

ATLAS OF AIRBORNE FUNGAL SPORES OF BIKANER CITY

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ABSTRACT

Human health is profoundly influenced by the quality of the air we breathe, which is laden with various particles, including allergens such as pollen grains and fungal spores. Fungal aerosols, in particular, have been identified as significant contributors to respiratory allergies. However, despite their prevalence, fungal spores have received less attention compared to pollen in aerobiological studies. This paper presents an extensive aeromycological survey conducted in Bikaner City, located in the Thar Desert, to assess the diversity of fungal spores in the region's atmosphere. Using the gravity slide method, fungal spores were collected and analyzed over a two-year period. The study identified a wide range of fungal genera, including *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium*, and *Alternaria*, among others. Understanding the distribution and characteristics of airborne fungi is crucial for effective diagnosis and management of respiratory ailments in the region. The study provides a comprehensive key to identify airborne fungal spores, aiding future aerobiological research and allergen monitoring efforts.

Keywords: Fungal spores, Aeromycology, Fungal aerosols, Allergenic

INTRODUCTION

Human breathes about 35 lb. of air per day as compared to consumption of 3 lb. water and 2.5 lb. of food. He cannot survive for a few minutes without air. Air carries an array of particles varying in size shape, density, and other features, which poses burden on the respiratory tract of humans. These include a few inorganic and organic contaminants; airborne bio-particles (bio-pollutants) such as microorganisms, pollen grains, fungal spores, hyphae of fungi, spores of mosses and ferns, insect debris, animal dander and mites etc. These are known to be the chief causative agents of allergic disorders such as bronchial asthma, allergic rhinitis and atopic dermatitis (Aalberse et al., 1989; Burge, 1990; Kang et al., 1979; Lacey & Crook, 1988; Singh & Kumar, 2003; Singh & Singh, 1994). Among all these agents pollen grains and fungal spores are the most predominant allergens in air (Verma, 2024). Detailed

information on daily, seasonal and annual variations of fungal spores in the atmosphere is of paramount importance in effective diagnosis and therapeutic management of allergic ailments (Verma, 2022).

Although pollen has been widely studied as aeroallergens throughout the world, far less is known about the fungal aerosols, which are present in much higher concentration than the pollen grains in the air. Early reports on fungal allergy attributed the symptoms to fungal spores prevailing in the patients home or occupational exposure to them (Barkai-Golan et al., 1977; Benton, 1930; Berman, 2000; Crook, B. and Lacey, 1988; Hopkins et al., 1930). Feinberg (1935) reported that outside air was an equally significant source of fungal spores and correlated it with the patients' symptoms. This gave impetus to the study of environmental conditions affecting prevalence of moulds in indoors and outdoors, all over the world. Since then, several researchers investigated the role of fungal spores in

allergic diseases (Durham, 1946; Feinberg, 1944; Hyde & Williams, 1956; Nilsby, 1949; Nilsson et al., 1977; Prince & Morrow, 1969; Rudert & Portnoy, 2017; Shivpuri, 1980).

There are more than 80,000 of fungi, which have elaborate mechanism for spore dispersal and dissemination. The spore size facilitates their suspension in the atmosphere for a long time, leading to sensitization, when inhaled by the population.

In outdoor environment the source of fungal spores includes cereal crops, decaying vegetables and organic wastes on which fungi thrive. While in indoor environment the sources are damp walls, dustbins, window pans, mattresses, leaking pipes and humidifiers. Most studies on fungi as aeroallergens have been limited to the fungi imperfecti (Deuteromycotina) with little information on the allergenicity of the members of other fungal groups. The literature is still insufficient regarding the development of allergic asthma by inhaled fungal spores and there is very little information available on the characterization of antigenic fractions of common fungal allergens.

METHODOLOGY

Study area:

The present study was conducted in Bikaner City, situated in the heart of the Thar Desert. Spanning an area of 30,247.90km², Bikaner is positioned between 27°11' N and 29°03' N latitude and 71°54' E and 74°12' E longitude. The landscape of Bikaner primarily includes sandy plains and dunes, with elevations ranging from 61m to 305m above sea level. It experiences a hot desert climate characterized by minimal rainfall and extreme temperatures. Summers (April to June) are exceptionally hot, with maximum temperatures surpassing 48°C, whereas in winters (November to February) the temperatures may drop below freezing point at night. Majority of rainfall occurs during the rainy season from July to September coinciding with high temperatures. Annual

precipitation does not exceed 352 mm. sand and dust storms during the hot and dry summers are very common, with wind speeds reaching up to 110 km/hr, causing changes in the landscape by shifting sand dunes. Due to low relative humidity and high evaporation rates, frequent famine is very common in the area. The construction of canals in Rajasthan has increased vegetation along their banks, providing habitats for various plant species. However, it also poses risks such as the introduction of invasive species, habitat fragmentation, and altered hydrology, which can have adverse effects on the region's flora.

Collection of Fungal Spores

Fungal spores were collected and studied using the gravity slide method. An aeroscope, based on the "slide exposure-apparatus" principles described by Lakhanpal and Nair in 1958, with some modifications, was utilized. In this apparatus, microslides were placed in a slanting position inside an aluminium tube open at both ends.

Two aeroscopes were positioned at a height of approximately 20 feet above ground level at two sites: one in the residential area of Bikaner City and the other in open fields. Both sites were fully exposed to air currents from all directions. The two selected sites were located around 10 km apart from each other.

Two standard microscopic slides (75 x 25 mm) were exposed daily at each site for two years (2000 and 2001). Before exposure, the slides were smeared with glycerine-jelly stained with cotton blue over an area of about 40 x 22 mm. To smear the slides, a small amount of glycerine-jelly was gently heated and melted over a slide, and a thin smear was made with the help of a needle. Slides were exposed at around 10 a.m. at the first site and about 11 a.m. at the second site. After 24 hours of exposure, the slides were removed from the sampling device, and large dust particles, soot, and sand were first removed with a dissecting needle under a hand lens. Then, a few drops of Cotton blue-stained jelly were placed on the slide and mounted under a cover slip of 40 x 22 mm.

The entire area (8.8 cm²) under the cover slip was scanned. The slides were examined to identify and count the fungal spores. Spores were primarily identified based on their morphology. Whenever possible, spores were identified up to the genus level. Group counts were taken for types like basidiospores and small round spores (e.g., *Aspergillus* and *Penicillium*).

RESULTS AND DISCUSSION

Two years extensive aeromycological survey of study area reveals that following airborne fungal spore producing species are dominant in and around the Bikaner (Rajasthan).

Zygomycotina

***Mucor Micheli* (Figure 1a)**

Spores ash colored to slightly black or grayish blue, wall smooth or dotted, rarely echinulated; globose to elliptical or rounded angular in shape, aseptate; 3-8 µm in diameter.

***Rhizopus Ehrenberg* (Figure 1a)**

Spores pale gray to grayish brown in color; wall smooth or marked by longitudinal striations, rarely echinulated; globose to oval or angular in shape, aseptate; 4-7 µm in diameter.

Ascomycotina

***Aspergillus Micheli ex Fries* (Figure 1b)**

Spores greenish, bluish or yellow in color; smooth verruculose or echinulated; small, globose or ovoid, aseptate; 4-7 µm in diameter.

***Chaetomium Kunze* (Figure 1c)**

Spores dark brown to olive brown; lemon shaped, somewhat flattened in one plane, non-septate with smooth wall and pointed apex; 10-13 µm long and 7-9 µm thick in the broadest part.

***Didymella Saccardo* (Figure 1d)**

Spores hyaline; ovoid to ellipsoidal in shape, 1-septate, with or without constriction at the septum; 12-18 µm long and 5-7 µm thick in the broadest part.

***Didymosphaeria Fuckel* (Figure 1e)**

Spores olivaceous brown to dark brown; smooth or minutely verrucose, 1-septate, with or without constriction at the septum, both cells equal or unequal in size; 15-20 µm long and 7-9 µm thick in the broadest part.

***Herpotrichia Fuckel* (Figure 1f)**

Spores pale to dark brown; ovoid to ellipsoidal in shape, 1-septate, with longitudinal ridges in each cell, may or may not be constricted at the septum, with or without mucilaginous sheath; 15-30 µm long and 8-17 µm thick in the broadest part.

***Leptosphaeria Cesati & de Notaris* (Figure 1g)**

Spores yellowish to yellow brown; smooth to finely verruculose; fusoid, straight or curved, 2-or more septate, usually with one cell wider than the other; with or without gelatinous sheath; 40-56 µm long and 9-14 µm thick in the broadest part.

***Melanospora Corda* (Figure 1h)**

Spores dark; smooth to finely verruculose; lemon shaped, aseptate; 10-16 µm long and 8-13 µm thick in the broadest part.

***Penicillium Link ex Fries* (Figure 1b)**

Spores yellowish to green in color; globose to ovoid, resemble glass beads with smooth wall; 4-7 µm in diameter.

***Pleospora Rabenhorst* (Figure 1i)**

Spores yellow brown to dark brown colored; smooth or verruculose, ellipsoidal, with several cross septa and one or more longitudinal septa in most or all of the segments (muriform), may or may not be constricted at the septum, 15-40 µm long and 7-16 µm thick in the broadest part.

***Rosellinia de Notaris* (Figure 1j)**

Spores gray to dark brown or black in color, cylindrical-fusiform, with a small longitudinal furrow, smooth walled; often with guttulation; 16-20 µm long and 8-13 µm thick in the broadest part.

***Sporormia* de Notaris (Figure 1k)**

Spores dark brown; cylindrical, straight or slightly curved, 3-8 septate, strongly constricted at the septum and readily separating into unicellular segments; 35-60 µm long and 8-12 µm thick in the broadest part.

Basidiomycotina**Basidiospores (Figure 1l)**

Spores free, hyaline, pale or gray; rough walled, globose to ovoid or ellipsoid, aseptate; 9-18 µm.

Smut spores (Figure 1m)

Spores grayish brown to dark brown; smooth walled or slightly verrucate; round, oval or shortly elliptical, aseptate; 10-12 µm.

Deuteromycotina***Alternaria* Nees ex Fries (Figure 1n)**

Spores pale to olivaceous or dark brown in color; surface smooth or warted or echinulated; variously shaped, obclavate to elliptical or ovoid, provided with beak of varying length; muriform with transverse, longitudinal and sometimes oblique septa; frequently borne acropetally in long chain; 30-90 µm long and 15-35 µm thick in the broadest part.

***Bispora* Corda (Figure 1o)**

Spores dark brown; oblong, 2-celled with thick black septa; catenulate; 12-18 µ long and 5-7 µ thick in the broadest part.

***Botrytis* Pers ex Fries (Figure 1p)**

Spores hyaline to pale brown; variable in shape and size, ellipsoidal-obovoid, spherical or subspherical, smooth, single, celled, finely apiculate at the base; 9-18 µm long and 7-12 µm thick in the broadest part.

***Cladosporium* Link ex Fries (Figure 1q)**

Spores subhyaline to dark brown colored; surface smooth, finely verrucose; polymorphous, ovoid to cylindrical and irregular in shape, 1-4 celled, scar present at one or both ends; occur single or in simple or branched chains; 4-17 µm long and 3-8 µm broad.

***Curvularia* Boedijn (Figure 1r)**

Spores olivaceous brown to dark brown in color; more or less fusiform in shape, typically bent or curved, 3-5 celled, with one or two of the central cells being distinctly enlarged and darker than the terminal cells; 17-34 µm long and 8-16 µm thick in the broadest part.

***Drechslera* Ito (Figure 1s)**

Spores straw colored or pale to dark brown, end cells paler than intermediate ones; mostly smooth walled, rarely verruculose; ellipsoidal, fusiform or obclavate in shape, straight or curved, one to many pseudoseptate; 25-90 µm long and 15-35 µm thick in the broadest part.

***Epicoccum* Link ex Wallroth (Figure 1t)**

Spores golden brown to olivaceous black; surface verruculose or finely echinulated; globose or subglobose, irregularly septate and muriform with up to 15 cells; 11-15 µm in size.

***Fusarium* Link ex Freis (Figure 1u)**

Spores hyaline; fusiform to falcate; 3 to many septate, slightly curved or bent at the pointed ends, 15-32 µm long and 3-5 µm thick in the broadest part.

***Helminthosporium* Link ex Fries (Figure 1v)**

Spores olivaceous brown to dark brown in color; wall smooth or verruculose; subcylindrical or lanceolate in shape, straight, curved or bent, one to many transversely pseudosepte (up to 20 septa), may be faintly constricted at one or more, septa, each spore with a basal scar; 30-105 µm long and 10-20 µm thick in the broadest part.

***Melanoconium* Link (Figure 1w)**

Spores yellowish brown to dark brown; smooth or verrucose; ovoid to ellipsoid or oblong, aseptate; 16-20 µm long and 8-13 µm thick in the broadest part.

***Memnoniella* Hohnel (Figure 1x)**

Spores yellowish brown to dark brown in color; rough surfaced; globose, ovoid or angular in shape, single celled in long persistent chain (catenulate); 4-6 µm in diameter.

***Nigrospora* Zimm. (Figure 1y)**

Spores opaque and shining black colored; smooth surfaced; depressed globose, single celled, typically quite round when viewed from the end and elliptical when seen from the side; 15-25 µm in diameter.

***Periconia* Toda ex Schweinitz (Figure 2a)**

Spores simple, pale to dark brown colored; wall verruculose or echinulated; usually spherical or subspherical in shape, aseptate; 8-15 µm in diameter.

***Phoma* Saccardo (Figure 2b)**

Spores hyaline; rounded or lens shaped or lenticular with smooth wall; 16-30 µm long and 5-10 µm in the broadest part.

***Pseudotorula* Subram. (Figure 2c)**

Spores dark brown; thick walled; elliptical to oval, 2-5 septate, usually constricted at one or more septa; 11-24 µm long and 5-7 µm thick in the broadest part.

***Stemphyliomma* Sacc. & Traverso (Figure 2d)**

Spores dark brown colored with pale ends cells; wall verruculose or finely echinulated; ellipsoidal in shape, 3-6 septate, constricted at the septa, septa and walls thick; 30-50 µm long and 9-14 µm in the broadest part.

***Stemphylium* Wallroth (Figure 2e)**

Spores pale to mid dark or olivaceous brown colored; surface smooth, verruculose or echinulated; globose to broadly ellipsoidal in shape, rounded at the ends, muriform with transverse, longitudinal and oblique septa; 50-70 µm long and 20-30 µm thick in the broadest part.

***Tetracoccusproium* Szabo. (Figure 2f)**

Spores simple, mid to dark brown or olivaceous brown in color; surface verruculose or minutely echinulated, spherical or subspherical in shape, divided cruciate by septa at right angles to one another; 12-18 µm and 10-13 µm in size.

***Tetraploa* Berkeley & Broome (Figure 2g)**

Spores appendaged, brown, verruculose or verrucose; muriform with 4 columns of cells, tend to diverge from one another apically and terminate each in a septate setiform appendage, shallow furrows between columns; 25-40 µm long and 18-30 µm broad, with 40-90 µm long appendages.

***Tilakiopsis* Tilak (Figure 2h)**

Spores olivaceous brown to dark brown; smooth, fusiform, 2-4 septate, septa thick and dark; basal and apical cells light colored whereas middle cells dark brown; 30-50 µm long and 18-35 µm thick in the broadest part.

***Torula* (Pers.) Link ex Fries (Figure 2i)**

Spores brown or olivaceous brown, with paler end cells; surface verruculose or echinulate; cylindrical or ellipsoidal with rounded ends, 2-4 septate, septa deeply constricted; 12-20 µm long and 4-12 µm thick in the broadest part.

KEY TO AIRBORNE FUNGAL SPORES OF BIKANER

I Spores 1-celled

1. Spores hyaline, pale or grey :A
 - A1. Spores free :B
 - B1. Spores smooth walled :C
 - C1. Spores rounded or lenticular, spore size 16-30 µm x 5-10 µm. :*Phoma*
 - C2. Spores variable in shape and size, finely apiculate at the base; spore size 9-18 µm x 7-12 µm. :*Botrytis*
 - B2. Rough walled :C
 - C1. Spores globose to ovoid or ellipsoid; spore size 9-18 µm. :Basidiospores
 - A2. Spores often in mass some time free :B
 - B1. Spores ash coloured, wall smooth or dotted; rounded angular in shape; 3-8 µm in diameter. :*Mucor*

- B2. Spores pale gray; wall smooth or marked by longitudinal striations; globose to oval; 4-7 μm in diameter. :*Rhizopus*
2. Spores coloured :A
- A1. Spores greenish yellow in colour :B
- B1. Spores smooth, verruculose or echinulate; globose or ovoid; 4-7 μm in diameter walled :*Aspergillus*
- B2. Spores globose to ovoid, resemble glass beads with smooth wall; 4-7 μm in diameter. :*Penicillium*
- A2. Spores yellowish brown to brown. :B
- B1. Spores smooth or verruculose; ovoid to ellipsoid; spore size 16-20 μm x 8-13 μm . :*Melanconium*
- B2. Spores in long, persistent chain (catenulate); rough surfaced; globose, ovoid or angular in shape, 4-6 μm in diameter. :*Memnoniella*
- B3. Spores verruculose or echinulate; usually spherical or subspherical in shape; 8-15 μm in diameter. :*Periconia*
- A3. Spores olivaceous brown to dark brown :B
- B1. Spores smooth walled :C
- C1. Spores lemon shaped, somewhat flattened in one plane; spore size 1-13 μm x 7-9 μm . :*Chaetomium*
- C2. Spores cylindrical-fusiform, with a small longitudinal furrow; often with guttulation; spore size 16-20 μm x 8-13 μm . :*Rosellinia*
- B2. Spores verruculose or echinulate :C
- C1. Spores lemon shaped, 10-16 μm x 8-13 μm . :*Melanospora*
- C2. Spores round, oval or shortly elliptical; 10-12 μm in size. :Smut spores
- A4. Spores opaque shining black, smooth, depressed globose; 15-25 μm in diameter. :*Nigrospora*
- II Spores 2-celled**
1. Spores hyaline, ovoid to ellipsoidal; 12-18 μm x 5-7 μm in size. :*Didymella*
2. Spores olivaceous brown to dark brown :A
- A1. Spores smooth or minutely verruculose; spore size 15-20 μm x 7-9 μm . :*Didymosphaeria*
- A2. Spores ovoid to ellipsoidal, with longitudinal ridges in each cell; spore size 15-30 μm x 8-17 μm . :*Herpotrichia*
- A3. Spores oblong, with thick black septa; catenulate; spore size 12-18 μm x 5-7 μm . :*Bispora*.
- III Spores 1- to many septate**
1. Spores with transverse septa A
- A1. Spores subhyaline to grayish brown :B
- B1. Spores often in chain, 1-4 celled polymorphous, scar present at one or both ends; spore size 4-17 μm x 3-8 μm . :*Cladosporium*
- B2. Spores free :C
- C1. Spores 3 to 6 septate :D
- D1. Spores with one cell wider than the other; spore size 40-56 μm x 9-14 μm . :*Leptosphaeria*
- D2. Spores slightly curved or bent at the pointed ends; 15-32 μm x 3-5 μm in size. :*Fusarium*
- C2. Spores 3 to many pseudoseptate with end cells paler than intermediate ones. :*Drechslera*.
- A2. Spores olivaceous brown to dark brown :B
- B1. Spores in chain :C
- C1. Spores cylindrical or ellipsoidal with rounded paler end cells, septa deeply constricted; spore size 11-24 μm x 4-12 μm . :*Torula*

- C2. Spores elliptical to oval, thick walled; spore Size 12- 18 μm x 4-7 μm . :*Pseudotorula*
- B2. Spores free :C
- C1. Spores 3 to 8 septate :D
- D1. Spores strongly constricted at the septum and readily separating into unicellular segments; spore size 35-60 μm x 8-12 μm . :*Sporormia*
- D2. Spores with one paler end cells. :E
- E1. Spores typically bent or curved; spore size 17-34 μm x 8-16 μm :*Curvularia*
- E2. Spores ellipsoidal, verrucose or finely echinulated; spore size 30-50 μm x 9-14 μm . :*Stemphyliomma*
- E3. Spores smooth, fusiform; 30-50 μm x 18-35 μm in size. :*Tilakiopsis*
- C2. Spores 3- to many pseudoseptate, each spore with a basal scar; 30-105 μm x 10-20 μm in size. :*Helminthosporium*
2. Spores with transverse, longitudinal and sometimes oblique septa (muriform) septa :A
- A1. Spores globose or subglobose :B
- B1. Spores divided cruciately by septa at right angles to one another; 12-18 μm x 10-13 μm in size. :*Tetracoccusporium*
- B2. Spores verrucose or finely echinulate; irregularly septate with up to 15 cells; 11-15 μm in size. :*Epicoccum*
- A2. Spores obclavate to elliptical or ovoid. :B
- B1. Spores provided with beak of varying length, frequently borne acropetally in long chain; 30-90 μm x 15-35 μm in size. :*Alternaria*
- B2. B2. Spores smooth or verrucose, constricted at 1 or 2 septum; 15-40 μm x 7-16 μm in size. :*Pleospora*
- B3. Spores smooth or verrucose, rounded at the ends; 50-70 μm x 20-30 μm in size. :*Stemphylium*
3. Spores with 4 divergent septate appendages. :*Tetraploa*

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