

A PRELIMINARY SURVEY OF AIR-BORNE FUNGUS FLORA IN THE VICINITY OF CENTRAL LABORATORIES, PAKISTAN COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, KARACHI

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A preliminary survey of air-borne mould flora in the vicinity of Central Laboratories, P. C. S. I. R., Karachi was conducted. Petri plates were exposed against the air current at 5 ft to 25 ft from the ground level. Thirty-two species, belonging to fifteen genera, namely *Acrospiera*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Cunninghamella*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Malustela*, *Paecilomyces*, *Penicillium*, *Rhinoctadiella*, *Scopulariopsis*, and *Streptomyces*, are reported.

A preliminary study of aerial-mycology was undertaken, partly because the mycoflora of West Pakistan is very poorly known, particularly from the atmospheric source. Up to 1947 it consisted of approximately 200 species. Most of these species were recorded by Butler (1931) and Mundkur (1946). In 1956, Sultan Ahmed published, "Fungi of West Pakistan" and added most of the 1000 new records since 1938. From 1956 onward he has been publishing a major part of the new records from West Pakistan and thus the number has increased to approximately 1500 species. Even this number is very low for an area of over 300,000 sq miles. It is considered necessary that a more detailed survey of the mycoflora of West Pakistan should be conducted. To achieve this objective, it is desired that the mycologists working in different parts of West Pakistan should contribute by reporting more and more fungi from their respective areas. If this practice continues, it would be possible to increase our knowledge about the fungi of this region and gradually accumulate a very large number of these organisms which do exist but have never been reported. By doing this, it would be possible to provide cultures required by our scientists in connection with different types of research problems, such as deterioration, fermentation, metabolic products, fungicides evaluation, and thus partly obviate the financial difficulties in procuring cultures from foreign sources.

Materials and Methods

The exposure experiments for tapping the atmospheric fungal spores were conducted from 15th May to 23rd July, 1966. Czapek's medium with Rose Bengal (1:30,000) was employed throughout. This medium was found suitable for screening fungi from air and restricting the growth of fast growing moulds and thus minimising the chances of overlapping. Small petri plates (9.5 cm dia) were exposed in tripli-

cates against the air current at different heights, 5 ft to 25 ft from the ground level. Each exposure was carried against the air current for about 5 minutes. The exposed petri plates were incubated at room temperature and the fungi identified and isolated in pure cultures. A total number of thirty-two species belonging to fifteen genera, namely: *Acrospiera*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Cunninghamella*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Malustela*, *Paecilomyces*, *Penicillium*, *Rhinoctadiella*, *Scopulariopsis*, and *Streptomyces* are reported. A brief description of twenty-four species based on our observations is provided. The purpose of such descriptions is to help the local mycologists (in the presence of actue paucity of literature) in diagnosing some of the common fungi from atmosphere.

The fungi for which a description has not been provided are : *Acrospiera fluctuata*, *Aspergillus charlesii*, *Cunninghamella bainieri*, *Helminthosporium australiense*, *H. hawaiiense*, *Melustella aerea*, *Rhinoctadiella* sp. and *Streptomyces* sp.

Description of Fungi

1. *Alternaria tenuis* Nees.—Conioidiophores short, olivaceous brown. Conidia in chains, muriform, with three to five cross walls, constrictions broad at the septa, olivaceous brown, opaque, varying in shape and size, usually ranging from $30-36 \times 14-15 \mu$.

2. *Aspergillus flavus* Link.—Colonies widely spreading, ranging from shades of yellow to citron-green or lime-green to mignorette-green. Reverse of the colony colourless to yellow or buff. Conioidiophores up to 1000 μ long and 15 μ in diameter, broadening upward, pitted, gradually enlarging upward to form a vesicle, up to 40 μ in diameter. Heads in every colony vary from small with a few chains of conidia to large, columnar masses or both. Larger heads partly with simple

phialides, partly with branched or double series, or both in the same head; primary phialides $7-10 \times 3-4 \mu$; secondary $7-10 \times 2.5-3.5 \mu$. Conidia pyriform to globose, colourless to yellow-green, usually roughened, $2-5 \times 3-6 \mu$ in diameter. Sclerotia usually seen; cleistothecia not found.

3. *Aspergillus fumigatus* Fresenius.—Colonies usually velvety, fast spreading, aerial mycelium commonly found in the old cultures, bluish-green, becoming almost black in age; reverse and substratum colourless to yellow. Conidiophores short, densely crowded, upto 300μ in length and $2-8 \mu$ in diameter, gradually enlarged upward with an apical, flask-shaped vesicle upto 30μ in diameter, usually fertile only in the upper half, bearing phialides in one series, $6-8 \times 2-3 \mu$, closely crowded. Conidial chains upto $400 \times 50 \mu$. Conidia dark-green in mass, globose, $2-3.5 \times 2.5-3 \mu$.

4. *Aspergillus niger* Van Tieghem.—Colonies rapidly growing with submerged mycelium. Reverse usually colourless. Conidiophores arising directly from the substratum, smooth, $200-400 \times 7-10 \mu$. Conidial heads usually carbonous black to brownish-black, occasionally purple brown or any other shade in between, from small columnar masses to the more common globose or radiate heads, up to 500μ long, vesicle globose, upto 100μ in diameter; primary and secondary phialides present, primary varying greatly in length, secondary $6-10 \times 2-3 \mu$. Conidia smooth but later spinulose, $2.5-4$ or upto 5μ in diameter. Superficial sclerotia produced in abundance.

5. *Aspergillus niveus* Blechwitz.—Colonies on Czapek's agar white, rather slow growing, forming a dense mat of mycelium with conidiophores, plane or radially furrowed, usually producing amber to brown exudate; reverse in dark yellow shades through greenish to brownish-black; Conidiophores colourless, smooth, slender, $4-6 \mu$ in diameter, enlarging into a hemispherical vesicle, chains of conidia in comparatively loose columns, most frequently $20-30 \mu$ in diameter, but in large heads up to 60μ . Phialides in two series, primary $5-8 \times 2.5-3 \mu$, secondary phialides $5-7 \times 2-2.5 \mu$. Conidia colourless, thin-walled, smooth, $2-2.5 \mu$ in diameter.

6. *Aspergillus ochraceus* Wilhelm.—Colonies in Czapek's medium ochraceous. Conidiophores rough or pitted, yellow, bearing large, radiate conidial heads. Vesicles globose, $60-75 \mu$ in diameter; phialides in two series, primary $15-30 \mu$ long, secondary $7-10 \times 1.5-2 \mu$. Conidia globose to elliptical, smooth to delicately spinulose, $3.5-5 \mu$ in diameter. Sclerotia usually present.

7. *Aspergillus quadrilineatus* Thom & Raper.—Colonies on Czapek's agar fast growing, central area gray with a pinkish tinge, olive-green conidial areas towards the margin. Reverse purplish red. Cleistothecia developing abundantly throughout, light brown, enveloped by hulle cells, $125-150 \mu$ in diameter. Ascospores purple-red, lenticular, smooth, $4-4.8 \times 3.4-3.8 \mu$ with two equatorial crests paralleled by a narrow secondary pair which is indistinct. Conidial heads short, columnar, green, $60-70 \mu$, stalks smooth-walled, slightly brownish, $50-75 \times 3.5-4.5 \mu$; vesicle $7.5-9 \mu$ in diameter. Phialides in two series, primary $5-6 \times 2-3 \mu$, secondary $5-7 \times 2-2.5 \mu$. Conidiospores globose, pale yellow-green, rugulose, $3-4 \mu$ in diameter.

8. *Aspergillus rugulosus* Thom & Raper.—Colonies on Czapek's agar slowly growing, buckled or wrinkled. Cleistothecia formed abundantly at different depths, purple-gray to purple-brown in age, with green conidial heads sparsely produced; reverse in shades of deep purple-red. Each cleistothecium surrounded by hyphae and dark brown hulle cells, globose, $225-350 \mu$ in diameter including mycelium covering, with dark reddish-purple walls; ascospores purplish red, lenticular, walls conspicuously rugulose, $4-4.4 \times 3.6-3.8 \mu$, with two plated equatorial crests, $0.5-0.6 \mu$ in width. Conidial heads short, columnar, $75-100 \times 30-40 \mu$, stalks sinuous, smooth, pale brownish, $50-80 \times 5 \mu$, enlarging into vesicles, $8-10 \mu$ in diameter. Phialides in two series, primary phialides $7-8 \times 3-3.5 \mu$, secondary phialides $6-7 \times 2.5-3 \mu$. Conidia globose, green, rugulose, $3-4 \mu$ in diameter.

9. *Aspergillus terreus* Thom.—Colonies on Czapek's agar from pinkish-cinnamon to deeper brown shades in age, spreading, velvety. Reverse and agar from pale or bright yellow to fairly deep brown. Conidiophores $150-250 \times 5-8 \mu$, more or less flexuous, smooth, with apex enlarged to form a vesicle up to 25μ in diameter; phialides in two series, primary phialides $7-9 \times 2-2.5 \mu$, secondary $5-7 \times 2-2.5 \mu$, closely packed. Heads becoming solid columnar masses up to $500 \mu \times 50 \mu$ in diameter. Conidia elliptical to globose, $2.2-3 \mu$ in diameter, smooth.

10. *Aspergillus sydowi* (Bainier and Sartary) Thom & Church.—Colonies on Czapek's agar blue-green velvety with some aerial hyphae. Reverse and substratum orange to red, later on becoming almost black. Conidiophores mostly arise from submerged hyphae, up to $500 \times 4-8 \mu$, colourless, smooth, thick-walled. Heads radiate or globose; vesicles $12-20 \mu$ in diameter. Phialides in two series; primary up to $7 \times 2-3 \mu$, second-

dary $7-10 \times 2 \mu$. Conidia globose, $2.5-3.5 \mu$, spinulose. No sclerotia or cleistothecia found.

11. *Aspergillus ustus* (Bainier) Thom and Church.—Colonies from white through shades of grey, towards fuscous, with often a greenish cast, but no true green colour; in old cultures purplish, vinaceous at times. Reverse through shades of yellow, orange and brown. Stalks few, septate, sinuous, smooth; vesicles $10-20 \mu$ in diameter; heads hemispherical to almost columnar; phialides colourless, semi-radiate, loosely arranged in two series, primary $5-8 \times 3 \mu$, secondary $7-9 \times 2-2.5 \mu$. Conidia globose, $3.5-4 \mu$, spinulose or with fine, faint bars of rosy, reddish-yellow or vinaceous colour with chains forming fairly compact columns in old cultures. Cleistothecia have not been found; hulle cells present.

12. *Chaetomium aureum* Chivers.—Grey or pale olive, becoming yellow to golden yellow. Perithecia globose to subglobose, often bluntly pointed at the base, small $120-150 \times 100-135 \mu$, without differentiated rhizoids, bearing long slender, arched or recurved cirrus. Terminal hairs straight to slightly recurved, at tip nearly straight or incurved, regularly septate, minutely roughened and in colour ranging from grey to golden yellow, lateral hairs slender, straight or flexed, regularly and distinctly septate, minutely roughened, 3.5μ in thickness, broadly arched at tips, colour grey to olive-yellow. Asci club-shaped, 8-spored, $40 \times 10 \mu$, ascospores when mature olive-brown, irregularly ovate, apiculate at both ends, $9.4-11 \times 4.5-5.6 \mu$.

13. *Caladosporium sphaerospermum* Penz.—Erect or creeping, branched and septate hyphae on the surface as well as inside the substrate, brown in colour. Conidiophores erect, branched, septate, frequently aggregated together, forming a velvety layer, brown in colour, $140-310 \mu$ long and $3.5-4.5 \mu$ broad; conidia borne at tip or laterally near tip, arising in chains, round or oval in shape, rarely uniseptate, size variable, smaller ones $3-3.4 \times 4-4.5 \mu$, bigger ones $6.5-14 \times 3.5-4.5 \mu$, hyaline or olive coloured, smooth walled.

14. *Cunninghamella echinulata* Thaxter.—Turf white, becoming yellowish with age; filaments interwoven. Conidiophores erect, branching not regular and definite. Terminal vesicles very variable in size, nearly spherical to obovoid, up to 65μ maximum; lateral branches similar to terminal but smaller. All conidia oval to elliptical, finely echinulate, up to 25μ .

15. *Curvularia siddiqui*, Ahmad and Quraishi.—Colony when grown on Czapek's agar grows

fast and is buff coloured in the beginning, changing to olive in the absence of conidial formation and finally dark grey or black when conidia are formed. Older colonies are covered with greyish mycelium which spreads on the surface. Mycelium with profuse branching, the branches closely interwoven and twisted, forming a tough leathery layer on the surface of the medium. Hyphal diameter $3.44-7 \mu$, chlamydospores frequently seen in cultures, stroma not seen. Conidiophores multiseptate and of two types; one short and unbranched, $34-172 \mu$, the cell at the tip wider and club-shaped, $5.6-6.88 \mu$ in diameter, bearing a solitary conidium, the other type of conidiophores are long and dichotomously branched with short alternate bends, bearing a conidium at each bend, the tip of the cell bearing conidium is very variable, $206-413 \mu$, or more, the width is $5.160-7.74 \mu$. Conidia four-celled, slightly curved, second and third cell larger and darker than others. the size of the conidium is $20-38 \times 10.5-17.2 \mu$.

16. *Curvularia spicifera* (Bainier) Boedijn.—The conidiophores are brown, simple, unbranched, septate with geniculate markings at the tips, $2.6-5.2 \mu$ in width. Conidia borne at the tips of the conidiophores, brown, ellipsoidal, mostly straight, seldom curved, septa usually 3, occasionally 5-6 septate; middle cells broadest and darkest, while the upper and lower cells paler and narrower, $18.2-44.2 \mu \times 7.8-10.4 \mu$.

17. *Curvularia verruculosa* Tandon & Bilgrami.—Conidiophores pallid brown, erect to curved, geniculate at the apex, variable in length, $3-4.2 \mu$ broad. Conidia curved, 3 septate, septa transverse, conidia distinctly rough and verruculose, apical and basal cells as well as the third cell from the base colourless. Conidiophores measure $24-26 \times 10.5-13 \mu$.

18. *Fusarium solani* (Martius) Appel and Wollenweber.—Conidia scattered in false heads, brownish white to loam-yellow or from greenish to dark brown. Larger conidia strongly twisted, spindle-shaped, slightly curved, both ends rounded to acute, rarely pedicellate, conidia usually 3-5 septate, occasionally 0-2 septate; O-septate: $8.5-14.8 \times 4.2-4.4$; I-septate: $20.1-21.2 \times 4.7-5.3$; 2-septate: (occasional) 29.7×4.9 ; 3-septate: $27.6-33.9 \times 4.5-5.9$; 5-septate: (occasional) $31.8-44.5 \times 5.3-6.2 \mu$. Chlamydospores terminal and intercalary.

19. *Fusarium semitectum* Berkeley and Ravenel.—Aerial mycelium more or less white; the lower part of the stroma usually bright brown, rarely violet carmine. Chlamydospores intercalary; Sporodochia lacking. Conidia scat-

tered on aerial mycelium, spindle to sickle-shaped, not pedicellate, often with an attachment-wart at the base. Smaller conidia none to two septate, larger three septate, seldom four to five septate, occasionally six or seven septate: O-Septate, $4-16 \times 2-4 \mu$, 1-septate, $8-12 \times 2-4.5 \mu$, 3-septate, $23-50 \times 3.2-6.25 \mu$, 7-septate, $36-77 \times 4-6 \mu$.

20. *Penicillium citrinum* Thom.—Colonies bluish-green to clear green, becoming olive to brownish-olive, reverse yellow. Aerial part of colony consists of densely standing conidiophores except in the centre, where tufts of aerial hyphae arise. Conidiophores usually up to 150μ in length. Metulae $16-30 \times 3 \mu$, each producing a compact verticil of phialides, $6-7 \times 2-3 \mu$. Conidial chains in columns, a separate column arising from verticil of cells, so that the fructification may appear double, triple or more complex. Conidia globose, $2.4-3.5 \mu$ green, slightly granular.

21. *Penicillium charlesii* Smith.—Colonies rather restricted, strongly buckled and wrinkled with central area depressed, with thin basal felt, close textured, more or less velvety, in dull-green, lilly-green, becoming olive-grey in age with thin margin, reverse in dull greenish shades. Conidiophores arising from closely interwoven aerial hyphae with conidial chains forming a compact column, 150μ long; conidiophores variable in length, $20-25 \mu$ in diameter with enlarged tip, $4-5 \mu$ in diameter, walls smooth. Phialides in compact clusters, ten to twelve in a verticil, $7.5-9 \times 2.2-2.5 \mu$, conidia ovate to slightly elliptical, roughened, $2.5-3 \times 2-2.5 \mu$, dark green in mass.

22. *Penicillium janthenillum* Biourge.—Colonies bluish-green or bright green, azonate, with surface growth consisting of net works, of hyphae and ropes of hyphae, becoming reddish, reverse yellow to ochraceous; conidiophores $30-40 \times 2 \mu$, arising from creeping hyphae with smooth walls. Metulae $7-10 \times 1.5-2 \mu$, mostly in threes; phialides $5.5-9 \times 1.5-2 \mu$, in pairs or threes, conidia globose, $2.4-3 \mu$.

23. *Scopulariopsis brevicaulis* Bainier.—Colonies white at first, then yellowish-brown or chocolate, consisting of short, closely crowded conidiophores making powdery-areas overgrown by loose, trailing floccose hyphae and ropes of hyphae, with broadly spreading, indeterminate margin. Conidiophores short, $10-30 \mu$ long, conidial fructifications either simple chains terminating in unbranched or sparingly branched conidiophores, $12-15 \times 4 \mu$, tapering at the apex. Conidia somewhat pear-shaped, slightly tuberculate at the apex, with broad base, $7.5-10.5 \times 7.5-9 \mu$,

in mass light brown to chocolate, smooth at first then with thick tuberculate walls.

24. *Paecilomyces varioti* Bainier.—Colonies on Czapek's agar slow growing, felty, with scattered, floccose aerial mycelium; surface olive to brownish-olive, reverse colourless. Conidiophores freely and irregularly branched, conidiophores up to 325μ long. The conidial fructification is typically in two stages, the branches of the conidiophore bearing a terminal verticil of divergent metulae, with divergent phialides. Metulae extremely variable in length, phialides $10-25 \times 2.5-4 \mu$. Conidial chains very long, divergent, seldom more than five or six in a head. Conidia elliptical, smooth, $4.5-6 \times 2.5-4 \mu$.

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