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Diversity of halophilic mycoflora habitat in saltpans of Tuticorin and Marakkanam along southeast coast of India

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Abstract

Highly diverse biological system of solar salterns with different salinities, often provide high densities of mycofloral populations, makes the salterns excellent model systems for both its diverse and activity. In this study, diversity of halophilic fungi in six stations which includes reservoir, evaporator and crystallizer pond of both Marakkanam and Tuticorin saltpans in relation to environmental parameters were carried out for a period of two years. 95 species of halophilic fungi from water and sediment samples belongs to 41 genera were recorded in both saltpans. *Aspergillus* and *Penicillium* species were recorded as dominant, vast differences in growth of each isolate at different salt concentrations in the ponds were observed. This paper also elucidated the slight fluctuations in physico-chemical parameter among the ponds with respect to seasonal variations were also recorded.

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Introduction

Saltpans are man-made seasonal ponds constructed mainly for the production of raw salt. These ponds offer an experimental system with an extreme environmental conditions include high and low temperature, pH, pressure, salt concentration, low nutrient concentration, water availability and also conditions having high levels of radiation, harmful heavy metals, toxic compounds (organic solvents) and strong gradient in biodiversity of primary and secondary producers. (Satyanarayana *et al*., 2005). It is one such example for thalassohaline environment, it contains the salinity range of five to ten times saltier than seawater (150-300 g/l salt concentration). Life at high salt concentrations requires special adaptations of the cell's physiology. Microbes must sense environmental stresses, transduce these signals and mount protective responses to survive in hostile environments (Nikolaou *et al*., 2009).

Most microbial diversity studies in salterns have focused on halophilic Archaea bacteria of the order Halobacteriales, which comprise the main microbial component in these environments (Oren, 2002). Other organisms such as algae, protozoa, eubacteria and even fungi are also found in the salterns, even though it was thought that they could not survive under extreme salt conditions (Gunde-Cimerman *et al*., 2004). Fungi are ubiquitous in most ecosystems where they usually colonize a diverse range of substrates. Fungal cell adaptations to high saline environment are the promising biological process and the level of plasma-membrane fluid fluctuation are indicators of fitness for survival and adaptability in fungi obtained from extreme environments (Turk *et al*., 2007). Unique *in-situ* morphology was interpreted as a response to multiple stress factors which can adapt to extreme conditions. The accumulation of osmoprotective compounds such as polyols (glycerol) sugars (trehalose and manitol) and some unusual amino acids may also play an important role under salt stress (Griffith, 1994).

Enumeration of fungi in these habitats revealed their presence in relatively large numbers (up to 4×10^4 ml^{-1}), but the biodiversity appears to be limited to a small number of fungal genera. At present, 106 orders of fungi were known to tolerate at low water activity (Kirk *et al*., 2001). Within Ascomycota, the main orders with halophilic and halotolerant representatives are Capnodiales, Sporidiales, Dothideales and Eurotiales. Both orders Capnodiales and Dothideales have a xerotolerant tendency, as they contain a large number of extremotolerant species that can grow as epilithic or cryptoendolithic species at high or low temperatures (Selbmann *et al*., 2005) and hypersaline coastal areas worldwide.

This new ecological findings are not only important for our understanding of microbial processes in hypersaline environments worldwide, but also for not yet fully acknowledged. Though, the sequence of works regarding halophilic fungi from solar saltern environments has been carried out for the past two decades in many continents but the meager works were contributed by Indian subcontinent. Owing to the lack of studies on mycofloral in salterns along the Indian coast, the present study was carried out to understand the ecology and diversity, seasonal variations, frequency of occurrence and distribution of fungi in relation to physico-chemical parameters in Tuticorin and Marakkanam saltpans along southeast coast of India.

Materials and methods

Monthly samples were made to record the physico-chemical parameters and to check the fungal diversity. Rainfall data was obtained from the local meteorological unit under government of India located at Villupuram and Tuticorin. Field data like temperature, salinity, dissolved oxygen and pH were measured. Atmospheric and surface water temperatures were measured using standard mercury filled centigrade thermometer. Salinity was estimated by hand refractometer (Atago, Japan) and pH was measured using Elico pH meter (Model LC- 120).

Dissolved oxygen was estimated by the modified Winkler's method (Strickland and Parsons, 1972).

For the analysis of nutrients, surface water samples were collected in clean polyethylene bottles and kept in an ice box and transported to the laboratory. The water samples were filtered using a Millipore filtering system and analyzed for dissolved inorganic phosphate, nitrate and reactive silicate. Sediment samples were dried in an oven at 105°C for 6 hours and ground using mortar and pestle for breaking and were sieved before analysis. Sediment nutrients like Phosphate (TP), Nitrogen (TN) and Total Organic Carbon (TOC) by adopting the standard methods described by Strickland and Parsons (1972).

A calendar year was divided into four seasons viz., monsoon (October to December), post monsoon (January to March), summer (April to June) and pre-monsoon (July to September) based on the north east monsoon which is prevalent in the study area.

The fungal strains from water and sediment samples of all the six stations were isolated using standard pour plate technique. In mycological analysis, water and sediment samples were plated on to Potato Dextrose Agar + 20% solar salt for isolation of halophilic fungi, which were then incubated at room temperature of about 30°C, up to 15 days.

The isolates were picked based on dissimilarity in the colony characteristics, purified and numbered according to the stations and the samples. Fungal identification was done on the basis of colony and microscopic morphology characteristics with reference to identification keys (Ellis, 1971). Based on the results, statistical analyses were assist to make the cleared data output.

Statistical Analysis

Further, various statistical methods such as Univariate, graphical/distributional and multivariate were applied for the data analysis. The computer programme. Origin 8 and PRIMER (Ver-6) were sed for univariate and multivariate analyses.

Periodicity of occurrence

It denotes the number of samplings in which a particular fungus was recorded against the total number of samplings. Based on the percentage periodicity of occurrence, fungi were classified into four groups as Most common (76-100%), Common (51-75%), Occasional (26-50%) and Sporadic (1-25%).

$$H' = \frac{3.3219\,(N\log N - \sum ni - \log ni)}{N}$$

In the present study, the data were analyzed for diversity index (H') using the following Shannon-Wiener's formula (1949)

Species richness

Species richness (d) was calculated using formula given by Margalef

$$d = (S-1)/\log N$$

Pielou's evenness index

The equitability (J') was computed using the following formula of Pielou (1996):

$$J' = \frac{H'}{\text{Log}_2 S} \text{ or } \frac{H'}{\text{In}S}$$

Graphical/ distributional methods

K-dominace plot

Dominance plot is also called as the ranked species abundance plot developed by Lambshead *et al*. (1983).

Ellipse plot

Average taxonomic distinctness index (delta+) and variation in taxonomic distinctness (lambda+) were studied graphically. Being based on the presence or absence of species according to seasonal variations, they can be calculated using simple species list.

Average taxonomic distinctness (Δ+) and variation in taxonomic distinctness (λ+) were calculated for all staions during all the seasons from the study area.

Study area

Fig. 1. Map showing the sampling areas in Marakkanam and Tuticorin, India.

Results and discussion

The physico-chemical parameters and nutrients of both water and sediment samples in Tuticorin and Marakkanam saltpans showed the variations in all the six stations. In the present study, brine temperatures at different stations vary, maximum temperature was observed during summer due to the intensity of solar radiation heat the brine water of shallow ponds of salterns resulting in rapid evaporation of water causing speedy crystallization process. In case of sediment, minimum temperature was recorded compared with water. Slight increase in brine temperature was observed from reservoir to crystallizer ponds, which was highly collaborated with the study of Petrovic (1998). This might be due to the red pigmentation produced by phototrophic microorganisms and the photosynthetic primary producer in crystallizer ponds that cause β-carotene accumulation by the green alga *Dunaliella salina* which was the main or sole primary producer in these ponds (Litchfield, 1991). This red pigmentation increases light absorption by the brine and increases its temperature, thus enhancing the salt production process (Javor, 2002).

The pH of brine at different stages of the salt pan was alkaline in nature (Baati *et al*., 2008). In the present study, the pH encountered high during summer and minimum in monsoon season showed the moderate ranges between 7-7.8 (alkaline) in both Tuticorin and Marakkanam salterns. This study was highly supported by Manikandan *et al*. (2009) and Radhika *et al*. (2011), who observed the range from 7.35- 7.85 in Marakkanam salterns and 7.1-8.3 in Tuticorin salterns respectively. Butinar *et al*. (2005a) highlighted that pH in all the ponds were approximately low (7.2) from the beginning of December to the mid of February. Due to the uptake of CO_2 by the photosynthesizing organisms, especially phytoplankton and planktonic cyanobacteria from the seawater could have increased the pH level during the summer season (Subramanian and Mahadevan, 1999). The low pH observed during the monsoon due to the influence of freshwater influx and dilution of brines, reduction of salinity and temperature and decomposition of organic matter (Subramanian and Kannan, 1998). Gradual decrease of pH (7.1-7.65) from station-1 to station-3 was noted.

Femitha and Vaithyanathan (2012) observed the pH value increases from source (seawater) to reservoir because of the increasing concentration of iron oxide and calcium carbonate but there was a marginal decrease in the values at evaporator and crystallizer stages because of the removal of above mentioned salts before the brine pass on to the evaporator ponds. The soil samples were slightly acidic to neutral and the pH ranged from 6.9-8.3 reported by Biswas and Paul (2012). Nazareth *et al*. (2012) stated that the pH of sediments in the range of 5.3.

In this study, salinity fluctuated widely between stations. It went up high due to excessive evaporation in the summer period and dropped sharply during the monsoon season, the brine salinity ranged between 395-400‰ and 400-410‰ in case of saline soils during summer seasons of both salterns of Tuticorin and Marakkanam. Salinity in these ponds reaches as high as 400‰ during the peak salt producing season (summer) and as low as 5‰ during the monsoons (Kamat and Kerkar, 2011). Comparatively, the salinity showed vast variation among brine and saline soils this could be due to precipitation of halites from the water column due to evaporation could be responsible for the higher salinity of the sediment as compared to brine (Oren, 2003). Pedros-Alio *et al*. (2000) observed that as temperature increases, salinity also increase due to the higher calorific capacity of the brines. Gradual increase of salinity was noted from reservoir to crystallizer ponds, which might be due to the initial sea water pumped from the ocean directly, from there the water almost continuously flows into other ponds and so the salinity ranged not much difference from the adjacent inshore waters of the sea and the initial reservoir pond, the salinity ranged 35- 37‰. In second category, the water was allowed to pass on to the evaporator pond where the water was stored for some days for evaporation enhanced by strong local winds and temperatures and the salinity increases reached up to 100-200‰. Finally, to the crystallizer pond where the salinity maintained up to 300-600‰, in this water masses the salinity is considerably high and never gone below 250‰. This might be due to the water evaporates, gypsum and other minerals precipitate, eventually, sodium chloride (NaCl) precipitates and salinity increases above 300 psu (Oren, 2002).

Lower silicate was recorded during summer season, while it was higher in monsoon period. The study was supported by Kovac (2009) reported that lower concentration of silicate during summer, due to the considerable reduction in the freshwater input and greater utilization of silicate by the abundantly occurring phytoplankton and cyanobacteria for their biological activity (Nedumaran and Perumal, 2012). Nitrate concentrations were high (5.51µmol l^{-1}) in crystallizer ponds and evaporator ponds ranked second during monsoon season. Madkour and Gaballah (2012) recorded the data which supported the study and reasoned, organic and inorganic nutrients caused hyper-nitrification, possibly phytoplankton blooms, oxygen depletion and formation of anoxie situation which was observed in pond (Ehrlich, 1987). Anbazhagan (1988) have suggested that the addition of nitrogenous nutrients mainly through freshwaters and terrestrial runoff in the lagoon definitely increase the levels of nitrate. Lower concentration of nitrate was recorded which may be due to the utilization of benthic algae and phytoplankton. Inorganic phosphate concentration showed slight variation from reservoir to crystallizer ponds which may be due to decomposition of bacteria and further brine concentration (Dundas and Halvorson, 1966).

O_2 are often short supply in hypersaline environments, because of its limited solubility at high salt concentration (Nealson and Stahl, 1997) and other point of interest were under extreme conditions O_2 or organic nutrients become limiting, thus making this nutritional extremophily as a commonly encountered adaptation in harsh environments. In this present survey, oxygen values in the first pond reached as high and

steadily goes down, probably due to the high peak in diatoms and filamentous cyanobacterial populations that increased in biomass and occasional high values of nutrients, during the seasons at salinities of 2-10%. Lowest oxygen concentrations were detected in summer this situation probably occurs because respiration exceeds primary productivity in extremely strong brines (Sammy (1983)). Phosphate level gradually decreases from station 1 to 3 due to the changes in the levels of phosphorous in sediment can be linked with the influx of phosphorous from upstream region and also its regeneration into the overlying water column under suitable conditions and the production of H_2S by anaerobic microbes enhances the eutrophication of phosphorus.

In the present investigation, total nitrogen concentration was recorded high in monsoon season and also gradual increase from reservoir to crystallizer. This is due to freshwater inflow which brings in abundant N_2 rich terrigenous deposits and their subsequent settling in sediments and also statically significant correlation between sediment nitrogen and silt. Recent studies revealed that some proteinaceous material could be resistant to microbial degradation and that part of nitrogen may be preserved as such in sedimentary environments (Nguyen and Harvey, 2001). High organic carbon was recorded at station-2 during monsoon and low in summer this may be due to the terrestrial run-off that results in high levels of organic matter and inorganic nutrients (Rasheed *et al*., 2001). Similar observation were made during summer due to the decrease of organic carbon, total nitrogen and silicate content in Secovlje salt-pans located in Gulf of Trieste, Northern Adriatic (Kovac, 2009). The general decrease in TN and TOC contents might reflect an overall decrease in primary productivity as salinity increased (Liu *et al.*, 2004). Lowering range of phosphate content were moderate in terms of total organic carbon was observed in the study of Biswas and Paul (2012). Temperature, pH, salinity, silicate of both water and sediment showed positively correlated with fungal diversity and reactive silicate, inorganic phosphate, dissolved O_2 level, phosphate, nitrogen, total organic carbon showed a negative correlation. The data proposed the overall maximum and minimum averages among the stations was shown in Table 1.

Both Pielou's and Shannon indices were highest at Marakkanamin station-III (5.151 and 9.319) (Table 2). Species richness and diversity of fungi in all the sampling stations during the study period was in conformity with the diversity studies of Maria and Sridhar (2002). The K-dominance curves showed the cumulative dominance of species in rank order for each seasons. The fungal diversity showed variation in season as well as stations wise. The maximum diversity and density were observed in station 3 during summer seasons (crystallizer pond) of both Tuticorin and Marakkanam saltpans. The curve for the summer season of 2011 was lying at the bottom indicating highest diversity (Fig. 2).

In Tuticorin saltpans, based on the combination of delta+ and lambda+ values the number of species in the entire stations showed 95% confident limit except reservoir during summer in the year of 2012, premonsoon and monsoon showed the exception in the year of 2012 in crystallizer ponds. Low fungal counts were recorded as 20 species whereas, in crystallizer pond during postmonsoon and summer had richest diversity in both years which contains 90 fungal species. Simultaneously, In Marakkanam saltpans, number of species in every stations showed 95% confident limit except in crystallizer pond during pre-monsoon in the year of 2011 and 2012 which contains 10 of fungal species. In case of reservoir and evaporator ponds, summer season showed the exception in the year of 2012 which contains 40 of fungal species and above. In crystallizer pond, summer season had richest diversity in both years which contains 60 of fungal species (Fig. 3).

Sediments harbour more fungal counts than in water samples. Density of halophilic fungi ranged from 3.0×10^3 to 5.4×10^4 CFU/ml in water sample and 3.6×10^3 to 5.7×10^4 CFU/g in sediment sample of both Tuticorin and Marakkanam salterns. In both the saltpans, the higher fungal density was recorded in station-III. Overall, maximum fungal counts were observed in summer season (2011) while minimum in monsoon season (2012). In Tuticorin saltpan, 80 species belongs to 32 genera were recorded, In Marakkanam saltpan, 83 species belongs to 36 genera were recorded.

Altogether, 95 species of halophilic fungi from water and sediment samples belongs to 41 genera was recorded in both saltpans. Of these, 26 species in 21 genera falls under Ascomycota and 4 species under 3 genera belongs to Basidiomycota, 2 species in 2 genera fit in to Zygomycota and the remaining 63 species in 15 genera belongs to mitosporic fungi which included 56 species in 13 genera of Hypomycetes and 2 species in 2 genera of Coelomycetes respectively. 22 species of fungi were common to both the stations of Tuticorin and Marakkanam saltpans. 16 species of fungi present only in Marakkanam and 12 species of fungi was recorded in Tuticorin saltpan (Table: 3 & Fig. 3).

The predominance of *Aspergillus* species such as *A. niger, A. terreus, A. fumigatus, A. candidus, A. wentii, A. flavus, A. sydowii, A. versicolor, A. ochraceus, A.Tamarii* and *Penicillium chrysogenum* were the common isolates identified during this survey. *A. niger* was ranked first among the halophilic fungal.

Table 1. Physico-chemical parameters of water and sediment samples.

Parameters	Tuticorin Saltpan	Marakkanam Saltpan
Water		
Rainfall	2.13 to 124.1 mm	0.66 to 128.7 mm
Atmospheric temperature	22.8 to 37.5ºC	22.5-37ºC
Temperature	22 to 34ºC	20 to 35.8ºC
pH	7.23 to 7.7	7.2 to 7.6
Salinity	21 to 395‰	19 to 400‰
Dissolved Oxygen	1.43 to 4.35 mg/l	1.28 to 4.92 mg/l
Inorganic phosphate	2.62- 4.33 µM/l	2.62- 5.55 µM/l
Nitrate	1.2- 5.1 µM/l	1.32- 5.19 µM/l
Reactive silicate	34.8 to 59.82 µM/l	31.9 to 60.64 µM/l
Sediment		
Temperature	21 to 34ºC	20 to 35.6ºC
pH	7.2 to 7.65	7.1 to 7.59
Salinity	24 to 400‰	20 to 410‰
Total Nitrogen (TN)	4.43 to 8.52 µg/g	4.36 to 8.58 µg/g
Total Phosphate (TP)	0.95 to 1.782 µg/g	0.951 to 2 µg/g
Total Organic Carbon (TOC)	2.37 to 7.48 mg/g	2.51 to 7.59 mg/g

Diversity, this may be due to the ability of this fungus to grow in the absence of oxygen and the production of pigmented spores that are more resistant than hyaline spores to extreme conditions (Domsch *et al*., 1993). *Penicillium* was also in fairly high proportion that can able to dwell in low water potential and low to high temperature. The active mycota were dominated by these two genera because of their osmoregulative mechanism and hence species of *Aspergillus* and *Penicillium* could be isolated even from the deep sea environment (Jones, 1988).

Other species of *Aspergillus* in teleomorphs found in haline environments include *Eurotium* genus. Kis-Papo *et al*. (2001) reported that the most frequently isolated species of halotolerant were *E. amstelodami, E. chevalieri, E. echinulatum, E. halophilicum, E. herbariorum*, *E. intermedium*, *E.repens, E.rubrum, E. Spiculosum* and *E. umbrosum* both from arid, saline soil and salt marshes, in Israel, Syria and Kuwait. Butinar *et al*. (2005) reported that *E. Rubrum* were recorded in the salterns only occasionally at lower salinities (5–15% NaCl) at Dead Sea, Adriatic and

Eilat salterns. In this study, similar results were observed that *Eurotium amstelodami* occurred commonly from moderate to high concentration of NaCl and in case of *Eurotium rubrum* showed their presence occasionally. Kis-Papo *et al*. (2003) reported that *in vitro* studies showed that the spores and mycelium of these species are able to survive long-term exposure in solutions within a broad range of salt concentrations (0–30%). Similarly, 75% of all *Penicillium* sp. could tolerate 20% NaCl and more than half of these survived 25% NaCl (Tresner and Hayes, 1971). In this study, 13 species were identified and showed there occurrence at the salinity range of 19-400‰. Kogej *et al*. (2005) reported that minimal addition of NaCl slow down the growth rate of these species. Redkar *et al*. (1998) stated that *Emericella nidulans* can adapt to gradually higher concentrations of NaCl. Here, in this study, *Emericella nidulans* showed their occurrence occasionally at high concentration of salinity in both brine and saline soil. Thamizhmani *et al*. (2013) reported the presence of *Emericella* both in Marakkanam saltpan and marine ecosystem in east coast of Tamilnadu. At high salinities the black yeast *Hortaea werneckii,* showed their dominance in the hypersaline waters of the salterns. In this present work, this species were found to be common in both evaporation and crystallizer ponds at maximum salinity range. The fungal membranes are more fluid over a wide ranges of NaCl concentrations which might be the possible reason that indicating high intrinsic salt stress tolerance.

The highly halotolerant fungus *Gymnascella marismortui* can well adapted to extreme hypersaline environments. It was recorded in water of the Dead Sea (range of 600‰) and was never recorded on other localities (Buchalo *et al*., 1998). Interestingly, in crystallizer pond at Marakkanam saltpan, the isolates of ascomycete *Gymnascella marismortui*, were isolated at the salinity range of 331-400‰. It is the first record that this species were isolated in Marakkanam saltpan area in this study. Grishkan *et al*. (2003) reported that only species of *Gymnascella marismortui* was shown to be an obligate halophile, which grown in the presence of 0.5-2 M of NaCl or 10-30% in Dead Sea water. In this study, *W. sebi* and *W. Ichthyophaga* were isolated at high concentration of NaCl and they showed their presence very rarely. Zalar *et al*. (2005) reported that *W. ichthyophaga*, *W. muriae* and *W. sebi* can adapt in low concentration of NaCl, 5% for *W. Muriae* and *W. sebi* as in case of *W. ichthyophaga* it was found to be 15%. They can also withstand at high NaCl concentrations.

Table 2. Species richness, diversity and evenness of Halophilic fungi recovered from all stations of saltpans.

SEASONS	Shannon Wiener Diversity (H')						MargalfSpecies richness(D')						Pielou'sEvenness (J')					
	TUTICORIN			MARAKKANAM			TUTICORIN			MARAKKANAM			TUTICORIN			MARAKKANAM		
	STATIONS (RESERVOIR-I, EVAPORATOR-II, CRYSTALLIZER-III)																	
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Pos-11	4.326	4.352	4.757	4.513	4.679	5.014	5.45	6.209	8.403	5.21	7.904	9.319	0.8129	0.7924	0.8054	0.8538	0.7988	0.8236
Sum- 11	4.267	4.438	4.872	4.376	4.784	5.151	5.148	6.324	7.89	5.286	7.878	9.201	0.8191	0.808	0.8353	0.828	0.8201	0.8462
Pre-11	4.355	4.026	3.937	4.347	4.243	4.193	5.234	4.55	4.277	5.005	5.738	5.179	0.8239	0.7982	0.8023	0.8284	0.7869	0.8049
Mon-11	4.348	3.836	3.833	4.371	4.103	4.038	4.905	4.452	3.771	4.827	4.885	4.271	0.8347	0.7605	0.8061	0.839	0.7877	0.815
Pos-12	4.366	4.226	4.552	4.230	4.113	4.511	5.521	6.167	8.129	5.185	7.09	7.667	0.8204	0.7741	0.7803	0.8121	0.7288	0.7876
Sum-12	4.16	4.241	4.714	3.999	4.017	4.632	4.907	6.368	7.791	4.397	6.157	7.443	0.8111	0.7722	0.8154	0.8072	0.7402	0.8125
Pre-12	4.124	3.828	3.809	4.032	3.75	3.82	4.892	4.432	4.189	4.539	5.02	4.212	0.7977	0.7657	0.7841	0.7992	0.7254	0.7864
Mon-12	4.231	3.687	3.73	4.076	3.742	3.678	4.974	4.223	4.13	4.622	5.093	3.539	0.8122	0.7443	0.7678	0.8012	0.7183	0.792

Periodicities of occurrences of halophilic mycoflora diversity were tabulated based on its frequency in various stations of Tuticorin and Marakkanam saltpans (Table 4). Hooley *et al*. (2003) reported the halophilic and halotolerant yeast belong to genus *Wallemia* and *Debaryomyces, Pichia, Rhodotorula, Hortaea*, *Basipetospora, Polypaecilum*, *Scopulariopsis* and *Cladosporium* could tolerate much saline extremes. In the present investigation, true obligate halophilic fungal species and black yeasts like *Basidiobolus haptosporus, Bipolaris spicifera, Botryophialophora marina, Candida* sp., *Cryptococcus neoformans, Dabaryomyces*

hansenii, Dreschlera halodes, D. hawaiiensis, Eurotium rubrum, Exophiala xenobiotica, Exserohilum rostratum, Paecilomyces sp., *Phaeotheca fissurella, Scopulariopsis brevicaulis, Stachybotrys* sp., *Stenella araguata, Trichophyton verrucosom, Trimmatostroma* sp., *T. salinum, Wallemia sebi* and *Yarrowia lipolytica* were isolated that can tolerate upto

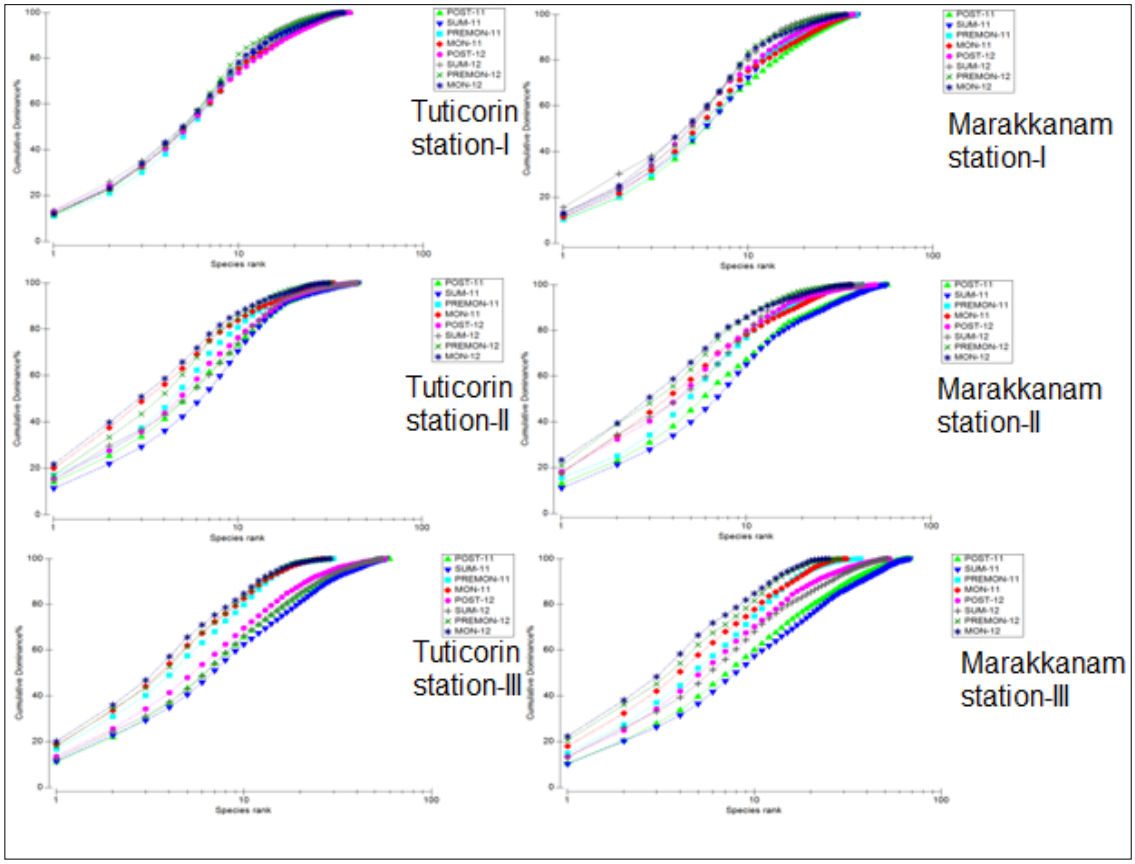


Fig. 2. K-Dominance curve representing the fungal diversity in season wise in both Tuticorin and Marakkanam salterns.

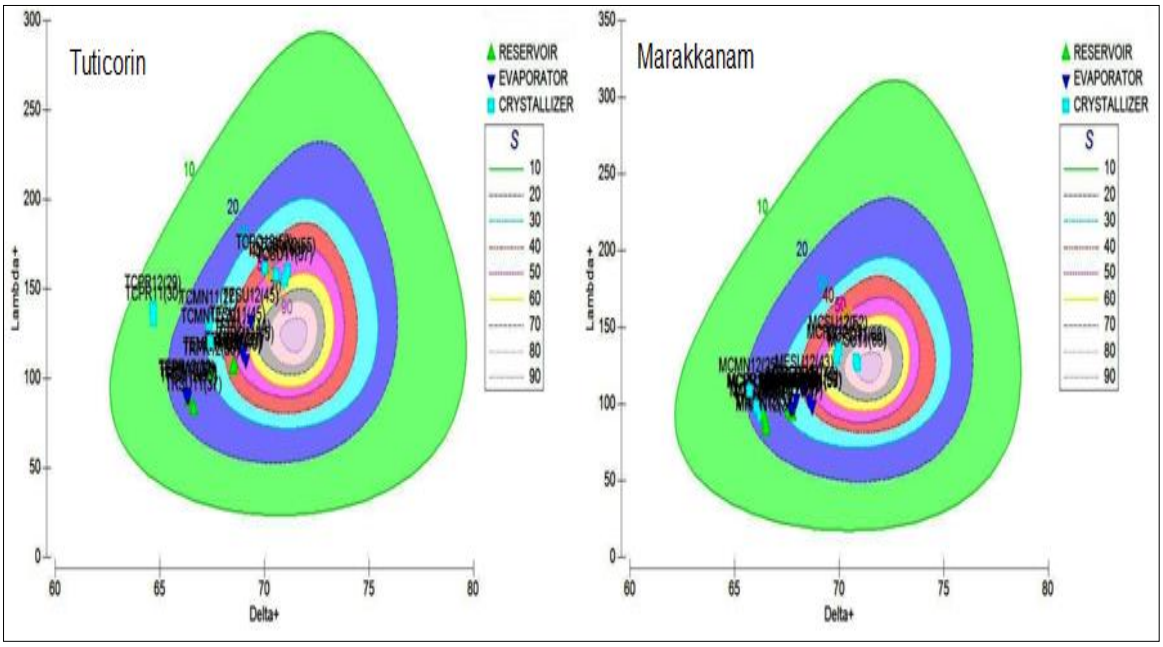


Fig. 3. Ellipse plot representing overall fungal diversity of both Tuticorin and Marakkanam salterns.

Table 3. List of fungi from saltpans of Marakkanam and Tuticorin.

S. No	Fungi		
	Halophilic Fungi common to both Stations	Halophilic Fungi present only	
		Marakkanam	Tuticorin
1.	*Aspergillus candidus*	*Alternaria alternata*	*Botryophialophora marina*
2.	*Aspergillus flavus*	*Aspergillus* sp.	*Dabaryomyces hansenii*
3.	*Aspergillus fumigates*	*Basidiobolus haptosporus*	*Exserohilum rostratum*
4.	*Aspergillus niger*	*Cryptococcus neoformans*	Non-sporulating fungi-3
5.	*Aspergillus sydowii*	*Exophiala jeanselmei*	*Paecilomyces* sp.
6.	*Aspergillus melleus*	*Exophiala xenobiotica*	*Phaeotheca triangularis*
7.	*Aspergillus nidulans*	*Gymnascella marismortui*	*Scopulariopsis brevicaulis*
8.	*Aspergillus ochraceus*	*Hyphospora agavaciensis*	Sterile Black mycelium 3
9.	*Aspergillus restrictus*	Non-sporulating fungi-4	*Syncephalastrum racemosum*
10.	*Aspergillus sulphurous*	*Penicillium polonicum*	*Trimmatostroma salinum*
11.	*Aspergillus tamarii*	*Phaeotheca fissurella*	*Wallemia ichthyophaga*
12.	*Aspergillus terreus*	*Scedosporium apiospermum*	*Aureobasidium* sp.
13.	*Aspergillus versicolor*	*Stachybotrys* sp.	
14.	*Aspergillus ustus*	*Stenella araguata*	
15.	*Aspergillus wentii*	*Trichophyton verrucosum*	
16.	*Cladosporium cladosporioides*	*Exserohilum rostratum*	
17.	*Curvularia lunata*		
18.	*Penicillium chrysogenum*		
19.	*Penicillium fellutanum*		
20.	*Penicillium digitatum*		
21.	*Penicillium expansum*		
22.	*Penicillium oxalicum*		

the salinity range of 395-410‰ in both evaporator and crystallizer ponds throughout the season of salt production (summer) at Tuticorin and Marakkanam saltpan. In the extreme hypersaline conditions of both ponds, the environment is too harsh and biodiversity is consequently limited while many taxonomic groups are absent, but obligate halophilic and halotolerant taxa persist and thrive. This confirmed result was supported by Rodriguez-Valera (1988). Melanized fungi, a selective advantage over the mycoflora in saline environments, representing 85-100% of the total isolated mycobiota from highly saline waters and partially replaced by non-melanized fungi at lowered salinities, being detected only occasionally with NaCl concentrations below 5%.

In the present investigation, sediment acts as a good anchor for the fungi to dwell than water. Nearly 80 species of fungi isolated from the saline soil of both study area this may be due to the precipitation of halite from the water column by evaporation and so the salinity ranges high in sediment than water this could be a possible reasons for obligate halophiles to survive more readily greater numbers in sediment, while the facultative halophiles were found more in the water column (Oren, 2003). This can be also due to a physiological stress to stenohaline algal species resulting mortality during increased salt concentration. The accumulated dead algae contributed to the high organic content of the soil. Thamizhmani *et al*. (2013) reported among the 46 species of fungi isolated, 35 species were isolated from sediment samples followed by water with 31 species. In this present survey, Marakkanam showed the maximum fungal diversity than Tuticorin, this may be due to comparatively longer concentration process of salts in addition to spatial changes would have caused the occurrence of lower species diversity and population density in the Tuticorin saltpans. Similar observations were reported by Rajalekshmi (2001) in Marakkanam and Tuticorin saltpans, Tamilnadu.

In this study, the fungal density showed massive progress in crystallizer and evaporator ponds during summer in this survey (2011-2012). Likewise, fungal diversity and density showed huge diverse at increased salinity. The

hypothetical reasons behind may be at high salinities, often high densities of phototrophic microorganisms, planktonic as well as benthic, makes the salterns excellent living biological system of primary production and other microbial processes. In evaporator ponds, most of the primary production occurs in benthic microbial mats seal ponds against water leakage and infiltration, permanently remove excess quantities of nitrogen and phosphate from the overlying water and maintain desired thicknesses in all ponds (Davis, 2000) and dominated by different types of unicellular and filamentous cyanobacteria sometimes in association with diatoms. Diatom diversity decreased noticeably with increasing salinity, the number of benthic diatom taxa was much higher at lower salinities (salinity 58–114g/l; 12 to 18 taxa) than in high salinities (salinity 157-206g/l; 1-5 taxa) in the salterns of Eilat, Israel (Oren *et al*., 2009). The planktonic and benthic communities of marine organisms (e.g. bacteria, algae, copepods, molluscs, worms) that develop along with the increasing salinity gradient in the evaporating ponds and crystallizers of saltworks create a biological system that can either help nor harm the salt production (Davis, 1993).

In crystallizer ponds, the unicellular green algae *Dunaliella* are the sole primary producer that lives in association with dense communities of halophilic archaea as well as extremely halophilic bacteria that color the brines red. Costa *et al*. (1996) stated that solar salterns are not just salt production plants, they also function as integrated saline wetlands of a unique coastal aquatic ecosystem that combines considerable environmental heterogeneity with a steep salinity gradient. In the summer seasons, increased brine temperature and longer day length, both due to increased solar radiation, apparently promote biological productivity in solar salt ponds. While the unadapted species gradually decreases while increasing of salinity, thus the sole nutrients may comes from the dead and decaying organic matter which helps the fungi to thrive well in such extreme hypersaline environments. Pedros-Alio (2004) concluded that these ponds provide a diversity of environments where different conditions of salinity, pH, temperature, light intensity, oxygen and nutrient concentrations are found, allowing the study of different microbial communities.

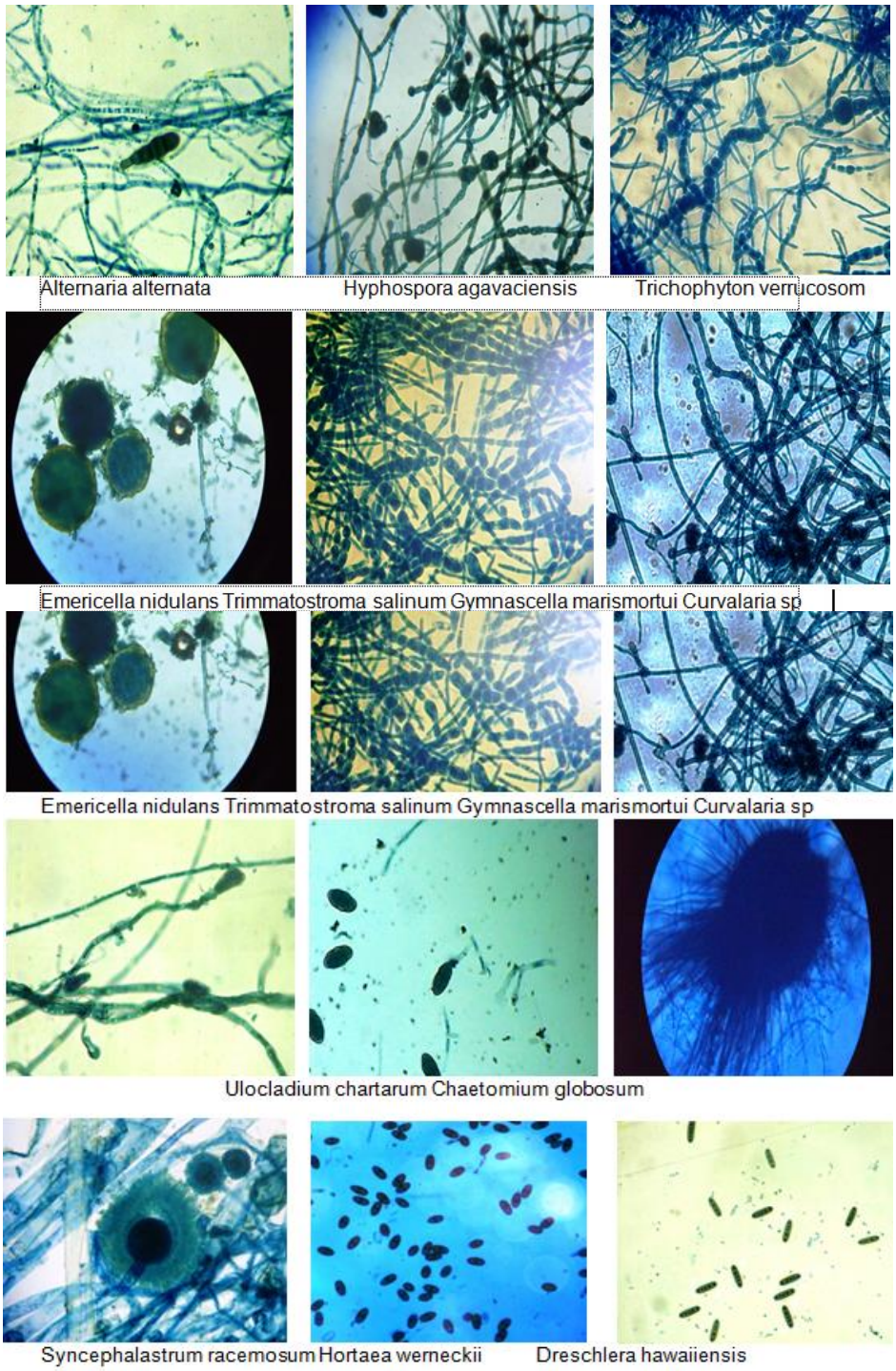
The findings showed the number of obligate halophilic fungi isolated in the present work seems to be higher as compared to that isolated on media without high salt concentrations. Buchalo *et al*. (1998) stated that obligate halophilic fungi did not grow in freshwater medium and showed increased growth with increasing salinity at 35ºC and the ionic composition of the medium had little effect on growth. During monsoon season, microbial growth was observed only in the salinity range of 19-35‰. However, during the entire study period it showed the occurrence of common marine forms in the saltpans due to the inflow of marine water mixed with rain water and from post monsoon onwards flourishing growth on the all salinity ranges were observed. Extensive studies were required for exploiting and acknowledge the fungi from solar salterns habitats especially in India.

Table 4. Periodicity of occurrence of halophilic mycoflora in various stations of Tuticorin and Marakkanam saltpans.

FUNGI	TUTICORIN						MARAKKANAM					
	S-1 (RESERVOIR)		S-2 (EVAPORATOR)		S-3 (CRYSTALLIZER)		S-1 (RESERVOIR)		S-2 (EVAPORATOR)		S-3 (CRYSTALLIZER)	
	W	S	W	S	W	S	W	S	W	S	W	S
ASCOMYCOTA												
Bipolarisspicifera	-	-	-	-	-	S	-	-	-	-	-	S
Botryophialophora marina	-	-	-	-	-	S	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	S	-	-	S	S	S	S
Candida sp.	-	-	-	-	S	-	-	-	-	-	S	S
Chaetomiumglobosum	-	-	-	-	O	-	-	-	O	S	S	-

FUNGI	TUTICORIN						MARAKKANAM					
	S-1 (RESERVOIR)		S-2 (EVAPORATOR)		S-3 (CRYSTALLIZER)		S-1 (RESERVOIR)		S-2 (EVAPORATOR)		S-3 (CRYSTALLIZER)	
	W	S	W	S	W	S	W	S	W	S	W	S
ASCOMYCOTA												
Chrysosporiumtropicum	-	-	-	S	-	S	-	-	S	S	S	S
Dabaryomyceshansenii	-	-	-	-	S	-	-	-	-	-	-	-
Eurotiumamstelodami	-	-	C	C	C	-	-	-	-	-	C	C
Emericellanidulans	-	-	S	S	-	O	-	-	-	S	-	O
Eurotiumrubrum	-	-	-	-	-	O	-	-	-	-	O	O
Exophialajeanselmei	-	-	-	-	-	-	-	-	-	-	S	S
Exophialaxenobiotica	-	-	-	-	-	-	-	-	-	-	S	S
Exserohilumrostratum	-	-	-	-	S	-	-	-	-	-	-	-
Gymnascellamarismortui	-	-	-	-	-	-	-	-	-	-	S	-
Hortaeawerneckii	-	-	S	C	C	C	-	-	S	C	C	C
Hyphosporaagavaciensis	-	-	-	-	-	-	-	-	S	O	S	-
Phaeothecafissurella	-	-	-	-	-	-	-	-	-	-	S	-
Phaeothecatriangularis	-	-	S	S	-	S	-	-	-	-	-	-
Scedosporiumapiospermum	-	-	-	-	-	-	-	-	-	-	S	-
Saccharomyces cerevisiae	-	-	-	-	-	S	-	-	S	S	S	S
Stenellaaraguata	-	-	-	-	-	-	-	-	-	-	S	-
Trichophytonverrucosum	-	-	-	-	-	-	-	-	-	-	S	-
Trimmatostromasalinum	-	-	-	-	S	S	-	-	-	-	-	-
*Trimmatostroma*sp.	-	-	-	-	-	S	-	-	-	-	S	-
Ulocladiumchartarum	-	-	S	-	S	S	-	-	S	-	S	C
Yarrowialipolytica	-	-	-	-	S	S	-	-	-	-	S	O
BASIDIOMYCOTA												
Cryptococcus neoformans	-	-	-	-	-	-	-	-	-	-	S	-
Rhodosporiumsphaerocarpum	-	-	S	S	-	-	-	-	-	-	S	S
Wallemiaichthyophaga	-	-	S	S	-	S	-	-	-	-	-	-
Wallemiasebi	-	-	-	-	-	S	-	-	-	-	S	S
MITOSPORIC FUNGI (HYPOMYCETES)												
Alternariaalternata	-	-	-	-	-	-	-	-	S	S	S	-
*Alternaria*sp.	-	-	S	S	O	O	-	-	S	O	O	O
Aspergilluscandidus	-	O	S	S	-	S	S	O	O	S	-	S
Aspergillusflavus	O	O	S	O	C	C	O	O	C	O	C	C
Aspergillusfumigatus	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC
Aspergillusniger	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC
Aspergillussydowii	C	C	C	C	MC	C	C	C	MC	C	MC	C
Aspergillusflavipes	O	O	-	-	S	-	O	O	O	-	O	O
Aspergillusglaucus	O	O	-	O	-	-	-	O	O	O	-	-
Aspergillusjaponicus	O	O	-	-	-	-	-	O	-	-	-	-
Aspergillusmelleus	-	S	-	O	O	-	O	O	-	O	-	O
Aspergillusnidulans	-	S	S	S	-	S	-	O	O	O	O	O
Aspergillusochraceus	MC	MC	MC	MC	C	C	MC	MC	MC	MC	C	C
Aspergillusoryzae	C	O	S	S	-	-	S	O	S	S	-	-
Aspergilluspenicilloides	-	-	S	S	O	O	-	-	S	S	O	O
Aspergillusrestrictus	C	C	C	C	C	C	C	C	C	C	C	C
Aspergillussclerotium	O	-	-	S	-	-	S	O	-	S	-	-
*Aspergillus*sp.	-	-	-	-	-	-	-	-	S	S	-	-
Aspergillussulphureus	O	-	-	O	-	S	S	O	O	O	-	S
Aspergillustamarii	O	C	O	O	O	O	O	C	O	O	O	O
Aspergillusterreus	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC
Aspergillus unguis	-	-	O	O	O	O	-	-	O	O	O	O
Aspergillusversicolor	C	C	MC	MC	C	C	C	C	MC	MC	C	C
Aspergilluswentii	C	C	C	C	C	C	MC	MC	C	C	O	O
Aspergillususstus	C	C	C	C	C	S	C	C	C	C	S	C
Aureobasidiumpullulans	-	-	O	O	O	O	-	-	O	O	C	C
*Aureobasidium*sp.	-	-	O	O	C	-	-	-	-	-	-	-
Cladosporiumcarrionii	-	-	S	C	S	-	-	-	S	S	-	S
Cladosporiumcladosporioides	C	C	MC	MC	MC	C	C	C	MC	MC	MC	MC
Cladosporiumherbarum	-	-	-	S	-	-	-	-	S	S	-	S
Cladosporiumsphaerospermum	-	-	-	-	-	C	-	-	C	C	C	C
Curvularialunata	C	C	O	O	S	S	C	C	O	O	O	O
*Curvularia*sp.	-	-	-	O	O	O	-	-	-	-	-	O
Dreschlerahalodes	-	-	-	-	O	-	-	-	-	-	-	O
Dreschlerabiseptata	-	-	-	-	O	O	-	-	S	S	-	O
Dreschlerahawaiiensis	-	-	-	-	S	S	-	-	-	-	S	S
Fusariumsolani	-	C	-	-	-	-	S	C	-	O	-	-
Fusariumverticilliodes	-	O	-	-	-	-	O	O	O	O	-	-
Nigrosporasphaerica	-	-	S	S	-	S	-	-	-	S	S	S
*Paecilomyces*sp.	-	-	-	-	-	S	-	-	-	-	-	-
Penicilliumbrevicompactum	O	C	-	O	-	-	-	O	C	C	C	C
Penicilliumchrysogenum	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC
Penicilliumfellutanum	C	C	MC	C	MC	C	C	C	MC	MC	MC	MC
Penicilliumcrustosum	O	O	O	O	-	-	O	O	O	O	-	-
Penicilliumcyclopium	O	O	-	O	-	-	-	O	C	C	-	C
Penicilliumdigitatum	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC
Penicilliumexpansum	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC	MC
Penicilliummarneffei	-	O	-	-	-	-	S	O	S	S	-	-
Penicilliumoxalicum	MC	MC	C	MC	MC	C	MC	MC	MC	C	MC	C
Penicilliumpolonicum	-	-	-	-	-	-	-	-	S	-	O	-
*Penicillium*sp.	-	-	-	-	-	O	-	-	O	S	O	S
Penicilliumvariabile	S	S	-	-	-	-	-	S	-	-	-	-
Penicillumcitrinum	S	S	-	-	-	-	S	O	O	O	-	-
Scopulariopsisbrevicaulis	-	-	-	-	S	-	-	-	-	-	-	-
Stachybotrys sp.	-	-	-	-	-	-	-	-	-	-	S	-
Trichodermapiluliferum	-	-	-	S	-	-	-	-	S	S	-	S

FUNGI	TUTICORIN						MARAKKANAM					
	S-1 (RESERVOIR)		S-2 (EVAPORATOR)		S-3 (CRYSTALLIZER)		S-1 (RESERVOIR)		S-2 (EVAPORATOR)		S-3 (CRYSTALLIZER)	
	W	S	W	S	W	S	W	S	W	S	W	S
ASCOMYCOTA												
MITOSPORIC FUNGI (COELOMYCETES)												
Non-sporulating fungi-1	C	-	-	-	-	-	-	C	C	C	-	-
Non-sporulating fungi-2	-	C	-	-	-	-	-	C	-	O	-	O
Non-sporulating fungi-3	S	-	-	-	-	-	-	-	-	-	-	-
Non-sporulating fungi-4	-	-	-	-	-	-	S	-	-	-	-	-
Sterile Black mycelium-1	-	-	-	-	O	-	-	-	S	-	-	S
Sterile Black mycelium-2	-	-	S	S	-	S	-	S	-	-	-	-
Sterile Black mycelium-3	-	S	-	-	-	-	-	-	-	-	-	-
ZYGOMYCOTA												
Basidiobolushaptosporus	-	-	-	-	-	-	-	-	-	-	-	S
Syncephalastrumracemosum	-	O	-	-	-	-	-	-	-	-	-	-



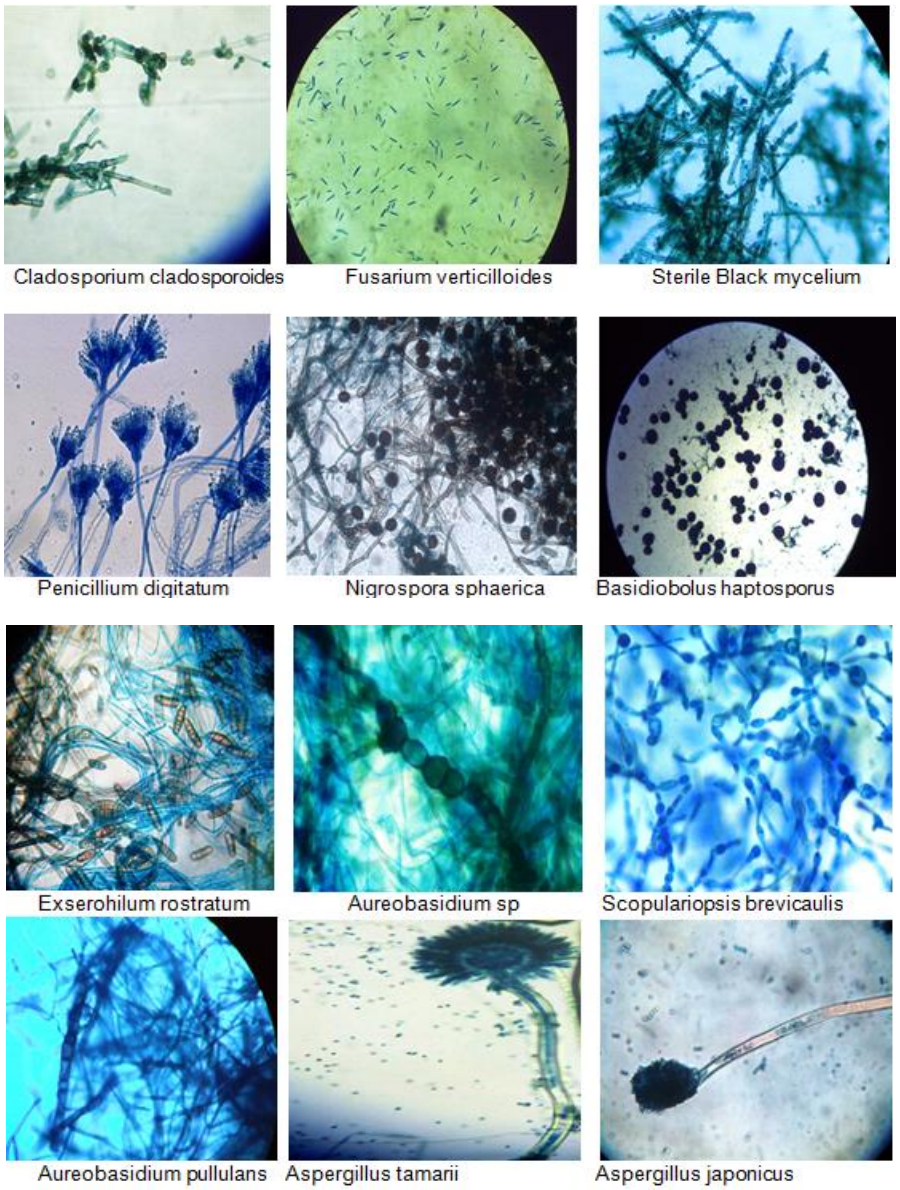


Fig. 3. Microscopic observation of halophilic fungi.

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