# A PRELIMINARY SURVEY OF ASPERGILLUS SPECIES FROM KARACHI (WEST PAKISTAN)

S. IFTIKHAR AHMED & YAZDANA MIRZA RIZKI
*Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi.*

**Abstract**

*A preliminary survey of* Aspergillus *species from Karachi was undertaken. The sources from which these were isolated consist of soil, air, spoiled fruits and leather products. Malt extract agar and Czapek-Dox agar with Rose Bengal* (1: 30,000) *were utilized for culturing these fungi.*

*A total of* 23 *species were recorded. Out of these,* Aspergillus *near* flavo-furcatis, A. puniceus, A. unguis *and* A. variecolor *have been reported for the first time from Karachi. The species not reported from West Pakistan as such are* A. *near* flavo-furcatis, A. puniceus *and* A. variecolor. *Brief descriptions of only these three species have been provided.*

*It has been ascertained that out of a total of* 49 *species of the genus* Aspergillus *reported by the previous workers, only* 23, *beyond doubt, are the accepted ones. The remaining* 16 *species include synonyms, probable synonyms and non-recognisable species. These cases have been discussed.*

It was not before the middle of nineteenth century that the species of *Aspergillus* were recognised as the organisms for decay of innumerable substances, as the causes for human and animal diseases and as the agents capable of producing industrially valuable metabolic products. In addition to several other substances produced by the species of *Aspergilli*, citric acid, oxalic acid and itaconic acids are of considerable importance. The initiation of the present work on the genus *Aspergillus* is based mainly on two considerations: 1. Pakistan is industrially underdeveloped and so one may anticipate that these organisms would be required for the industries to be developed for metabolic products. 2. The species of *Aspergillus* are very poorly represented from West Pakistan and, therefore, the most important effort would be to build up a more comprehensive picture of the genus in this part of the world.

The mycologists who reported *Aspergillus* species from the part now known as West Pakistan include Thaukur and Norris (1928), Mahju (1933),

Chaudhuri and Sachar (1934), Chaudhuri and Umar (1935), Galloway (1936), Hukam Chand (1937), Chaudhuri and Umar (1938), Sultan Ahmad (1956), Ahmed and Quraishi (1960), Ahmed, Quraishi and Murtuza (1960), Mirza and Nasir (1965), Quraishi (1966), Mirza and Husain (1966), Husain Hasany and Ahmed (1966), Rizvi (1966), Ahmed (1967), Husain, Hasany and Ahmed (1967), Nishat, Ahmedunnisa and Ahmed (1968) and Ahmedunnisa, Ahmed and Nishat (1968).

The *Aspergillus* species included in these reports are: *Aspergillus aculeatus* Ilzuka, *A. albicans* de Mello & Vas, *A. amstelodami* (Mang.) Thom & Church, *A. aguiri* de Mello & Vas, *A. asperescens* Stolk, *A. awamori* Nakazawa, *A. calyptratus* Oudemans, *A. candidus* Link, *A. carneus* (Van Tiegh.) Blochwitz, *A.* (st.) *castanea* Patterson, *A. chevalieri* (Mang.) Thom & Church, *A. corolligena* Massee, *A. ficuum* (Reich.) Hennings, *A. flavipes* (Bain & Sart.) Thom and Church, *A. flavus* Link, *A. fumigatus* Fresenius, *A. fumigatus* var. *tumescens* Blumentritt, *A. fuscus* Amons, *A. herbariorum* Wigger, *A. humicola* Chaudhuri and Sachar, *A. japonicus* Saito, *A. luchuensis* Inui, *A. nidulans* (Eidam) Wint., *A. niger* Van Tiegh., A. *niveus* Blochwitz, *A. ochraceus* Wilhelm, *A. oryzae* (Ahlb.) Cohn, *A. ortae* de Mello, *A. phaeocephalus* Durien & Montagne, *A. polychromus* de Mello, *A. quadrilineatus* Thom and Raper, *A. repens* (Corda) de Bary & Woronin, *A. restrictus* Smith, *A. ruber* Thom & Church, *A. rugulosus* Thom & Raper, *A. sachari* Chaudhuri and Sachar, *A. sclerotiorum* Ruber, *A. stellatus* Curzi, *A. sulphureus* (Fr.) Thom and Church, *A. sydowi* (Bain. & Sart.) Thom and Church, *A. tamarii* Kita, *A. terreus* Thom, *A. unguis* (Emile-Weil & Gaud.) Thom and Raper, *A. ustilago* Beck, *A. ustus* (Bain.) Thom and Church, *A. versicolor* (Vuill.) Tiraboschi, *A. violaceo-fuscus* Gasperini, *A. wentii* Wehmer and *A. zonatus* Kwon and Fennell.

Most of the above species were repeatedly reported by different mycologists of West Pakistan. A thorough scrutiny of these reports indicated that out of these 49 species, 33, beyond doubt, were the accepted ones. The remaining included synonyms, probable synonyms and non-recognisable species. These cases have been included in the discussion portion. In the present work a total of 23 species of *Aspergillus* have been recorded. The species not reported from West Pakistan, include as such, *A. puniceus*, *A. variecolor* and *A.* near *flavo-furcatis*.

**Materials and Methods**

*Aspergilli* for the present studies were isolated from diverse sources: soil, air, deteriorated fruits and leather goods. The depth at which soil samples were collected ranged from 1 to 5 inches. One g of each sample was mixed thoroughly with 10 ml of sterilised water, one ml of this mixture was poured into four sterilized Petri plates and 0.1 of the same mixture in another set of four similar

plates. Malt agar medium was used in two Petri plates from each set and Czapek-Dox agar (with Rose Bengal in a proportion of 1 : 30,000) in the remaining dishes. For uniform mixing all the plates were gently rotated. Spores were collected by exposing the Petri plates for 4-5 min against the air current at a height of 5-10 ft from the ground level.

Incubation was carried out at a temperature of 28-30 C. After 3 to 4 days the Petri plates were examined under the dissecting microscope for *Aspergillus*-like colonies. For obtaining pure cultures, these were picked up and inoculated on Czapek's agar slants. Detailed studies of micro-structures were carried out after a week. For this purpose, a small portion of the culture was taken with the help of a sterilised needle, washed in a drop of 75% methyl alcohol on a slide and mounted in Amman's solution by Raper and Fennell (1956). Based on our studies, brief descriptions of *A.* near *flavo-furcatis*, *A. puniceus* and *A. variecolor*, which were not reported as such from West Pakistan have been provided. All measurements are given in $\mu$.

**Observations**

A total of 23 species, namely: *Aspergillus amstelodami*, *A. awamori*, *A. candidus*, *A. chevalieri*, *A.* near *flavo-furcatis*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. ochraceus*, *A. puniceus*, *A. restrictus*, *A. rugulosus*, *A. sclerotiorum*, *A. sulphureus*, *A. sydowi*, *A. tamarii*, *A. terreus*, *A. unguis*, *A. ustus*, *A. variecolor*, *A. versicolor* and *A. wentii* were recorded by us from Karachi. Out of these, *A.* near *flavo-furcatis*, *A. puniceus*, *A. unguis* and *A. variecolor* have been reported for the first time from Karachi. The species not reported from West Pakistan as such are *A.* near *flavo-furcatis*, *A. puniceus* and *A. variecolor*. Brief descriptions of only these three species have been provided in the following.

1. *Aspergillus* near *flavo-furcatis* Batista & Maia: Colonies greenish yellow at first, finally becoming olive-brown, margins thin, somewhat irregular; exudate inconspicuous, almost colourless in the beginning, finally becoming yellowish brown, cleistothecia, sclerotia and hulle cells not observed; conidiophores short, erect, slightly roughened, faintly yellowish pink in water mounts; conidial heads of two types - large and small, globose to loosely radiate, 300-600 in dia.; vesicle of the large heads flask-shaped, 20-40 in width, fertile over most of the surface, vesicles of the small heads flask-shaped to clavate, incompletely fertile; sterigmata 2-seriate in the large heads, 1-seriate in the small ones, primary 10-14 x 5-8 secondary 7.5-10 x 4.5-7; conidia elliptical to pyriform when young, finally sub-globose to globose, conspicuously tuberculate, 5 - 7.5 in dia.; garden soil of Central Laboratories, P.C.S.I.R., Karachi (IMI. No. 12590).

The above strain is interesting in the sense that though it resembles *A. flavo-furcatis* Batista & Maia and *A. tamarii* Kita in several respects, it does not agree completely with any of these two. It has more characters in common with *A. flavo-furcatis* as compared to *A. tamarii*. The points in which it differs from these two species are precisely mentioned in the following.

1. It does not agree well with any one of the two as regards the colour of the colony which is very clearly inclining towards yellow in the young cultures.

2. Its conidiophores are faintly yellowish pink whereas *A. flavo-furcatis* and *A. tamarii* possess colourless conidiophores.

3. The reverse in our strain is yellowish brown whereas in *A. tamarii* it is colourless to occasionally pinkish.

4. In our specimens sclerotia are not present whereas they could be present in *A. tamarii*.

2. *Aspergillus variecolor* (Berk. & Br.) Thom and Raper: Growth not rapid, attaining a dia. of 3-4 cm in 8 days on Czapek's agar at the room temperature, green heads produced in the centre of the colony, cleistothecia formed in the middle and the marginal portion of the colonies, reverse yellow, young colonies show purple colouration at the margin, which gradually darken with age; conidiophores arising from the basal felt, smooth, brownish, 140-200 x 3-5; conidial heads green, 8-10 in diam.; sterigmata in two series, primary 7-8 x 3-4 and secondary 8-9 x 2.5-3; conidia globose, rugulose, 2.5 - 3.5 in dia.; cleistothecia 300-400, surrounded by hyphae and hulle cells which are globose, about 30 in diam. soil, North Nazimabad, Karachi (IMI. No. 125914).

3. *Aspergillus puniceus* Kwan and Fennell: Colonies 4-5.5 cm in diam. at room temperature in about 7-8 days on Czapek's agar, at first creamy in shade, yellow later on and finally light pinkish in colour; reverse yellow to reddish brown, exudate reddish, conidiophores arise from the submerged mycelium and the trailing aerial hyphae, mostly 150-250 x 5-6, light brown in shade, wall thick and smooth; conidial heads hemispherical; vesicles globose to elliptical, 14-17 x 12.5-15, three-fourths of these elliptical vesicles are fertile; sterigmata double in series, primary 4-7 x 3-4, secondary 4-7 x 2.5; conidia globose, roughened, yellowish-green in shade, 2.5-3.5 in diam; hulle cells in masses, curved, irregular, elongated-garden soil, Nazimabad 4, Karachi (IMI. No. 130755).

**Discussion**

In the reports on *Aspergilli* from West Pakistan, 16 species are considered doubtful. These include synonyms, probable synonyms and non-recognisable

species. These have been discussed in the following. The numbers quoted within brackets refer to the page numbers of "The Genus *Aspergillus*" by Raper and Fennell (1965).

1. *Aspergillus aguiri* de Mello & Vas (p. 584). According to Mohanty (1948) this species is inadequately described and not identifiable.

2. *A. albieans* de Mello & Vas (p. 584). This species is also unidentifiable vide the same reference.

3. *A. castanae* Patterson (p. 330). According to Raper & Fennel (1965) this seems to be a strain of *A. niger* group with conidia of 3.5 - 4.0 but occasionally 4.5 dia.

4. *A. corolligena* Massee (p. 578). This species has never been described under this genus by Massee. He has described it under the genus *Sterigmatomyces*. Positive identification is impossible; the large and coarsely roughened conidia seem to place it more near to *A. flavus* than *A. sulphureus*.

5. *A. fumigatus* var. *tumescens* Blumentritt (p. 245). Raper & Fennell have expressed that the cultures described produced a slow growing, dense, black, pseudoparenchyma like dense felt of mycelium bearing conidia structures. These structures were commonly aberrant but did not differ significantly from measurements of *A. fumigatus*, and the variety is not recognisable.

6. *A. fuscus* Amons (p. 572). According to Raper & Fennell (1965) this species is a probable synonym of *A. terreus* as indicated by its description.

7. *A. humicila* Chaudhuri & Sachar (p. 547). This species was described from Panjab by Chaudhuri & Sachar (1934). It seems to appropriate *A. ustus* except for its smooth conidia. Since the roughening of conidia is a delicate character to observe, it is suspected that it might have escaped observation. On this ground *A. humicola* is considered a probable synonym of *A. ustus*.

8. *A. herbariorum* Wiggers. There is no such species of *Aspergillus* described by Wiggers. However, he is the authority for *Eurotium herbariorum* as well as *Mucor herbariorum*.

9. *A. luchuensis* Inui (pp. 317, 318). Inui described this black *Aspergillus* and the distributed material consisted of single and double sterigmata in the same head. It is difficult to differentiate it from *A. awamori*, and therefore, it is treated as a synonym by Raper & Fennell (1965).

10. *A. ortae* de Mello (p. 592). According to Mohanty (1948), this species is unidentifiable.

11. *A. phaeocephalus* Durieu & Montagne (p. 309). It is stated by Raper and Fennell (1965) to be a member of *A. niger* group, probably approximating *A. phoenicis*.

12. *A. polychromus* de Mello (p. 499). The information given in the description of this species suggests relationship with *A. nidulans* except the 4-spored ascus. Since no 4-spored species of *Aspergillus* has ever been reported, there is more likelihood of *A. polychromus* being the synonym of *A. nidulans*.

13. *A. sachari* Chaudhuri & Sachar (p. 273). This species was also described from Punjab by Chaudhuri & Sachar (1934). Raper and Fennell (1965) have placed it under the probable synonymy of *A. sclerotiorum*. *A. sachari* seems to differ from *A. sclerotiorum* only in possessing smooth conidia. It is likely that the roughenings might have escaped observation and therefore, *A. sachari* is considered as a probable synonymy of *A. sclerotiorum*.

14. *A. stellatus* Curzi (p. 514). According to Fennell & Raper (1965), this has now been treated under the synonymy of *A. variecolor*.

15. *A. ustilago* Beck (p. 307). This has now been treated under the synonymy of *A. ficuum*-Fennell & Raper (1965).

16. *A. violaceo-fuscus* Gasperini (p. 330). No black *Aspergillus* with globose conidia is known to possess single secondary sterigmata, and therefore, a misinterpretation by Gasperini is suspected. Since the other measurements of *A. violaceo-fuscus* agree reasonably well with *A. aculeatus*, the former is considered as a probable synonymy of the latter.

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