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# ISOLATION AND IDENTIFICATION OF SOIL MYCOFLORA OF RIVER INDUS AT KOTRI

**M. SUHAIL, S. AKHUND, T. JATT, ISHRAT RANI AND H. ABRO**

*Institute of Botany,*
*University of Sindh, Jamshoro, Pakistan.*

**Abstract**

During the period from June 2004 to May 2005, a survey of the soil mycoflora was carried out at 3 sites (Right bank, Center and Left bank) from the bed of river Indus at Kotri. A total of 24 soil samples were observed by using three different methods; soil plate, soil dilution and baiting technique. Out of 73 fungal strains, 10 species viz., *Absidia glauca* (1.83%), *Cunningamella echinulata* (4.09%), *C. elegans* (2.96%), *Mortierella exigua* (10.86%), *M. ramanniane* (7.76%), *M. zonata* (5.08%) *Mucor hiemalis* (13.12%), *M. mucedo* (18.19%), *Rhizopus nigricans* (14.81%), and *R. stolonifer* (21.29%), belonging to the group Zygomycetes were isolated which have not hitherto been recorded from bed of river Indus. The fungal counts were generally high in right bank when compared to those of the samples of left bank and center. In addition, upper soil layers not only contained the largest amount of fungi, but also by far the highest species diversity.

## Introduction

After insects, fungi are the largest group of organisms (Hawksworth, 1979). They are remarkable for their antiquity, diversity, ubiquitous distribution and longevity and are known to occur in almost all environments. Many fungal species can be isolated non-destructively from their native habitats and can grow in culture to be perpetuated and used indefinitely (Rossman, 1994). There are about 72,000 known species and new species are being added at the rate of about 1500 each year. Additional evidence to support the world estimate of 1.5 million fungal species is based on the rates of newly described species of 50-66% in some fungal groups over the last twenty years (Hawksworth, 1993). Fungi are generally considered to have a central role in soil ecology and to produce beneficial pharmaceuticals; as for instance some members of Zygomycetes are used to produce commercially important products, such as organic acids, pigments, fermented oriental foods, alcohols and modified steroids (Certik *et al*., 1997). However, the distribution and abundance of soil microfungi in various habitats is still insufficiently understood (Christensen, 1989). This stems primarily from the lack of information on the identification of fungal species assemblages from different habitats. The few studies done on fungal assemblages within different soils indicated that some species occur in great frequency but are restricted to a specific habitat, while other species occur in lower frequency but are distributed over a wider range of habitats (Bhatt, 1970; Christensen, 1969; Visser & Parkinson, 1975; Wicklow & Whittingham, 1974). Keeping this view in mind, the present study was under taken to characterize the mycoflora in three different habitats in the bed of river Indus at Kotri (Right bank, Center and Left Bank) during the period from June 2004 to May 2005. The specific emphasis was on Zygomycetes.

## Material and Method

**Study area and collection of samples:** The study area is located at longitude 68.°22'E, latitude 25.°22'N. Air temperature ranges between 9.3°C to 40.4°C. Five types of vegetation were observed in the area: *Tamarix, Saccharum, Acacia arabica, Salvadora persica* and *Prosopis* (Chaudhri *et al*., 1966). The area consists of deep, poorly drained soils formed in clayey calcareous, glacial lacustrine sediment. Mean annual precipitation is about 24 inches (Suhail *et al*., 2006). The pH value generally ranges from 8 to 8.50. The investigation covers right bank, center and left bank of river Indus. The samples were collected from surface, 10 cm, 20 cm and 30 cm depth by sterilized vials and were stored in polythene bags until they reach laboratory. The samples immediately underwent isolation using Soil dilution (Waksman, 1922), Soil plate method (Warcup, 1950) and baiting technique (Booth, 1971)

**Soil dilution plate method:** The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.5ml volumes were pipetted onto Potato Dextrose Agar and incubated at 26°C for three days.

**Soil plate method:** About 0.005 g of soil was scattered on the bottom of a sterile Petri dish and molten but cooled (45°C) agar medium (PDA) was added, which was then rotated gently to disperse the soil particles in medium. The plates were then incubated at 26°C for three days.

**Baiting technique:** The soil samples were placed in sterilized Petri dishes, approximately half filled the dishes, moistened with sterile water, and small pieces of baiting material previously sterilized by autoclaving were scattered over the soil surface (Booth, 1971). The dishes were then kept at room temperature and observed for appearance of any fungal growth on baits for 7 days. In order to obtain pure cultures, very small inocula were taken from several parts of the growth on the bait, and spread out on Potato dextrose agar containing chloramphenicol.

Counts of fungal colonies were conducted and screening of fungi was made from mixed isolates and subcultured on MEA. Sub culturing was continued until a pure isolate was obtained. Identification was performed according to Mirza (1979) and Gilman (1945).

## Result and Discussion

Out of 24 soil samples analysed for the presence of fungi, 10 strains viz., *Rhizopus nigricans, R. stolonifer, Mucor mucedo, M. hiemalis, Cunninghamella elegans, C. echinulata, Absidia glauca, Mortierella ramanniane, M. zonata* and *M. exigua were* isolated. The most prevalent genus in all sampling sites was *Rhizopus* (36.15%) followed by *Mucor* (27.69%). *Mortierella* was recovered from the samples of Right bank and Left bank in a frequency of 26.29. *Cunninghamella* (7.82%) and *Absidia* (2.03%) were isolated only from one sampling site i.e. right bank and center respectively. The distribution and frequency of all species in different sites (Right bank, Center and Left bank) isolated by using 3 different methods is shown in Table 1.

**Table 1. Prevalence and distribution of the species in different sites by using three methods from the bed of river Indus.**

| Fungi isolated | Left bank | | | Centre | | | Right bank | | |
|---|---|---|---|---|---|---|---|---|---|
| | S.D.P | S.P | B.T | S.D.P | S.P | B.T | S.D.P | S.P | B.T |
| *Absidia glauca* | ….. | ….. | ….. | ….. | 1.40 | 0.42 | ….. | ….. | ….. |
| *Cunninghamella echinulata* | ….. | ….. | ….. | ….. | ….. | ….. | ….. | 1.40 | 2.69 |
| *Cunninghamella elegans* | ….. | ….. | ….. | ….. | ….. | ….. | …… | 0.85 | 2.12 |
| *Mortierella exigua* | 4.23 | ….. | 0.56 | ….. | ….. | ….. | 4.80 | ….. | 1.27 |
| *Mortierella ramanniane* | 2.96 | ….. | 0.70 | ….. | ….. | 0.14 | 3.95 | ….. | ….. |
| *Mortierella zonata* | ….. | 2.12 | ….. | ….. | ….. | ….. | ….. | 2.96 | ….. |
| *Mucor hiemalis* | ….. | 6.35 | ….. | 3.95 | ….. | ….. | 2.82 | ….. | ….. |
| *Mucor mucedo* | 2.53 | ….. | 1.55 | ….. | 2.40 | 1.13 | 7.05 | 3.53 | ….. |
| *Rhizopus nigricans* | ….. | 3.52 | 0.56 | 1.83 | 2.82 | ….. | 1.27 | 2.53 | 2.26 |
| *Rhizopus stolonifer* | 2.12 | 1.69 | 0.85 | 1.40 | 2.12 | ….. | 5.92 | 4.09 | 3.10 |

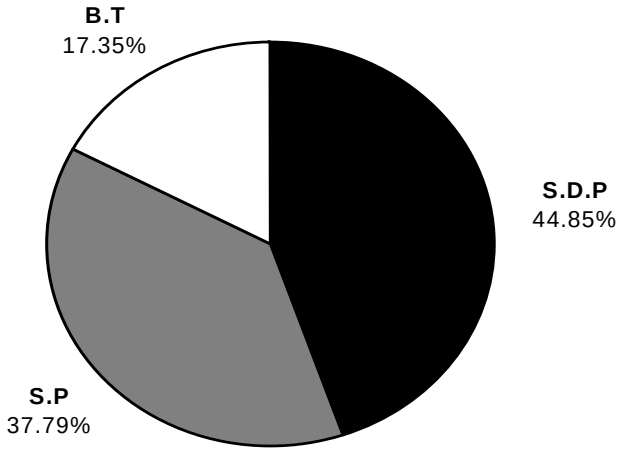


Fig. 1. Rate % of occurrence of fungi in three methods
B.T= Baiting technique, S.D.P= Soil dilution, S.P= Soil plate

With the soil plate method, the richest genera in terms of the number of colonies were *Rhizopus* (16.78%) and *Mucor* (12.27%). *Mortierella* (5.08%) was recovered in moderate frequency whereas *Absidia* (1.41%) was less frequently isolated.

The most predominated genus from soil dilution plate method was *Mucor* (16.36%) followed closely by *Mortierella* (15.94%) and *Rhizopus* (12.55%), while *Absidia* and *Cunninghamella* were never isolated by this method.

Baiting technique yielded variety of species by using different baits. The predominant genera were *Rhizopus* (6.78%) and *Cunninghamella* (4.79%). Three genera showed low incidence viz *Mortierella* (2.68%), *Mucor* (2.68%) and *Absidia* (0.42%).

The results of the present study provide the comparative analysis of the diversity of fungal species available at Right bank, Center and Left bank (Table 1). The higher number of isolates was obtained at Right bank, which is associated with deforestation, agricultural activities and human communities. Out of three different methods, Dilution plate yielded greater number of colonies (Fig. 1). Moreover, the qualitative studies

revealed that upper soil layers not only contained the largest amount of fungi, but also by far the highest species diversity. No report on the prevalence and distribution of members of this group in the soil of River Indus is found (Sultan *et al*., 1997).

Information concerning fungal assemblages helps to gain an understanding of the ecological dynamics of soil microbial community. This report can serve as a comparative model for documentation in microfungal assemblages, relative distribution and abundance in the study area.

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