929	1.240	1.840	1.906	1.417
4	*F.culmorum*	2.359	0.362	1.968	0.287	0.270
5	*F.solani*	1.687	0.340	0.990	0.202	0.146
6	*F.semitectum*	2.118	2.109	1.928	1.644	1.173
7	*Fusarium sp.1*	2.551	2.106	1.854	1.819	0.978
8	*Fusarium sp.2*	2.871	1.882	2.159	1.402	0.835
9	*Fusarium citri*	2.318	2.145	2.130	1.534	0.793
10	*F.subulatum*	1.809	0.985	1.712	1.490	1.426
11	*Fusarium sp.3*	2.132	1.478	1.743	1.670	1.301
12	*Fusarium sp.4*	2.229	2.181	2.046	1.417	0.988
13	*F.verticillioides*	1.821	1.255	1.044	1.318	1.013
14	*Fusarium proliferatum*	2.259	2.093	2.090	1.933	1.552
15	*Fusarium equiseti*	1.999	1.255	1.982	1.414	1.032
16	*F.roseum*	2.256	2.011	2.190	1.933	1.552
17	*F.napiforme*	2.239	1.460	1.840	1.653	1.332
18	*F.sambucinum*	2.340	2.204	2.039	2.014	0.682
19	*Fusarium sp.5*	2.122	1.819	1.734	1.591	1.143
20	*Fusarium sp.6*	2.815	2.540	2.080	1.941	1.137
21	*F.fusarioides*	2.344	1.340	1.728	1.544	1.231

Table. 13 Effect of carbon and nitrogen sources on fresh weight *Fusarium oxysporum*
(The values are represented in mg/ g)

S.No	Name of the fungi	CMC (1%)	Starch (1%)	Mannitol (1%)	Amm. Nitrate (1%)	Cal. Nitrate (1%)
1	*Fusarium oxysporum*	2.010	0.73	0.89	1.86	1.07
2	*F.moniliforme*	0.98	0.89	0.66	0.81	0.53
3	*F.graminearum*	0.69	0.61	0.49	1.82	1.21
4	*F.culmorum*	0.75	0.48	0.70	0.91	0.57
5	*F.solani*	0.87	0.87	0.76	0.98	0.90
6	*F.semitectum*	1.99	1.28	1.62	1.35	1.54
7	*Fusarium sp.1*	1.84	1.42	1.06	1.91	1.02
8	*Fusarium sp.2*	1.21	1.15	1.23	1.45	1.36
9	*Fusarium citri*	1.53	1.07	1.40	1.15	0.95
10	*F.subulatum*	0.89	0.61	0.71	1.24	1.18
11	*Fusarium sp.3*	1.95	1.28	1.83	1.68	1.50
12	*Fusarium sp.4*	1.21	1.04	1.21	1.73	1.23
13	*F.verticillioides*	1.07	1.00	1.04	1.57	1.43
14	*Fusarium proliferatum*	1.70	1.28	1.05	1.57	1.49
15	*Fusarium equiseti*	0.98	0.75	0.83	1.22	1.02
16	*F.roseum*	1.15	0.95	1.10	1.05	0.95
17	*F.napiforme*	1.72	1.51	1.25	1.38	1.31
18	*F.sambucinum*	1.45	1.22	1.20	1.03	0.97
19	*Fusarium sp.5*	1.03	0.95	0.95	1.11	1.05
20	*Fusarium sp.6*	0.95	0.75	0.81	0.90	0.82
21	*F.fusarioides*	1.76	1.10	0.87	1.20	1.12

Table 14. Effect of carbon and nitrogen sources on dry weight of *Fusarium oxysporum*
(The values are represented in mg/ g)

S.No	Name of the fungi	CMC (1%)	Starch (1%)	Mannitol (1%)	Amm. Nitrate (1%)	Cal. Nitrate (1%)
1	*Fusarium oxysporum*	0.32	0.10	0.09	0.38	0.10
2	*F.moniliforme*	0.19	0.11	0.13	0.13	0.10
3	*F.graminearum*	0.19	0.11	0.11	0.18	0.15
4	*F.culmorum*	0.29	0.07	0.10	0.04	0.06
5	*F.solani*	0.27	0.15	0.10	0.13	0.12
6	*F.semitectum*	0.15	0.12	0.13	0.26	0.16
7	*Fusarium sp.1*	0.18	0.11	0.09	0.13	0.12
8	*Fusarium sp.2*	0.12	0.12	0.07	0.14	0.13
9	*Fusarium citri*	0.17	0.09	0.09	0.19	0.11
10	*F.subulatum*	0.13	0.12	0.12	0.26	0.15
11	*Fusarium sp.3*	0.15	0.12	0.14	0.36	0.26
12	*Fusarium sp.4*	0.09	0.09	0.09	0.21	0.12
13	*F.verticillioides*	0.16	0.11	0.11	0.18	0.17
14	*Fusarium proliferatum*	0.12	0.08	0.08	0.30	0.20
15	*Fusarium equiseti*	0.10	0.09	0.08	0.18	0.16
16	*F.roseum*	0.13	0.07	0.07	0.19	0.12
17	*F.napiforme*	0.19	0.11	0.08	0.23	0.16
18	*F.sambucinum*	0.15	0.12	0.09	0.19	0.13
19	*Fusarium sp.5*	0.12	0.10	0.09	0.15	0.13
20	*Fusarium sp.6*	0.09	0.09	0.08	0.11	0.10
21	*F.fusarioides*	0.19	0.03	0.18	0.23	0.16

Table. 15 Effect of metals on *Fusarium oxysporum* (The values are represented in OD at 610 nm)

S.No	Name of the fungi	FeSo4 (1 %)	Zn So4 (`1%)
1	*Fusarium oxysporum*	2.071	0.792
2	*F.moniliforme*	2.069	0.964
3	*F.graminearum*	1.701	1.284
4	*F.culmorum*	1.087	0.375
5	*F.solani*	1.349	0.864
6	*F.semitectum*	1.616	0.982
7	*Fusarium sp.1*	1.466	0.879
8	*Fusarium sp.2*	1.425	1.000
9	*Fusarium citri*	1.136	0.874
10	*F.subulatum*	1.573	1.358
11	*Fusarium sp.3*	1.433	0.574
12	*Fusarium sp.4*	1.046	1.359
13	*F.verticillioides*	1.443	0.654
14	*Fusarium proliferatum*	1.687	1.033
15	*Fusarium equiseti*	1.321	0.709
16	*F.roseum*	1.423	0.696
17	*F.napiforme*	1.358	0.752
18	*F.sambucinum*	1.607	0.677
19	*Fusarium sp.5*	1.536	0.191
20	*Fusarium sp.6*	1.540	0.303
21	*F.fusarioides*	1.464	0.769

Table. 16 Effect of metals on dry and fresh weight of *Fusarium oxysporum* (The values are represented in mg/ g)

S.No	Name of the fungi	FeSo4 (1%)		ZnSo4(1%)	
		FW	DW	FW	DW
1	*Fusarium oxysporum*	1.75	1.12	0.14	0.13
2	*F.moniliforme*	1.39	1.12	0.15	0.14
3	*F.graminearum*	0.96	0.78	0.15	0.12
4	*F.culmorum*	1.29	0.87	0.16	0.15
5	*F.solani*	1.71	0.96	0.16	0.15
6	*F.semitectum*	1.36	0.21	1.01	0.33
7	*Fusarium sp.1*	1.41	0.12	0.92	0.41
8	*Fusarium sp.2*	1.10	0.15	1.15	0.13
9	*Fusarium citri*	1.19	0.21	1.46	0.22
10	*F.subulatum*	1.15	0.12	0.59	0.21
11	*Fusarium sp.3*	1.13	0.11	1.42	0.10
12	*Fusarium sp.4*	1.25	0.11	1.29	0.11
13	*F.verticillioides*	0.81	0.31	0.59	0.31
14	*Fusarium proliferatum*	1.07	0.21	1.21	0.31
15	*Fusarium equiseti*	1.06	0.22	0.79	0.32
16	*F.roseum*	1.48	0.11	1.12	0.31
17	*F.napiforme*	1.52	0.22	1.11	0.21
18	*F.sambucinum*	0.67	0.31	0.81	0.31
19	*Fusarium sp.5*	1.28	0.23	0.91	0.21
20	*Fusarium sp.6*	0.83	0.31	1.10	0.31
21	*F.fusarioides*	1.13	0.12	0.93	0.33

and Jennings (1964) can be extended 'the reduced vegetative growth, reproduction and spore germination in terrestrial fungi under saline conditions may be the factors in maintaining the fungus flora of the sea distinct from that of non-marine habitats. Studies on the salinity tolerance of marine fungi have preoccupied many mycologists as can be seen from the following papers (Borut and Johnson, 1962; Jones, 1963; Jones *et al*., 1971).

Effect of carbon and nitrogen sources on the growth of fungi

In this study, the maximum growth was observed in carboxy methyl cellulsoe after 8 days of incubation (Table.12). In this study, *F.oxysporum* showed maximum growth with 2.931(OD). Fresh weight of the fungi were also maximum in *F.oxysporum* (2.010 mg/g) and Dry weights of the fungi was maximum in *F.oxysporum* (0.32 mg/g). In ammonium nitrate after 8 days of incubation, *F.oxysporum* showed maximum growth with 2.180 (OD)**.**Fresh weight of the fungi was also maximum in *F.oxysporum* (1.86 mg/g) and dry weight was observed with 0.38 mg/g (Table.13&14).Swart (1958) studied that the mycoflora in the soil of mangrove swamp of Inhaea Island has suggested that these swamp are rich in simple carbohydrate and nitrogen and the dominance of the species of *Aspergillus*, *Penicillium* and *Fusarium* indicates their preference for simple organic compounds.

Effect of metals on the growth of fungi

In ferric sulphate after 8 days of incubation. In this study, *F.oxysporum* showed maximum growth with 2 (Table.15). Fresh weight of the fungi were also maximum in *F.oxysporum* (1.75 mg/g) and Dry weight of the fungi was also maximum in *F.oxysporum* (1.12 mg/g) (Table.16). Various researchers (Gourdon *et al*., 1990) have studied the mechanism of heavy metal biosorption and reported involvement of different mechanism such as intracellular uptake and storage via active cationic transport system, surface binding and other undefined mechanisms. Since most metal microbes interactions are initiated at the level of uptake, the uptake mechanism is likely to be closely linked to the mechanism of metal resistance in the microorganisms (Yilmaz, 2003).

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