A STUDY OF FUNGUS FLORA OF KARACHI SOILS

S. Shahid Husain, S.M. Hasany and S.I. Ahmed

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received October 25, 1965)

A study of fungus flora of Karachi soil was carried out to determine the frequency of occurrence of various fungi at different places and at different soil depths. A total number of 58 species belonging to 25 genera were isolated from these samples. Five genera and 7 species were recorded for the first time from West Pakistan. Eight genera and 13 species are new to Karachi region. The fact that more fungus species were isolated from surface and upper levels of soil than at lower levels may be due to poor aeration and antagonistic affects of certain microorganisms.

Introduction

The study of soil fungi gained momentum in the beginning of the present century and has opened a new vista in the field of Mycology and Plant Pathology. Mycologists are engaged in the fundamental problems of fungi in the soil. Different workers have adopted different techniques for isolating fungi from the soil and for other synecological studies.

Butler[1] was the first who initiated the investigation of soil fungi in Indo-Pakistan sub-continent and gave an account of the genus *Pythium* and some Chytridaceae. Thakur and Norris[2] reported 22 species of fungi from Madras soil. Mason[3] reported 4 species of *Aspergillus* and one species of *Aerothecium* isolated from the paddy soils of Sind and Burma. Chaudhuri and Sacher[4] reported 32 species isolated from Lahore soils. Butler and Bisby[5] and Mundkar and Ahmed[6] reported 198 species from the region now included in West Pakistan. Galloway[7] in 1935 gave some generic descriptions of soil fungi collected from Pusa and hilly districts of North India.

Hukam Chand[8] reported 198 species from Lahore soils. Sultan Ahmed[9] reported 1219 species of fungi isolated from soil and other sources from the area now comprising West Pakistan. Lodhi and Mirza[10] added 43 species in the list of West Pakistan soil fungi.

Sind, Kalat, Baluchistan and Karachi regions are very poorly surveyed and meagre records are available. It was, therefore, considered necessary to carry out a comprehensive survey of Karachi soils in order to investigate the prevalence of soil fungi and to ascertain their frequency in relation to the depth of soil.

Materials and Methods

Collection of Soil Samples.—The soil samples were collected from different places randomly. The places selected were Bizerta lines, Mohd. Ali Housing Society, Air Port, Malir, Azizabad and North Nazimabad. A hole, one foot in diameter and two feet in depth, was dug to expose the soil profile. The samples were collected at various depths by means of sterilized iron tubes, 3-inch long and 1-inch in diameter, with separable lids at both ends. The lid was tightly closed at one end. The other end was pushed horizontally into soil to a depth of one to two inches of its length. The iron tube was withdrawn along with the soil and the lid was replaced immediately in order to avoid any contact between this soil and atmosphere. Samples were then collected in this manner from 2, 4, 8, 12, 16, 20 and 24-inch depths and from surface soil.

Inoculation.—Inoculation of soil samples was carried out on the same day on Czapeck's Dox Agar medium by using soil plate method.[11] One thousand ml. of Czapeck's Dox Agar medium was prepared, and sterilized in an autoclave at 15 lb. pressure per sq. inch for 20 minutes. The pH of the medium was adjusted between 4.3-4.5 by adding required quantity of sterilized 10% phosphoric acid. The medium was kept acidic to inhibit the soil bacteria appearing in petridishes.

One to 1.5 g. of soil lump was taken by a sterilized spatula and placed into a sterilized petridish. Five ml. of sterilized distilled water was poured over the soil lump and shaken gently to obtain a thin film of soil suspension. Fifteen ml. of melted, but cooled Czapeck's Dox Agar was added to this soil suspension. To avoid fast growing Mucorales which are more prevalent in the upper soil levels, hot medium (50-55°C.) was poured on samples taken at depths of two inches, four inches and surface soil. Cool medium was added in rest of the samples. Five petridishes were inoculated with each soil sample and when the medium solidified petridishes were incubated at 28°C.

Isolation.—The petridishes were examined every 24 hours. Mucorales were isolated on third day

of incubation from all petridishes in Czapeck's Dox Agar slants. The same petridishes were again incubated for 14 days and various organisms were isolated in pure cultures. Colonies which were similar in colour, growth pattern, fruiting bodies and other characters were discarded.

Identification.—For detailed investigations, the cultures were reinoculated in petridishes on different media with variable pH and incubated at various temperatures. These factors (temperature, pH and media) generally are of great help in the correct identification of genera and species of fungi. The colour of colony, growth pattern, measurements of mycelium, conidia and conidiophores alongwith presence or absence of transverse septa were taken into account for specific identification of these fungi. Mycelium and fruiting bodies were mounted in lactophenol and stained either in cotton blue, lugol's iodine or Acid Fuschin.

Results

Members of various groups of fungi, Phycomycetes, Ascomycetes and Fungi Imperfecti were isolated during these studies. Fifty eight species belonging to 25 genera were recorded. Out of these five genera and seven species are new records for West Pakistan. These are listed below.

Phycomycetes.—*Cunninghemella echinulata* Thaxter; *Cunninghemella* sp.; *Mucor globosus* Fischer; *M. sphaerosporus* Hagen; *M. mucedo* (Linne) Brefeld; *M. racemosus* Fresenius; *Rhizopus nigricans* Ehrenberg.

Ascomycetes.—*Eurotium* sp.; *Microascus* sp.; *Neocosmospora vesinfecta* E. F. Smith;

Fungi Imperfecti; *Alternia tenuis* Nees; *Alternaria humicola* Oudemans; *Aspergillus candidus* Link; *A. flavus* Link; *A. flavipes* Bainer and Sartory; *A. fumigatus* Fresenius; *A. humicola* Chaudhuri; *A. niger* Van Tieghem; *A. nidulans* (Eidams) Winter; *A. ochraceous* Wilhelm; *A. sachari* Chaudhuri; *A. terreus* Thom; *A. tamarii* Kita; *A. ustus* (Banier) Thom and Church; *Botrydiplodia* sp.; *Botryotrichum* sp; *Cladosporium herbarium* (Persoon) Link; *Cladosporium* sp; *Curvularia lunata* Walker (Boedijn); *C. pallescens* Boedijn; *Dactylium fusaroides* Frag and Ciff; *Fusarium culmorum* (W. G. Smith) Saccardo; *F. dimerum* Penzig; *F. semitectum* Berkeley and Revenel; *F. solani* (Martius) Appel, Wollenweber; *Fusarium* sp. (three species); *Geliocladium pencilloides* Corda; *Helminthosporium anomalum* Gilman and Abbott; *H. nodulosum* (Berkeley and Curtis) Saccardo; *H. sativum* Pammel, King and Bakke; *Humicola brevis* (Gilman and Abbott) Gilman; *Monilia stophila* (Montagne) Saccardo; *Monosporium* sp.; *Penicillium chrysogenum* Thom; *P. notatum* Westling; *P. oxalicum* Thom; *P. oxyalicum* Currie and Thom; *P. variabile* Sopp; *P. expansum* (Link) Thom; *Paecilomyces fusisporus* Saksena; *Spicaria fusispora* Saksena; *Trichoderma lignorum* (Tode) Harz; *T. glaucum* (Abbott); *T. viride* Pers. ex Fr.; *Torula alli* (Harz) Saccardo; *Verticillium latritium* Berkeley.

Microascus, *Dactylium*, *Monosporium*, *Spiceria*, *Verticillium* and *Penicillium variabile* are reported for the first time from West Pakistan while *Paecilomyces*, *Botrydiplodia*, *Botryotrichum*, *Geliocladium*, *Humicola*, *Monilia*, *Torula*, *Neocosmospora vesinfecta*, *Aspergillus humicola*, *A. nidulans*, *A. sachari*, *A. tamarii*, and *Penicillium oxyalicum* are the first reports for Karachi.

Frequency of occurrence of isolated organisms differed in each sample and at different depths. A gradual reduction in the number of different colonies was observed as the depth increases. At surface levels the frequency of fungal population was high and 21 different species were isolated. As the depth increases the frequency shows a gradual fall and finally at 24-inch depth only 11 different species were isolated. From surface downward upto 8-inch depth the fall is rapid, but then it levels of at higher depths (Fig. 1).

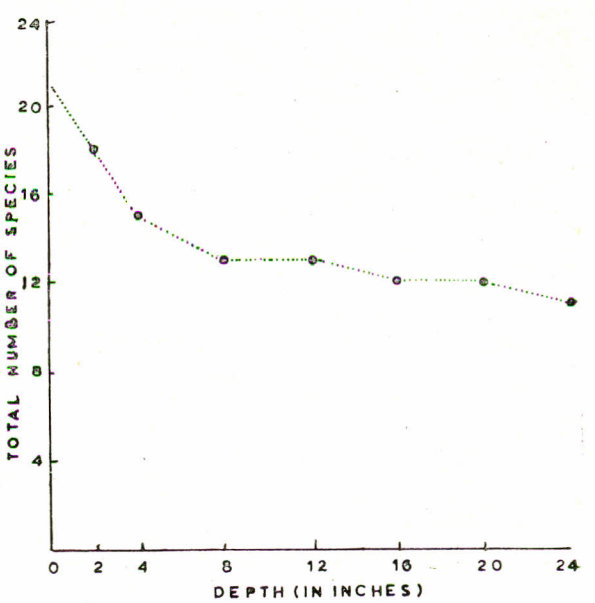

Fig. 1.—Frequency of occurrence of different fungi at various soil depths.

Discussion

The nature of soil, ecological conditions and habitat of plants growing near the soil was more or less the same. The soil was mostly barren, uncultivated and certain Angiosperms were growing in the vicinity. More common among them were *Prosopis spicigera* Linn., *Suaeda fruticosa* Forssk, *Suaeda monoica* Forssk, *Salsola foetida* Del., *Atriplex* Linn., and *Cassia* Linn.

The members of mucorales especially *Rhizopus* and *Mucor* were found at surface upto 4-inch depth but *Cunninghemella* was isolated only from 2-4-inch depths. Two species of *Cunninghemella* were isolated, one having spiny conidia, *C. echinulata*, while the other without spines at any stage of development.

Aspergillus niger, *A. terreus* and *A. flavus* were isolated from all samples and mostly at all depths, however the frequency of distribution of *A. niger* was higher than the other two. *A. ustus*, *A. nidulans*, *A. flavipes*, *A. candidus* and *A. fumigatus* were occasionally isolated from most of the soil samples and mainly from surface to middle levels of soil. *Aspergillus tamarii*, *A. ochraceous* and *A. humicola* were rarely isolated.

The distribution of *Penicillium* was significantly variable in all samples. No *Penicillium* was found at 24-inch depth. It is worth noticing that *Fusarium* was absent where *Penicillium* was found. This absence of *Fusarium* may be due to some antagonistic effects of *P. notatum* which is known for producing antibiotics. *Penicillium variabile* was reported for the first time from West Pakistan and was isolated from second and fourth samples (Mohd. Ali Housing Society and Malir respectively) at 2-inch depth. *P. notatum* was not as frequent as *P. expansum*.

Fusarium was found almost at all depths and in all samples. Its presence in all samples may be due to its resistant nature and tolerence to different conditions such as moisture, pH, temperature and parasitic as well as saprophytic mode of life.

Paecilomyces fusisporus was found ten times in third and fourth samples (Air Port and Malir respectively) at 2, 4, 8, 20 and 24 inch depths. *Spicaria fusispora* was isolated twice in fourth sample (Malir) at 24-inch depth. Gilman[12] advocates *Spicaria* a synonym of *Paecilomyces* while Burnett[13] gives an independent position to *Paecilomyces* equal to the status of *Spicaria* and *Penicillium*. Raper and Thom[14] show some affinity between *Penicillium* and *Paecilomyces*.

Trichoderma viride, *T. lignorum* and *T. glaucum* were rare and isolated from second and fourth sample (Mohd. Ali Housing Society and Malir respectively) at surface and 2-inch depth. *Humicola brevis*, *Botryotrichum* sp. and *Torula alli* were occasionally isolated from second, third and fourth samples. *Cladosporium*, *Geliocladium Penicilloides* and *Monilia stophila* were rarely isolated from second, fourth, fifth and sixth samples at 4-inches and middle levels of the soil. *Curvularia* was found only at surface and 2-inch depth while *Helminthosporium* was isolated from surface and lower depths of soil and not from middle levels.

Only one representative, *Botrydiplodia* of Sphaeropsidales was isolated twice from third and fourth samples at 24-inch depth among all the samples. *Monosporium* sp., *Verticillium latritium* and *Dactylium fusarioides* were reported for the first time from West Pakistan. Their prevalence was rare and only 1-2 colonies were isolated from fifth, sixth and second samples at above levels of soil. Only two perithecial fungi (Ascomycetes) were isolated from the total samples collected from Karachi. *Microascus* sp., a new record from West Pakistan was isolated from fourth sample (Malir) at surface soil. The fruiting bodies of this fungus consisted of a carbonaceous, globose and long neck perithecia. *Neocosmospora vesinfecta*, a common perithecial fungus of soil was isolated from second, third and fourth samples at 2-inch depth. Its degree of prevalence was high in third sample.

The distribution of fungal colonies at different depths depends upon many factors. These factors individually or collectively affect the growth and distribution of fungi in the soil. Spores and conidia are easily dispersed at surface levels and therefore, majority of genera and species isolated in all samples were found on surface soil. There is gradual decline in the number of organisms isolated at lower depths. This decline may be due to the difference in pH at various depths. The physical and chemical nature of the soil also plays an important role in the distribution of fungi. Since there is more perculation of water at surface levels than at lower depths, more organisms were found on the surface. At lower levels lack of aeration and antagonistic effects of soil bacteria and Actinomycetes reduce the frequency of fungal colonies and hence fewer number of fungi were isolated at lower depths than at surface and upper depths of soil.

Acknowledgements.—The authors wish to thank Dr. Salimuzzaman Siddiqui, F.R.S., Chairman, Pakistan Council of Scientific and Industrial Research, for his interest and encouragement in the progress of this work. Thanks are also extended to Commonwealth Mycological Institute, Kew Gardens, Surrey, England, for identifying and confirming some of our isolates.

References

1. E. J. Butler, Mem. Dept. Agr., India, Bot. Ser., 1, No. 5 (1907).
2. Thakur and Norris, Indian Inst. Sci., Golden Jubilee Res. vol., **11A,** 141 (1928).
3. E. W. Mason, Bureau of Mycol., List II (1928).
4. H. Chaudhuri and G. S. Sacher, Ann. Mycol., 90 (1934).
5. E. J. Butler and G. R. Bisby, Imp. Coun. Agri. Res., India Sci. Mono., I, Calcutta, **18,** 237 (1931).
6. B. B. Mundkur and Sultan Ahmed, Imp. Mycol. Inst., Mycol. papers, **18,** 1-11 (1946).
7. L. D. Galloway, Indian J. Agri. Sci., **6,** 13 (1936).
8. H. Chand, Proc. Ind. Acad. Sci., Sect. B, **5,** 324 (1937).
9. Sultan Ahmed, *Fungi of West Pakistan* (Published by the Society at Biological Laboratories, Govt. College, Lahore, 1956); Monograph I. First Edition.
10. S. A. Lodhi and F. Mirza, *Some Fungi of Lahore Soils* (Botany. Department, Panjab University, Lahore, 1961).
11. J. H. Warcup, Nature, London, **166,** 117 (1950).
12. J. G. Gilman, *The Manual of Soil Fungi* (The Collegiate Press Inc. Ames. Iowa, U.S.A., 1945), pp. 392.
13. H. L. Barnett, *Illustrated Genera of Imperfect Fungi* (Burgess Publishing Company Minneapolis, Minnesota, 1960), p. 225.
14. K. B. Raper and C. Thom, *Manual of Penicillia* (The William and Wilkins Co., Baltimore Md., 1949), p. 875.