Voyage Journal of Scientific Research and Exploration (VJSRE) *Vol . 1 Issue 1*
ISSN (Online): 3007-3898, ISSN (Print): 3007-388X, March 2024

GROUPING OF AIRBORNE MANGLICOLOUS FUNGI, PAKISTAN

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Abstract

This study deals with the grouping pattern of air-borne micro biota (fungi species and bacteria) in some mangrove forests of Pakistan. The study was undertaken to quantitatively and qualitatively assess the air-borne microorganisms of mangrove forests using agar plate culture technique. Six different sites i.e. Port Qasim, Kemari, Korangi, Mai Kolachi, Sonmiani and Ketti Bunder were selected to collect samples during June to August. During study, a total 26 fungal species viz., *Aspergillus niger, A. fumigatus, A. sulphureus, A. terreus, A. candidus, A. wentii, A. flavus, Alternaria alternata, A. maritima, A. porri, Alternaria sp., Rhizopus varians, Mucor sp., M. mucedo, Penicillium sp., P. notatum, Dreshellera nodulosa, D. dematioidea, Exosporiella fungorum, Botrytis cinerea, Cladosporium oxysporum, Acrodictys bambusicola, Chetomium sp., Fusarium solani, Trichoderma viridae* and *Curvularia sp.* were recorded from different mangrove sites through microscopic method. The one-way and two-way dendrogram were obtained from Ward's clustering method based on relative densities resulted into two and four main groups respectively. It may be concluded that Deutromycetes were widely distributed in the mangrove forest, while Ascomycetes were rare whereas Phycomecetes were entirely absent during the whole study period. This study will aid to know the abundance and assemblage of the mico-biota in other mangrove habitats.

Keywords: air- micro-biota, mangrove, fungi, bacteria, agar plate

Introduction

Approximately 25 % of the world's coastline is dominated by mangroves, distributed in 112 countries and territories encompassing an area of 181,000 sq km. worldwide. Mangrove forests being detritus-based ecosystem supports many food webs and a diverse group of animals ranging from microorganisms to the higher groups. Litter inputs are decomposed and utilized as energy sources by the diverse group of organisms (Suberkropp and Klug, 1981). Of all fungi and bacteria are the wide spread organisms in mangrove, originate from different

habitats i.e. soil, plant tissues and water. The mangrove fungi's geographic distribution differed across the oceans, with only 109 taxa shared by the Atlantic, Indian, and Pacific Oceans. India has the greatest number of mangrove fungus (339) followed by Thailand (303), Malaysia (171), Florida Everglades (134), USA (134), and Brunei (134) (Devadatha *et al*., 2021). They contribute in the decomposition of mangrove material and to the transformation and cycling of nutrients (Kathiresan and Bingham, 2001). According to Rajendran, (1997) fungi are the essential litter intruders, arriving at their pinnacle excess in the beginning stages of decay because of presence of high convergence of tannins in leaves on while microscopic organisms are the secondary intruders because of reverse association with tannins. Fungi in conjunction with bacteria break-down leaf litter quickly (Fell *et al*., 1975). In fact, they are more compelling decomposers than bacteria since they can enter in the tissues of host (Tomlinson, 1986).

Various environmental factors inundation, wave, wind etc. are responsible to transfer soil-borne fungal spores or conidia into the air. Kohlmeyer and Kohlmeyer, (1979) described the mangal as a home to a group of fungi and nominated as manglicolous fungi. Different researchers such as Mehdi and Saifullah, (1992a,b); Mehdi *et al*., (2000); Tariq and Mehdi, (2006) have reported the presence of manglicolous fungi on roots, leaves, fruits, rhizosphere and soil but no one studied the airborne fungi and bacteria in mangrove environment. The fungi colonizing mangrove substrata can be divided into terrestrial mycota, colonizing the plant part above the water column and marine fungi colonizing the parts inundated either completely or partially by sea water. Thus, terrestrial fungi and lichens occupy the upper part of the trees and marine species occupy the lower part. At the interface there is an overlap between marine and terrestrial fungi (Kohlmeyer and Kohlmeyer, 1979).

The major objectives of this study were to,

1. Determine the distribution and grouping pattern of the airborne fungi in different mangrove areas.
2. Evaluate the fungal species in highly polluted, polluted and less polluted mangrove forests.
3. Estimate the bacterial colonies in mangrove areas.

Materials & Methods

Six mangrove sites *i.e*. Port Qasim, Korangi creek, Kemari, Mai Kolachi, Sonmiani and Ketti Bunder were selected to conduct this study. Topbas *et al*., (2006) agar plate culture technique was used with five replications in selected mangrove sites. The standard laboratory techniques were followed according to Kohlmeyer and Kohlmeyer, (1979) and Hyde and Jones, (1988) for the isolation of maximum number of microbial colonies. The colonies were identified on the basis of morphological characters following Ellis, (1971); Booth, (1971); Thom and Reper, (1945) and Refai, (1987).The one-way and two-way dendrogram were obtained from Ward's clustering method based on relative densities.

Results

The culture plates produced total 566 fungal and bacterial colonies throughout the study period. The fungal populations were very high in the atmosphere of mangrove forests compared to bacterial count. Twenty six fungal species belonging to fourteen genera viz., *Aspergillus niger, As. fumigatus*. *As .sulphureus*, *As. terreus*, *As. candidus*, *As. Wentii*, *As. flavus*, *Al. alternata*, *Al. maritima*, *Al .porri*, *Alternaria sp.*, *R. varians*, *Mucor sp.*, *M. mucedo*, *P. notatum*, *Penicillium sp.*, *D. nodulosa*, *D. dematioidea*, *E. fungorum*, *B. cinerea*, *C. oxysporum*, *A. bambusicola*, *Chetomium sp.*, *F. solani*, *T. viridae*, *Curvularia sp.* were recorded with bacterial colonies.

Table 1 showing the number of fungal species at all sites. The maximum numbers of species were found at port Qasim while minimum was recorded from Ketti Bunder. *A. niger & P. notatum* were the most common species recorded from all sites.

Table 1. Airborne manglicolous fungi recorded from different mangrove swamps.

Species	PQ	KE	KO	SO	KB	MK
A.niger	+	+	+	+	+	+
A. fumigates	+	+	NF	NF	NF	+
A .sulphureus	+	NF	+	NF	NF	NF
A. terreus	+	+	NF	NF	NF	NF
A. candidus	NF	+	+	NF	NF	+
A. wentii	+	NF	+	NF	NF	NF
A. flavus	+	+	+	+	NF	+
Al. alternate	+	NF	+	NF	NF	NF
Al. maritime	+	+	+	NF	+	+
Al .porri	NF	NF	NF	NF	NF	+
Alternaria sp.	+	NF	NF	NF	NF	NF
R. varians	+	NF	+	+	+	+
Mucor sp.	NF	NF	NF	+	NF	NF
M. mucedo	+	NF	+	+	NF	NF
P. notatum	+	+	+	+	+	+
Penicillium sp.	+	NF	NF	NF	NF	+
D. nodulosa	NF	NF	NF	+	NF	NF
D. biseptata	+	NF	NF	NF	NF	NF
E. fungorum	+	+	NF	NF	NF	NF
B. cinerea	+	NF	+	NF	NF	NF
C. oxysporum	NF	+	NF	NF	NF	NF
A. bambusicola	+	+	NF	NF	NF	NF
Chaetomium sp.	NF	+	NF	NF	NF	NF
F. solani	+	+	NF	NF	NF	+
T. viridae	+	+	+	NF	NF	NF
Curvularia sp.	NF	+	NF	NF	NF	NF
Total number of species	18	14	12	7	4	10

Note: SP= Sandspit, PQ=Port Qasim, KE= Kemari, KO=Korangi, SO=Sonmiani, KB=Ketti Bunder, MK=Mai Kolachi, NF= not found, + = showing the presence of fungi

Spatial & temporal variations of micro biota in different mangrove swamps.

The dendrogram were obtained from Ward's clustering method based on Euclidean distance matrix (a space contracting strategy) resulted in two main groups (Fig.1).

Group A

Group A is the purest and the largest group contained all fungal species. Four important air-borne fungi included *A. niger*, *A. flavus*, *P. notatum* and *R. varians* grouped in cluster separately whereas the other species formed a unique chaining in the same group. These four species were recorded from all sites (except *R. varians* which did not find from Ketti Bunder) with higher values of relative density %. The maximum relative density (43.3%) of *P. notatum* was found at Sonmiani in September whereas least (17.1%) was observed in Korangi during August. The *A.niger* with the second highest relative density (40.54%) was recorded at Port Qasim in April. *R. varians* and *A. flavus* were the third and fourth members of this group with maximum relative density 26.4% and 25.8% reported from Ketti Bunder and Port Qasim respectively.

Group B

This group consisted of bacterial colonies with 100% relative density found throughout the study period at all sites.

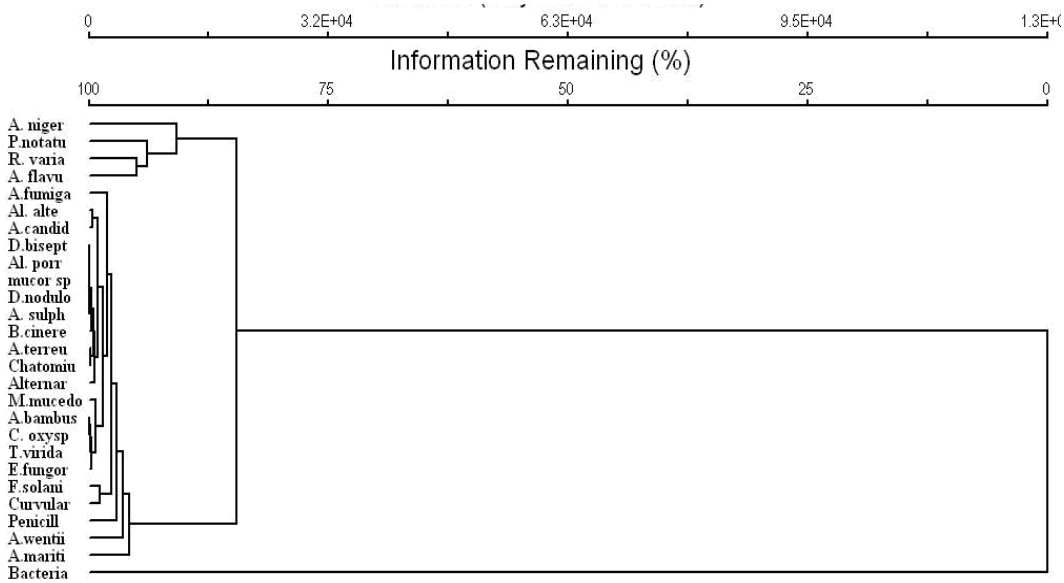

Figure 1. Cluster Analysis of aero myco flora in different mangrove forests.

Two way cluster analysis of micro-biota in different mangrove swamps.

The resulting dendrogram of airborne fungal species based on relative density exhibited four major groups (Fig.2). The groups were described named after first two dominant species,

Group I – A.niger– R. varians

This group consists of eight most abundant species recorded from all sites. It was sub-divided into two sub-groups; sub-group I a & I b. The I a consists of five species, *A.niger*, *P. notatum, A. flavus, Alt. maritima* and *A. fumigatus.* All these species were recorded from all sites except *A.niger* which was absent in Ketti Bunder and Sonmiani from August to September. Sub- group IIa contains three species, *R.varians*, *Curvularia* sp. and *Mucor* sp., the *R. varians* was a prolific and diverse species, witnessed from April to June and August to September at all locations except Kemari. The maximum mean value (8±2) of this species was recorded from Ketti Bunder. The *Mucor* sp. was reported once a year in August from Ketti Bunder with average % 4.5 ± 0.5 whereas *Curvularia* sp. was recorded with a high mean% (6 ± 1) from Port Qasim and Ketti Bunder during June and August.

Group II- *F. solani- Al. alternate*

This group incorporated 8 fungal species and was also sub-divided into two sub-groups as II a & II b. The sub-group IIa consists of four species namely *Al. alternata, A. candidus, D. biseptata an*d *Al. porri* recorded from three sites *i.e*. Port Qasim, Korangi and Mai Kolachi. The sub- group IIB consists of four species *i.e*. *E. fungorum, F. solani, A. terreus* and *Chatomium* sp. The *F. solani* was observed from April to August from three sites; Mai Kolachi, Port Qasim and Kemari with the highest mean value (5.3 ± 1.2) whereas *Chatomium* sp. was recorded once a year in June with mean % 2.7 ± 1.8 from Port Qasim.

Group III- *Penicillium* sp. - *Alternaria* sp.

It is evident from the dendrogram that group III is a small group compared to previous groups and consists of only five species i.e. *Penicillium* sp. *Alternaria* sp., *B. cinerea, A. sulphureus* and *A. wentii* with maximum mean % 7.0 ± 1.1, 4 ± 2, 1.6 ± 0.6, 2 ± 1.1 and 1.3 ± 0.3 respectively. These species were encountered from four main sites, Korangi, Port Qasim, Mai Kolachi and Ketti Bunder from April to June and in August.

Group IV- *T. viridae- M. mucedo*

Group IV also comprised of five species, *M. mucedo, T. viridae, A. bambusicola, C. oxysporum* and *D. nodulosa.* This group is the least abundant group, the species found only in March, June, July, August and September from Port Qasim, Kemari, Korangi and Sonmiani. *T. viridae* was the most dominant species with the highest mean % (3.6 ± 1.2) whereas *D. nodulosa* recorded once from Port Qasim. March was the period when all these species were present in highly polluted areas of Port Qasim.

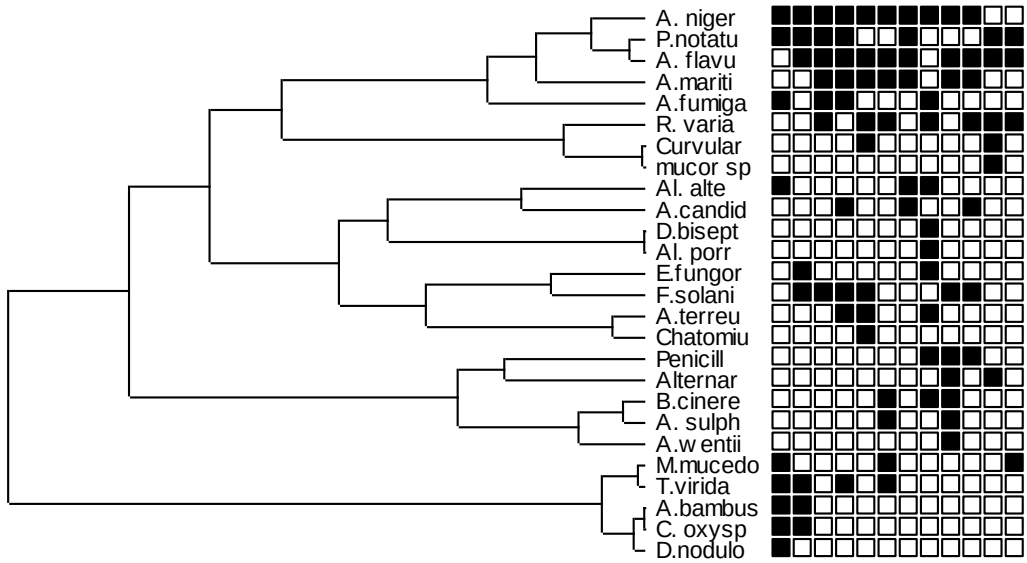

Figure 2 Two-Way Cluster Analysis of airborne manglicolous fungi.

Discussion

Cluster analysis showed significant grouping between fungal species and bacteria. The results indicated highly significant differences in the distribution of fungal species and bacteria in six different locations. It was noted that the genera of parasitic and saprophytic fungi recorded from mangrove canopy were generally large, dominant and wide spread often with species pathogenic to a number of hosts like *Cladosporium; Aspergillus; Penicillium; Dreshellera; Fusarium* etc. all have species affecting terrestrial plants. In some instances, the same species affect both mangrove and terrestrial plants (Kaushik and Hynes, 1968) except few which were restricted to their particular habitats. The prevailing climatic conditions of the coastal areas are generally high as a result of the dryness of the surrounding area. The maximum number of fungi and bacteria were recorded from Port Qasim the reason is that the climatic conditions of Port Qasim are generally hot and relatively humid especially in summer when the direction of wind is from South-West. The higher wind during the South-West monsoon has a tendency to carry airborne contaminants inland during the summer

months. In winter the winds is moderate and carried airborne pollutant out of the sea. The main sources of air pollution in Port Qasim area steel mills and the KESC oil power station. Particulates and dust are also released from the port itself during the unloading of grains and fertilizers handling etc. Dominance of airborne fungi on mangrove is dependent on the duration and severity of rains during southwest monsoon in the Pakistan. Usually heavy rains occur during July to August which decrease the salinity of mangrove waters, brought high amount of sediments and deposited on the substrate, resulted in colonization of fungi.

Some fungi *Aspergillus niger, A. flavus, Penicillium* sp., and *Rhizopus varians* also had overall RD % occurrence in the air of mangrove areas. These fungi may be able to tolerate a wide range of temperature and habitat and other abiotic factors. Other fungi although present in most of the sampling sites were not abundant in term of their overall RD % occurrence. Some fungi that appeared on one site were absent on other sites during the same sampling period. Infect such condition obscured the aspect of seasonality of individual fungi in mangrove forests. The major observations of the above study advocated that more number of fungal colonies were recorded during July and August. This showed that mangrove fungi prefer more dampness and moderate to high temperature, low values of pH and salinity due to tidal influence. The high mean values of *Aspergillus niger* and *Aspergillus flavus* by all locations clearly showed that despite pollution, unhygienic conditions, industrial or domestic wastes these two fungal species were successfully widely distributed. *Aspergillus niger* was very common in almost all locations except Ketti Bunder $P<0.05$).

The present study accords well with the findings of Mehdi and Saifullah (1992) and Shaukat *et al*., (2014) who also reported high abundance of *Aspergillus* species (i.e., *A. niger* and *A. flavus*) on grey mangrove *Avicennia marina* growing at Clifton and Korangi Creek. This species grows well over a wide range of temperature and are commonly found in decomposing compost. This is in accordance with the statement of Barron, (1968) that *Aspergillus* spp. biologically most successful in all fungi and expected to occur on all sorts of

organic debris. The higher mean value of this species was recorded from Sandspit (stand 3) may be due to high rate of organic matter about 31%. The fungal population in soil depends greatly on the amount of decomposable organic matter present. Fungal density is generally high as long as readily decomposable organic matter is present in sufficiently high amount. However, when organic matter declines fungal populations disappear because of competition for food and energy with other soil microbes. Bacteria are ubiquitous in all kind of environments. A moist soil with ample decomposable material may contain as many as a billion or more bacteria per gram of soil. Anaerobic bacteria obtain energy by reducing ions such as nitrate or sulphate and compounds like sugars. Heterotrophic bacteria obtain their energy by decomposing organic material by oxidizing some inorganic material.

Twelve fungal species were recorded from Korangi mangrove region with larger number of bacterial colonies. The rate of distribution of the dominant fungal species was influenced by climatic factors, but the other fungal species of the same point were unaffected. Korangi is close to waterfront region with varieties of industries like tanneries, oil, textiles and pharmaceuticals etc (Rao *et al*., 2009). In addition, this region is additionally occupied by underprivileged and nastiest inhabitants. This region remained polluted throughout the year due to its large population and various industries, particularly tanneries. In spite of the fact that tanneries squander water treatment plant is likewise worked in this space yet the contamination is as yet expanding (Rehman and Iqbal, 2008, 2009, 2011). Ten fungal species were recorded from Mai Kolachi which is packed, thickly populated and taking off traffic region because of the focal point of the city.

Enormous populace, high traffic outflows, residential waste, deficient sewerage framework and trash cause contamination. Likewise, the hazardous gases and metallic particles of the fumes from the autos additionally influence the natural ecological conditions of the mangrove forests. Most of the inhabitants in the area belong to lower-class creating unsafe issues for clean climate. These dirty circumstances established favorable climate for the

growth of airborne micro organism's particularly fungal species and bacterial colonies. The similar results were reported by Mehdi and Saifullah, (1992a, b). According to Nazim *et al*., (2012), the rapid decomposition of leaf litter, high temperature, low pH, and salinity values may all have contributed to the rapid colonization of the mangrove swamp by airborne pathogenic fungi.

Conclusion

It may be concluded that Phycomecetes were missing all through the study period though Ascomycetes were uncommon while Deutromycetes were found abundantly in the air of mangrove forest. This study will help in assessing the diversity, abundance and assemblage of mico biota in different mangrove habitats.

Acknowledgement

The author is highly grateful to Prof. Dr. Abdul Hakeem Sheikh, Department of Botany for critically reviewing this paper and provided valuable suggestions. The author is highly thankful to Port Qasim Authority (PQA) for providing the field facilities during study.

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