# SEVEN NEW RECORDS OF ASPERGILLUS SPECIES FROM LAHORE SOIL

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Six species and one variety of *Aspergillus* alongwith allied strains have been reported and described for the first time from three different soils of Lahore. Comparison with the type species is given.

## Introduction

During the last few decades mycologists have been taking interest in the exploration of soil microflora. Different workers have adopted different techniques for the isolation of fungi from soil. Butler [1] used hemp seeds floating on soil suspension as baits for isolating species of *Pythium* and related fungi from the soil. Waksman [2] used the method of direct inoculation of soil crumb. For the estimation of the number of soil-micro-organisms per gram of soil the dilution method is widely used. Waksman [3] and Rossi [4] introduced and Cholodny [5] modified the method of burried slide technique for studying soil microflora without isolating in pure culture. Chester [6] used immersion tubes for isolating fungi from soil. Warcup[7,8] devised the soil plate method, he also subjected the soil to partial sterilization by steam and thus eliminated fast growing fungi and succeeded in isolating the slow growing Ascomycetes. Thornton [9] used screened immersion plate which was a modification of Rossi-Cholodny's burried slide technique for the study of soil fungi.

Thavur and Norris [10] reported 22 species of fungi from Madras soil. Mason[11] reported 4 species of *Aspergillus* and one species of *Aerothecium* isolated from paddy soils of Sind and Burma. Chaudhuri and Sacher [12] mentioned 32 species isolated from Lahore soils. Galloway [13] in 1935 gave some generic descriptions of soil fungi collected from Pusa and hilly districts of North India.

The present investigation was undertaken to find out the species of fungi which are present in Lahore soil and to ascertain, if the depth of soil, soil conditions and seasonal variations have any influence on their distribution.

## Materials and Methods

Three soil types were selected for this study. Soil type "A" was taken from a richly manured potato field situated at Sanda Shamas Din; type "B" from a paddy field surrounded by Ichara main, Rehmanpura and Samanabad and type "C" was taken from an uncultivated saline plot situated opposite Wahdat Colony.

Soil samples were taken every fortnight over a six-month period beginning from first November. A 6″×6″ hole was manually dug to a depth of 24″ through 'Chuaramba' to expose soil profile and samples were removed from depths of 1″, 2″, 6″, 12″, 18″ and 24 inches in sterilized iron tubes (3″×1″) with lids separable at both ends. Inoculations were made on Czapecks Dox agar containing 5% yeast extract on the same day using soil plate method.[7] pH of this medium was adjusted, at the time of pouring plates, from 4.2 to 4.4 by adding 10% sterilized phosphoric acid. 0.5 to 0.75 grams of soil from iron tubes was placed in sterilized petridishes with preheated, spoon tip nichrome needle. Five ml. of sterilized distilled water was poured over this soil in dishes which were gently shaken to obtain a thin film of soil suspension. Thirty ml. of media (Czapecks Dox agar + .5% yeast extract) was poured over the soil suspension at a temperature of 50-55°C. to avoid fast growing members of Mucorales. The plates were marked for soil type, number of sample, depth and date of inoculation and were incubated at 28°C. for seven days. After this period different fungi appearing on the plates were isolated on (Czapecks Dox agar + .5% yeast extract) slants. These petridishes were further incubated for another seven days and the fungi which appeared at this stage of incubation were again isolated on the slants containing the same culture media. These slants were incubated for 5-14 days. The incubation period was adjusted according to the growth rate of particular fungus. When the fungus attained maturity, they were labelled and stored at 7°C. Purification of impure cultures was done by transfer and retransfer technique. When about 100 such cultures were

accumulated they were primarily sorted out, purely on the basis of texture, colour of upper and lower surfaces of the colonies, and presence of fruiting bodies and sclerotia. The frequency of occurrence of a particular fungus at a particular depth was also recorded and the results are shown in Fig. 1.

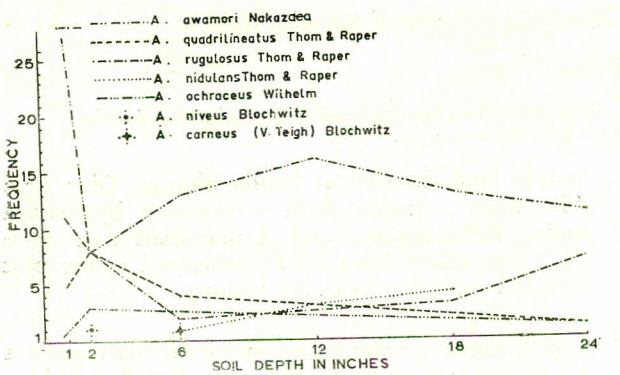


Fig. 1.—Graph showing the frequency of seven different species of *Aspergillus* at various soil depths.

All measurements were taken after washing the material in rectified spirit and hence the size of conidial heads indicates the size of the vesicle and sterigmata in one or two series. Colour of the conidial heads, vesicle and conidiophores is mentioned as seen under the low power microscope.

## Results and Discussion

Amongst the fungi isolated, *Aspergillus* Micheli was most frequent at a depth of 1″. Fifteen species, one variety and 28 different strains of *Aspergillus* have been identified and described during the course of this investigation. Seven of them are new records which are mentioned in this paper and are listed below.

1. *Aspergillus carneus* (V. Teigh.) Blochwitz
2. *Aspergillus nivens* Blochwitz
3. *Aspergillus awamori* Nakazaea
4. *Aspergillus ochraceus* Wilhelm
5. *Aspergillus nidulans* Var. latus. Thom and Raper
6. *Aspergillus rugulosus* Thom and Raper
7. *Aspergillus quadrilineatus* Thom and Raper

*Aspergillus Micheli* is a variable genus represented by a large number of species. Strains of some species are described and compared with type cultures.

## Key to the Groups of Aspergillus Based upon Colour and Morphology

a. Species not producing perithecia
  b. Conidial heads definitely in blue or yellow green shades
    c. Conidial heads in yellow green shades
      head radiate ..... *A. flavus*
    cc. Conidial heads in blue green shades
      d. Head compact columnar, vesicle flask shaped, colonies fast growing ..... *A. fumigatus*
      dd. Head radiate, pea green or sage green reverse in a shade of maroon. ..... *A. versicolor* (group)
  bb. Conidial heads lacking green colour
    c. Head on long compact columns in wood brown, pale buff, or light flesh colour ..... *A. terreus* (group)
    cc. Head radiate, sulphur yellow to ochre shades ..... *A. ochraceus*
      d. Head purple brown to black shades conidiophores upto 2300μ long ..... *A. niger* (group)
      dd. Head of some other colour
        e. Young colonies showing greenish colour passing to brown .. *A. tamarii*
        ee. Head white, colonies with pink tinge ..... *A. candidus*
aa. Species producing peritheia, ascospores purlpe red ..... *A. nidulans*

### Sub Key to the Species of Aspergillus Terreus Group

a Conidial heads in dull cinnamon shades ..... *A. terreus*
b Conidial heads dirty green in colour
  Colonies not brownish ..... *A. carneus*
  Colonies fasiculate in texture .... *A. niveus*

*Aspergillus terreus* Thom has already been reported from West Pakistan by Chaudhuri and Umar[14] from Lahore soil, therefore, it is not described in the present paper.

*Aspergillus carneus* (V. Teigh.) Blochwitz.—Colonies growing slow on Czapeck's solution agar at 35°C., velvety in texture with totally submerged mycelium, upper lafrance pink (Ridgeway Pl. 1)[15] and reverse mustard yellow (Ridgeway Pl. XVI). Heads dirty green, compact columnar, ranging from 20-28μ in diameter. Conidiophores light greenish, smooth, 3-5μ in diameter and ranging from 518-639μ in length, vesicle fertile on only upper ¾ portion, not perfectly globose, ranging from 10-13μ in diameter. Sterigmata strictly in two series, dirty green, primaries ranging from 6-8μ and secondaries from 8-10μ in length. Conidia brownish, globose, rough, ranging from 1.6-2μ in diameter. (Culture No. BR. 40).

This strain differs from type strain described in 'A Manual of Aspergilli' in the following characters.

| *Type Culture* | *Culture No. BR 40* |
|---|---|
| 1. Texture more or less Floccose | Perfect velvety in texture |
| 2. Colour Vinaceous Faun (Ridgeway Plate XL) | Colour Lafrance pink (Ridgeway Pl. XVI) |
| 3. Odour often putrid | Odourless |
| 4. Conidiophores 250-400μ in length | Conidiophores 518-639μ in length |
| 5. Vesicle 5.5-9μ in diameter | Vesicle 10-13.5μ in diameter |
| 6. Conidia 2.8-3.2μ in diameter | Conidia from 1.6-2.5μ in diameter |

*Aspergillus niveus* Blochwitz.—Colonies grow well at 30-35°C. on Czapeck's solution agar. Texture of the colonies vary from zone to zone, the heads are aggregated on the margin and mycelium submerged in these zones. In the central region mycelium is superficial and therefore, the overall texture may be called as fasciculate. Colour also varies greatly from marginal wood brown (Ridgeway Pl. XL) to deep sea foam green (Ridgeway Pl. XXXI). The central zone is light buff (Ridgeway Pl. XXX). Heads compact columnar, yellowish brown to hyaline ranging from 28-36μ in diameter, conidiophores smooth, without foot cells, 84-200μ in length, ranging from 3-6μ in diameter. Vesicles globose, yellowish, ranging from 12-20μ in diameter. Sterigmata strictly in two series, primaries 3-5μ and secondaries upto 5μ in length. Conidia globose, brownish, rough, from 16-25μ in diameter (Culture No. BR. 30)

This strain differs from type strain described in 'A Manual of Aspergilli' in the following characters.

| *Type culture* | *Culture No. BR. 30* |
|---|---|
| 1. Conidiophore sinuate, rough more or less septate | Conidiophores smooth, 84-200μ in length |
| 2. Colonies producing abundant exudate | Colonies not producing exudate |
| 3. Conidia smooth, thin-walled, colourless, 2-2.5μ in diameter | Conidia rough, brown, 16-25μ in diameter. |

SUB KEY TO THE SPECIES OF ASPERGILLUS NIGER GROUP

a Sterigmata in single series . . . . *A. luchuensis*
b Sterigmata predominantly in two series . . . . . *A. awamori*

*A. luchuensis* Inui has already been reported from West Pakistan by Chaudhuri and Umar [14] and therefore, it is not described here.

*Aspergillus awamori* Nakazaea.—Colonies growing fast at 30-36°C. on Czapeck's Dox solution agar, velvety in texture with submerged mycelium, upper surface approximately dark purplish grey (Ridgeway Pl. LIII) and reverse deep olive buff (Ridgeway Pl. XL). Conidial heads brownish black, radiate, ranging from 36 to 99μ in diameter. Conidiophores yellowish green, smooth, wall thickness 1.4μ, from 600 to 2000μ in length and upto 17μ in diameter. Vesicles globose, light brown, ranging from 21-57μ in diameter. Sterigmata usually in two series, very rarely in single series, primaries ranging from 7 to 15μ and secondaries from 5 to 6μ in length. Conidia produced in long persistant chains, globose, rough, ranging 3 to 6μ in diameter. There is no difference in colour or measurements of this culture from type culture reported and described by Nakazaea and Watanbe which is also reproduced on page 220 in 'A Manual of Aspergilli' and therefore, no comparison is necessary. (Culture No. B22).

An allied strain of *A. awamori* isolated from Lahore soil designated as culture No. B12, differs from culture no. B22 in having upper surface fucous black (Ridgeway Pl XLVII) and reverse surface colourless with regular divergent furrows. Conidial heads large upto 114μ in diameter, conidiophores upto 2240μ in length.

Allied strain B36 differs from Culture No. B22 in its reverse side colour which approximates massicot yellow (Ridgeway Pl. XVII).

*Aspergillus ochraceus* Thom and Raper.—Colonies growing slow at 30-35°C. on Czapeck's solution agar, velvety in texture with submerged mycelium and abundant sclerotia. Upper surface deep colonial buff (Ridgeway Pl. XXX). Conidial heads dirty whitish yellow, globose and radiate, ranging from 60 to 80μ in diameter. Conidiophores smooth, wall thickness upto 1.6μ, greenish, ranging from 8 to 11μ in diameter and from 504 to 798μ in length. Vesicles globose, dirty white, fertile all over, ranging from 20 to 39μ in diameter, sterigmata strictly in two series, primaries from 4 to 8μ and secondaries from 4 to 8.5μ in length. Conidia slightly rough, globose to slightly oval, ranging from 3 to 4μ in diameter when globose and 3×4μ when oval. Sclerotia dark cream coloured, globose to oblong, ranging from 420 to 540μ in diameter when globose, 490 to 360μ when oblong (Culture No. BR 9). This strain differs from type culture in the following characters.

| *Type culture* | *Culture No. BR 9* |
|---|---|
| 1. Conidiophores pitted and rough | Conidiophores smooth |
| 2. Primary sterigmata 15-30μ in length | Primary sterigmata 4-8μ in length. |

SUB KEY TO THE SPECIES OF ASPERGILLUS NIDULANS GROUP

Ascospores with two equatorial ridges

a. Ascospores smooth all over

i Mycelium submerged . . . *A. nidulans*

ii Mycelium predominent . . . *A. nidulans* var. Latus.

b. Ascospores rough all over . . . *A. quadrilineatus*

*A. nidulans* (Eid.) Wint. has been reported from West Pakistan by Chaudhuri and Umar[14] from Citrus at Lahore and, therefore, it is not described here.

*Aspergillus nidulans var. Latus* Thom and Raper.—Colonies growing well at 30-35°C. on Czapeck's solution agar, floccose in texture with predominant sterile mycelium. Conidial heads developed in limited number, perithecia developed fairly late in age, ripening slowly, colour of the colony changes with age. In early days upper surface of the colony white and reverse bitter sweet pink (Ridgeway Pl. II). At maturity upper Claret brown (Ridgeway Pl. I) and reverse Victoria lake (Ridgeway Pl. I); at maturity conidial heads light greenish, loose, columnar from 18 to 40μ in diameter. Conidiophores light brown, smooth, occasionally branched with swelling near the base, ranging from 1.6 to 5μ in diameter and 20 to 80μ in length. Vesicles globose, only upper ¾ portion fertile, dirty greenish, ranging from 3.3 to 10μ in diameter, conidia globose, rough, nearly hyaline, upto 3.3μ in diameter. Perithecia scanty, dim red in colour, globose, ranging from 100 to 140μ in diameter. Asci orange red in colour, globose to slightly oval, containing 6 to 8 ascospores measuring 8×7-10μ in size. Ascospores red, with two equatorial ridges, 1.5 to 1.8μ apart, 4.3-3×5-3μ in size. Hulle cells abundant, ranging from 15 to 21μ in diameter (Culture No. BR 26)

The above described strain is almost similar to the type strain and hence no comparison is given.

*Aspergillus rugulosus* Thom and Raper.—Colonies growing well at 35°C. on Czapeck's solution agar with restricted cream coloured margin; conidial heads sparsely produced on the margins of the slants and hence not generally observed. Upper surface of the colony light greyish olive (Ridgeway Pl. XLVI). Perithecia in maroon brown shades. Reverse of the colony flesh pink (Ridgeway Pl. XIV). Conidial heads loose, columnar, ranging from 25 to 36μ in diameter. Vesicles not fertile all over, nearly globose in form, yellowish green in colour, ranging from 8 to 13μ in diameter. Sterigmata strictly in two series, light yellowish green, primaries ranging from 5 to 7μ and secondaries from 7 to 8μ in length. Conidia globose, rough, bluish green, from 2.5 to 4.3μ in diameter. Perithecia abundant, reddish brown, globose, ranging from 80 to 160μ in diameter. Asci globose to slightly oval, reddish, ranging from 9 to 11μ in diameter containing 6 to 8 ascospores each. Ascospores thickly biconvex, reddish with two equatorial ridges, conspicuously ruglose all over, uniform in size, 3.5×3.5μ (Culture No. BR 4).

This strain differs from type culture in having perithecia smaller 80 to 160μ whereas in the type strain they are 225 to 350μ. Rest of the characters are similar.

*Aspergillus quadrilineatus* Thom and Raper.—Colonies growing well at 36°C. on Czapeck's solution agar. Velvety in texture with a tendency towards floccosity. The production of conidial heads is rare and restricted to one area while perithecia restricted to the other. Upper surface of the colony when observed under the naked eye, occupied by conidial heads Roman green (Ridgeway Pl. XL) reverse surface Salmon green (Ridgeway Pl. II). Conidial heads yellowish green under microscope, loose, columnar, 13 to 14μ in diameter. Sterigmata strictly in two series, primaries 4 to 8μ and secondaries 8 to 10μ in length. Conidia globose, yellowish green, slightly rough, 3 to 6μ in diameter.

Perithecia globose to slightly irregular, dark maroon 210 to 280μ in diameter. Asci globose to slightly oval, reddish containing 6 to 8 ascospores each. Ascospores purple reddish, thickly biconvex in form with four equatorial ridges. Hulle cells abundant 10 to 25μ in diameter (Culture No. BR 6).

Culture BR 6 differs from the type culture in perithecial size which is smaller than the type culture. The size of perithecia of BR 6 is 125 to 150μ while that of type culture is 210 to 280μ.

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