

ENVIRONMENTAL MICROBIOLOGY

Atmospheric Environment & Health

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Introduction

India, with the teaming population of more than one and half billion and with the divergent geographical backdrop ranging from upland plain (Deccan Plateau) in south, flat to rolling plain along the Ganges, deserts in west, Himalayas in north, has a rich aerobiological diversity. This diversity is further enhanced with the climate, which varies from tropical monsoon in south to temperate in north. The latter part of the 20th century has seen an increase in the prevalence of allergic diseases, implicating changing environment and lifestyle as significant causes. With the alarming increase in allergic disorders, such as allergic rhinitis, bronchial asthma and atopic dermatitis covering as high as 30 % of the population worldwide, there is an increasing interest in the presence and movement of bioparticulate matter in the earth's atmosphere and their impact on human health. This interdisciplinary approach is known as aerobiology. The bioparticulates implicated to cause allergic symptoms are pollen grains, fungal spores, insect debris, house dust mites, animal dander, chemicals and foods etc. Among all these agents, pollen grains and fungal spores are the most predominant allergens in the air. However, for the effective diagnosis and therapeutic management of these ailments, a detailed information on the daily, seasonal and annual variations of various bioparticles is essential.

Airborne microbes (fungi) are implicated in the causation of allergic diseases and infections in immunocompromised patients. As the sister group of animals and part of the eukaryotic group that radiated about a billion years ago, the fungi constitute an independent group equal in rank to that of plants and animals. They differ from bacteria by having genetic material arranged on chromosomes, and a membrane surrounding the nucleus. The fungi that produce spores and get airborne are called 'aerospores'. They cause a number of infections in tropical countries including ringworm, athlete's foot in human, and rusts, smuts, and leaf, root, and stem rots in plants. *Aspergillus* spp can invade the lungs and cause serious pneumonia in people with an impaired immune system. They are also established to cause Type I hypersensitive diseases with IgE mediated response. The common symptoms of hypersensitivity are bronchial asthma, allergic rhinitis and atopic dermatitis.

Fungi are better known than bacteria, viruses and other smaller forms of life such as viroids. There are about 80,000 named species and new species are being added at the rate of about 1500 species each year. Fungi can be classified into two basic groups:

1. **Molds:** Fungi that grow in filamentous form
2. **Yeast:** These are characteristically single cells

Fungi, in general, and moulds in particular, can cause disease in humans and animals in three ways:

1. Fungi can produce an actual infection in the host involving growth on or in the person or animal. It is quite uncommon for environmental moulds to produce this sort of disease unless one has a very severe reduction in the function of the immune system (e.g., is undergoing intensive therapy for cancer or is taking high doses of corticosteroids for prolonged periods of time.). Examples of fungal invasion are Aspergillosis, Blastomycosis, Candidiasis, Coccidiomycosis, Cryptococcosis, Histoplasmosis, Paracoccidiomycosis, Sporotrichosis, Zygomycosis.
2. Fungi can produce toxins that make people or animals sick. Although some toxins can be inhaled, the toxin is most often introduced into the person or animal by ingestion of mould-contaminated foods. Some of the toxins are very powerful e.g. aflatoxins,

3. Fungi can produce allergic reactions in hypersensitive subjects e.g. eczema, allergic rhinitis, allergic asthma, atopic dermatitis etc

History of Fungal Allergy

Fungi possess highly evolved mechanism of spore liberation due to which the spores remain suspended in the air for a varying duration i.e. few hours to several days. The first case of fungal sensitivity was reported as early as 1726. More than a century later, Blackley suggested the association of species of *Chaetomium* and *Penicillium* with attacks of bronchial catarrh. Feinberg reported respiratory allergic reactions to fungi in his patients and attributed outdoor environment as a source of fungi.

With the studies establishing the role of fungal spores as a major causative agent for the respiratory allergic disorders, the seasonal and annual variations in the bioaerosols have been extensively studied in different parts of the world including India. Their knowledge is of paramount importance for diagnosis and therapeutic management of allergic diseases.

Fungi and fungal particles can clearly induce an allergic response in susceptible individuals. Typical symptoms include wheezing, cough, rhinorrhea, itchy nose, sore throat, sinus congestion etc. The development of allergies to fungi follows the same biological phenomenon as allergies to other environmental allergens. Dead fungi are able to produce symptoms just as well as live fungi. Hayward and coworkers reported a separation and characterization of antibodies to moulds in human sera and the role of human precipitins to common fungal antigens in allergic reaction, which was later proved by Pepys.

Fungi as Allergenic Agents

The prevalence of respiratory allergy to fungi is estimated at 20 to 30% among atopic individuals and upto 6% in the general population. The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis. These diseases can result from exposure to:

1. Spores
2. Vegetative cells
3. Fungal metabolites

More than 80 genera of the major fungal groups have been associated with symptoms of respiratory tract allergy. In addition, as the fungal spores are small (usually less than 10 μm) a majority of them are capable of penetrating the lower airways of the lung and mediate allergic reactions. The conidia and fungal spores associated with immediate type of hypersensitivity are usually larger than 5 μm , while those associated with delayed type of hypersensitivity are considerably smaller (approximately 1 μm) and can penetrate the smaller airways. The site of deposition of spores is dependent on, whether spores enter the respiratory tract as individual propagules or as aggregates for e.g. the clusters of small conidia of *Aspergillus* / *Penicillium* are usually deposited in the upper respiratory tract, while the smaller individual spores reach the lower airways.

Methods to Monitor Airborn Fungi

Fungal spores are present in both outdoor and indoor environment and contribute the major part of suspended bioparticulate matter of the air. They are liberated in very high concentration and remain suspended in the air for the extended period of time. Their presence, profile of species, concentration etc depends on various climatic factors such as temperature, humidity, wind direction, sunshine, substrate precipitation and other seasonal factors. Fungi are present in outdoor environment from sources such as cereal crops, vegetation, garbage and storage centers. This outdoor air enters indoors and make the houses and working / occupational places with microbial contamination by:

1. **Infiltration:** Outdoor air flows into the house through openings, joints, and cracks in walls, floors, and ceilings, and around windows and doors.
2. **Natural Ventilation:** Air moves through opened windows and doors
3. **Mechanical Ventilation:** Number of mechanical ventilation devices like outdoor-vented fans that intermittently remove air from a single rooms such as bathrooms and kitchen, to air handling systems that use fans and duct work to continuously remove indoor air and distribute filtered and conditioned outdoor air to strategic points throughout the house.

Continuous efforts by workers have lead to the development of a vast array of sampling instruments since the time gravity method was initiated by Durham (1946). Some of the sampling devices developed and prevalent today are briefly described here:

A. Gravimetric Sampler

This is based on the principal that bioparticulates settle down on a surface due to gravitational force. The Durham gravity-sampling device consists of two horizontal disks and it provides qualitative spore data. However, this sampler is no more used in developed world, but developing countries still report the use of gravity settlement method for fungal spores/colonies. Even in still air the number of large particles collected on the surface will be overestimated and smaller particles that have a slower setting velocity will be underestimated.

B. Rotorod Impaction Sampler

Rotorod sampler developed by Perkins (1951) has leucite rods of 1-3 mm coated with adhesive silicon grease are used to collect air borne particles. It is a lightweight portable sampler operated by DC power (Fig. 1). The exposure time can be adjusted according to requirement. There are three models available in the Rotorod Aeroallergen Models (40s, 85s and 95s) and the spore catch obtained by these three models is almost similar. These three samplers are used to study both culturable and non-culturable spore types on continuous basis for diurnal, seasonal and annual variations.

C. Hirst Trap

The method requires suction of certain volume of air according to a known velocity and for a chosen duration on trapping. The Hirst spore trap is the most commonly used in UK. In this trap bioparticulates adhere to slides coated with glycerin jelly and slides are replaced each day with fresh slides and provides qualitative data.

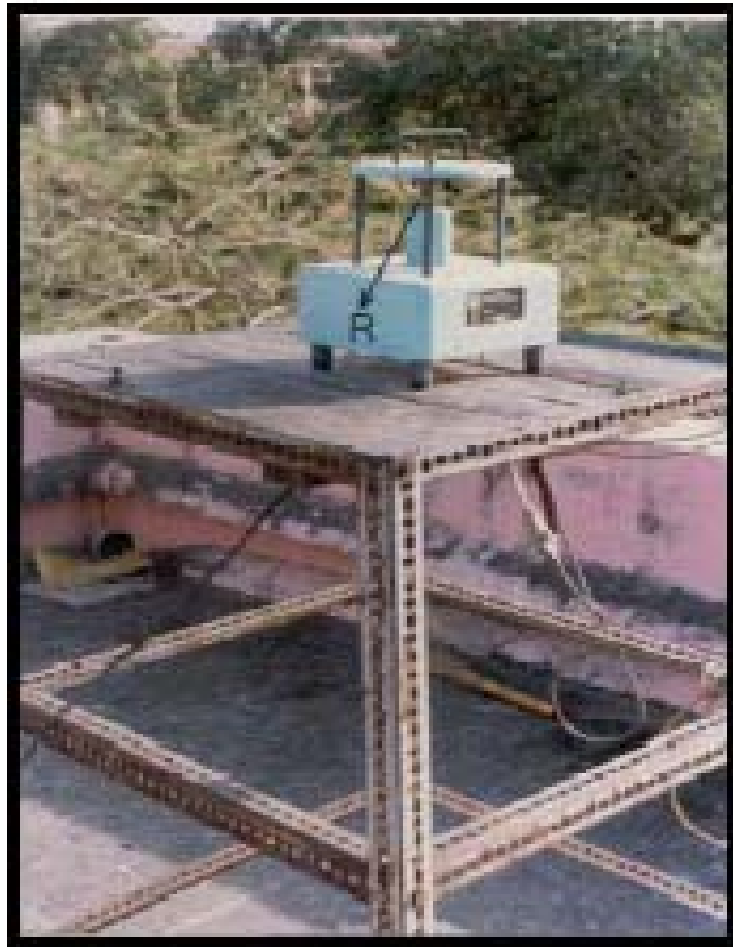


Fig. 1: Rotorod Sampler (Aeroallergen Model 40s) placed at the terrace of Institute of Genomics & Integrative Biology for continuous monitoring of aeroallergens

D. Burkard Seven Day Volumetric Sampler

The first trap was later modified to Burkard trap in which slides were replaced with a drum, which can rotate and run continuously for seven days with a definite speed with suction rate of 10 L of air per minute. Burkard continuous seven days sampler used in adhesive coated tape mounted on a rotating drum. The drum is connected to a timer and rotated at constant speed (Fig. 2). The tape is changed every seven days. Exposed tape is cut in seven strips corresponding to seven days and mounted on a slide. This is one of the best samplers to study diurnal or seasonal trends for fungal spores as well as pollen grains.

E. Burkard Slide Sampler

Burkard slide sampler is a compact battery operated sampler. It has a rectangular orifice at the top end and a slit on the slide to insert microslide (Fig. 3). The microslide is coated with glycerin jelly. The sampler sucks in 10 L of air per minute. The particles get impacted on the slide in the form of a streak. The slide is then mounted in glycerin jelly and scanned for fungal spore count under the microscope.



Fig. 2: Burkard Seven Day Volumetric Sampler for studying diurnal, seasonal and annual trends for fungal spores as well as pollen grains

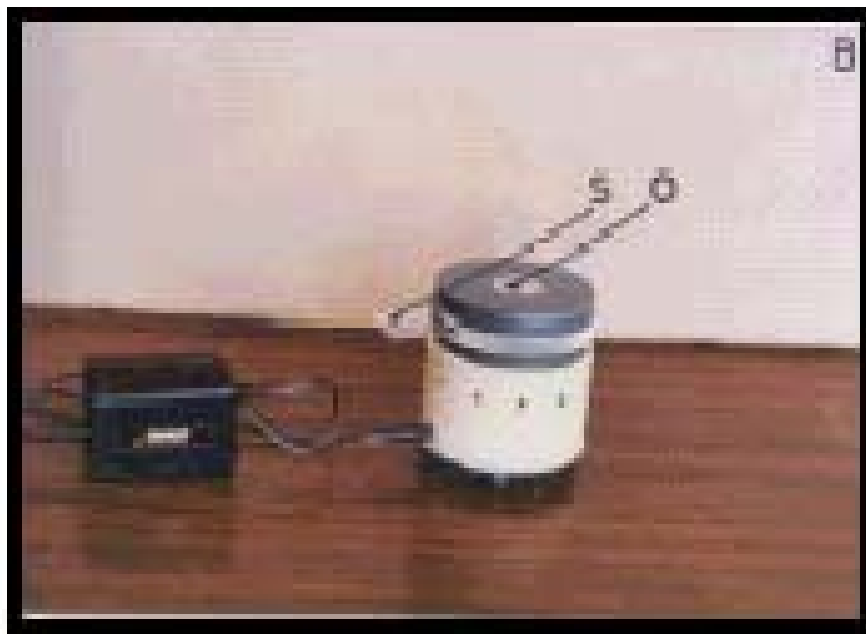


Fig. 3: Burkard Slide Sampler with inserted slide (S) coated with glycerine jelly on which the pollen and fungal spores get impacted through the orifice (O) for spot sampling

F. Burkard Petriplate Sampler

The Burkard Petriplate Sampler is similar to slide sampler except that it has a stage to hold the Petriplate and a sieve to cover the petriplate and on top a lid to cover the sieve. On the cover is an opening from where the air is sucked in (Fig. 4). These samplers are most convenient for outdoor and spot sampling and places where power connection is not available.



Fig. 4: Burkard Petriplate Sampler shown with a stage to hold the petriplate (P) containing nutrient media along with a sieve and a lid with an orifice (O) for fungal sampling (colony forming units)

G. Andersen Sampler

The best device to obtain culturable fungal spore count is the Andersen Six Stage Volumetric Sampler. It uses six petriplates in which media is kept under different sieve size present in decreasing order of pore size. Each sieve has 400 pores (Fig. 5). The sampler sucks in 28.3 L of air per minute. The air passes from the orifice to all the six petriplates before passing out. The particles of similar aerodynamic dimensions are impinged on the same plate. Now even two stage Andersen Sampler is available which is quiet efficient and less time consuming in number of plates to be examined.

H. Air-O-Cell Cassette

Air sampling cassette is a device for rapid collection and identification of wide range of airborne aerosols. The principle of the sampler is similar to that of Burkard Personal Sampler. The device is made up of plastic with two cells, namely upper cell and lower cell. The sampler can be run for 5 -10 minutes indoor at the suction rate of 5 L air per minute. After the sampling, the seal can be broken and the glass strip (trace) with the sample deposited is mounted in a clean glass slide with a suitable mountant. The slide can be scanned directly under the microscope. With this sampler the total spore in the aerosol can be counted. The quality and quantity of airspores within indoors can be determined. In this species level identification is not possible.



Fig. 5: Andersen Six Stage Volumetric Sampler with six petriplates kept under decreasing pore size of sieves for quantitative assessment of culturable fungi.

I. Liquid Impinger Sampler

Here the airborne spores are sucked in and suspended in a liquid in the sampler. The suction of the sampler enhances the airborne particles to get in to the flask, which contains sterile water. The suspended particles get dispersed into the liquid and further the liquid can be diluted and studied for the culturable molds using suitable medium. The multistage liquid impinger was devised to separate the collected particles into three fractions corresponding to the size in the upper respiratory tract, bronchi and bronchioles. In this sampler fungal spores can be identified upto species levels. Every principle for separating particle from air, from sedimentation, filtration, internal impaction, impingement in liquids, to thermal and electrical precipitation, has been applied to the collection of microorganisms. Changes in aerosol concentration with time can be followed with the rotation slit or slit-to-agar sampler in which a large petridish of medium is placed on a turntable beneath a stationary slit inlet.

Identification and Analysis of Airborne Fungi

Identification can be done by the following methods:

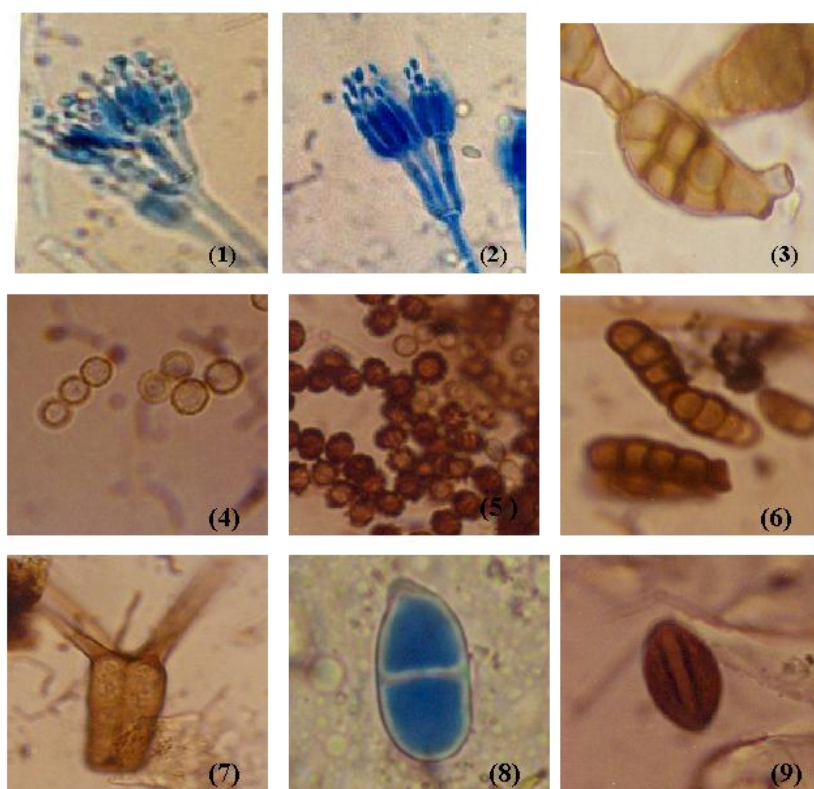
A. Culture Analysis (Petriplates)

This includes the tally of colonies produced in culture or semisolid media. In this the petriplates exposed for sampling are incubated at appropriate temperature and impacted spores are allowed to grow for a couple of days till colonies start forming. The colonies are identified based on their colony characteristics such as colour, shape and other morphological features of the mycelia and spores to the lowest taxonomic rank possible. Each colony represents one spore and considered colony-forming unit.

In addition, different atlases and literature can also be used for authentic identification.

B. Direct Microscopy (Slides)

The microscopic identification of distinctive particles is an approach validated by years of practical applications of both gravimetric and volumetric samplers. The exposed slides are examined directly by microscope because a variety of particles, including certain basidiospores, ascospores and spores of rust, smuts and downy mildew are recognizable but fails to grow on most laboratory media. The particles/spores are identified based on their characteristics such as shape, size and other morphological features of spores. Therefore it is a most dependable way to identify most of the fungal spores. Spores of some of the allergenically important fungi are shown in Fig. 6.



- | | | |
|---------------------------------|--------------------------------|---------------------------|
| 1. <i>Penicillium crinum</i> | 2. <i>P. oxalicum</i> | 3. <i>Alternaria spp.</i> |
| 4. <i>Aspergillus/penicilli</i> | 5. Smuts | 6. <i>Torula spp.</i> |
| 7. <i>Tetraploa spp.</i> | 8. <i>Trichothecium roseum</i> | 9. <i>Hypoxylon</i> |

Fig6. The photomicrograph of spores of some allergenically significant fungi

Analysis of data

After suspended particles have been collected on the slide or in a suitable medium, these particles can then be counted and identified. Environmental scientists looking for non-viable particles also used the techniques used to extract viable cells and particles carrying them from the air. The most efficient methods of removing suspended particles from the air, example, filtration through fine pore matrices, might be adequate for resistant forms of microorganisms, such as spores, but can be less damaged environmentally resistant vegetative cells. The absence of these sensitive cells from a sample could cause one to mistakenly conclude, thus, they were not present in the environment sampled. The total number of cells present can be estimated by microscopic examination, sometimes with the help of stains or fluorescent tags.

The concentration of fungal propagules counted from petriplates or on slides for spores are calculated as per the given formula:

$$\frac{\text{Total number of fungal colonies / Number of spores}}{\text{Total volume of air sampled}} \times 1000$$

The counts are expressed as number of colony forming units (CFU/m³) or number of spores per colony (spores / m³).

C. Immunoassay

Immunochemical analysis following descending elution, offers an analytic approach to dust without potential or defined form (e.g. – fungi, pollen, dander, seed pomace, arthropod effluvia etc). If micronic aerosols do carry pollen allergens, these fractions are also accessible to immunoassay in bulk samples obtained by high vacuum filtration. RAST and ELISA base procedures are followed. One advantage in immunoassay for airborne microorganisms is that the amount of materials needed to measure the concentration of viable air contaminants is much lower than that needed to gravimetrically quantitate nonviable particles where only one or a cluster of cell lands on an appropriate solid nutrient medium, or lawn of host cells, a microscopic fungal or bacterial colony, or viral plaque, will develop. These isolates can then be identified specifically using tests of biochemical and immunological reactions conducted on sub cultures of the original material. The researcher, therefore, is not limited to the original amount of the sample but can culture as much material as is needed for the various tests used for identification. The limitations are that a considerable experience is needed to identify particles by their morphology and to distinguish them from debris, and labeled antibodies for only a few clinically important microorganisms are readily available.

Atmospheric Microflora

The important airborne microflora from different parts of the world and India are broadly discussed in the following pages. The fungal spore counts in outdoor and indoor air vary considerably depending on various environmental and other factors. The prevalent weather conditions such as rain, humidity, wind speed and direction, temperature or the amount of sunshine may have direct and indirect effects on bioaerosols, the effect of which may be

immediate or cumulative. Dispersal of mold spores is linked intimately to precipitation and humidity. Certain ascospores and basidiospores require active rainfall for release of spores, whereas other Deuteromycetes are suppressed by precipitation. Fungal presence in indoor and outdoor air can be monitored with different samplers and samples can be analyzed by means of microscopy, culture, DNA probes, HPLC or immunodetection. Total fungal biomass can be estimated on the basis of measurements of ergosterol or glucan in environmental samples.

Outdoor Air Microflora

From USA, *Cladosporium* has been reported as the most dominant fungal genus on the West Coast along with *Alternaria*. In many countries basidiospores have been reported as predominant. In Spain and Denmark the dominant types that contributed more than 70% of the total catch were *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium*.

In Canada, Collins – Williams and Kachyk and Khan observed *Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus*, yeasts, smuts, rusts occurring most frequently in Canadian air. In UK, the major fungal forms observed in air are *Cladosporium*, basidiospores, ascospores, *Pullularia*, *Botrytis*, smuta, rusts, *Phoma*, *Epicoccum*, *Erisiphe* and *Alternaria*. A study from Derby showed that *Cladosporium* has the highest count followed by *Sporobolomyces*, *Tilletiopsis*, *Botrytis*, *Alternaria*, *Leptosphaeria*, *Ustilago* and basidiospores.

Spores of *Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Stemphylium* are chiefly encountered in Israel. Other common types are *Helminthosporium*, *Epicoccum*, *Fusarium*, *Mucor*, *Pullularia*, *Monilia*, *Botrytis*, *Rhizopus* and *Phoma*.

Ogunlana reported the prevalence of *Cladosporium*, *Curvularia*, *Fusarium*, *Aspergilli*, *Penicilli*, *Pithomyces*, *Aureobasidium*, *Geotrichum*, *Phoma*, *Rhizopus*, *Epicoccum* and *Neurospora* from Nigeria.

From China, the dominant forms reported are yeast, *Aspergilli*, *Penicillia*, *Hormodendron*, *Mucor*, *Curvularia*, *Alternata* and *Fusarium* in order of their prevalence. In Australia, *Cladosporium* spp have been found to be a major component of the airspora in Australia. It is followed by *Leptosphaeria*, *Epicoccum nigrum*, *Nigrospora*, *Geotrichum*, *Neurospora*, *Penicillium* and *Aureobasidium*.

In Almeria (Spain), Sabariego detected spores of *Alternaria* and *Cladosporium* throughout the year on air samples collected. The diurnal patterns of these taxons reflected a similar presence of spores during a 24-hr period. The correlation of meteorological parameters on fungal presence showed a positive association with temperature, hours of sunshine and accumulate rainfall, but negative with daily rainfall.

A survey carried on the culturable airborne fungi revealed higher fungal concentrations in the greener area around the Research Center for Eco-Environmental Sciences (RCEES) and Beijing Botanical Garden (BBG) than in the densely urban and highly trafficked area of Xizhimen, however the difference was not significant. *Penicillium* was the most abundant and *Cladosporium* species were the most dominant fungal group, followed by non-sporing isolates, *Alternaria*, *Pencillium* and *Asperigillus*.

In Germany, Wittmaack studied bioaerosols suspended in ambient air using single-stage impactors at a semiurban site in southern during late summer and early autumn. The observed

bioaerosols include fungal spores, hyphae, insect scales, hairs of plants and, less commonly, bacteria and epicuticular wax.

Airborne surveys for fungi have been reported from different parts of India as well. Dominant forms reported from Vishakhapatnam and Gulberga are *Cladosporium*, *Aspergillus*, *Nigrospora*, *Alternaria*, *Curvularia*, basidiospores, ascospores, *Helminthosporium* and *Periconia*. From Mysore, Ramalingam reported high concentrations of *Cladosporium* spp, smuts and *Epicoccum*.

Studies carried out in Gaya, Gauhati and Calcutta revealed that *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Curvularia*, *Helminthosporium*, *Aureobasidium*, *Neurospora*, *Mucor* and *Nigrospora* are the major types reported recorded from Eastern India.

In India, many reports provide information of prevalence of fungi in ambient air. *Alternaria* is reported as the dominant fungal type from Delhi.

From Pune and Kolhapur, the dominant fungal forms isolated are *Cladosporium*, *Alternaria*, *Curvularia*, *Nigrospora*, *Periconia*, *Helminthosporium*, smuts, rust, *Aspergillus* and *Penicillium*

In Delhi, a survey conducted for culturable and non-culturable fungi reported 98 fungal forms with *Cladosporium* contributing 25-40% of total airborne fungi followed by *Ustilago* (smuts) (24%) *Aspergillus flavus* (10-13%), *Alternaria* (11%) and *A. niger* (8%). Basidiomycetes contributed 7-13% at different sites.

A volumetric paired assessments of airborne viable and non-viable fungi in five outdoor sampling stations in a rural agricultural area of India concluded that: (i) a rich fungal airspora existed in the rural study area, (ii) to achieve representative information on the total airborne fungal spores of an area, the monitoring in multiple sampling stations is preferable over a single sampling station; for viable fungi, however, one station can be considered, (iii) the percentage of airborne fungal viability is higher in rural agricultural areas

Indoor Air Microflora

The spectrum of indoor airborne mold spores, such as in homes, offices, and other workplaces, differs from place to place due to the influx of spores from outdoor air through ventilation and air exchangers. Hence, it is difficult to arrive at any significant conclusion on the role of the indoor mold spore in the allergic response. Again, it is not always the quantity but allergenicity of the mold, which determines the overall development of clinical allergy. Sampling methods used to evaluate indoor environments include air sampling for spores, measurement of allergens, and determination of microbial generated volatile organic compounds, ergosterols, glucans, and mycotoxins, as well as environmental conditions that lead to fungal contamination

Ren concluded that presence of fungal propagules in indoor air couldn't be reliably predicted by home characteristics. Actual measurements are required for fungal exposure assessment, and the use of only one medium to collect samples in one location in a home might be adequate to represent residential levels of fungi in indoor air.

In United States, a very large study of airborne indoor and outdoor fungal species and concentrations conducted on fungal air samples from buildings showed that the culturable airborne fungal concentrations in indoor air were lower than those in outdoor air. *Stachybotrys chartarum* was identified in the indoor air in 6% of the buildings studied and in the outdoor air of 1% of the buildings studied. In US cities, O'connor examined the spectrum and concentration of fungi in the air inside and outside of the homes mold-sensitive children with asthma in urban communities. The concentrations of fungi were higher in homes with dampness problems, cockroach infestation, and cats.

Hayes analyzed air samples from offices in which DNA from air sample was extracted, DNA amplification of fungal and bacterial DNA was performed. The data showed that fungal fauna other than *A. niger* or *A. flavus* is present in 87.2% of the samples.

A comparison of MAS-100 and the Andersen air samplers' performances was made and a similar trend in both instruments was observed in the microbial contamination levels in samples of offices, hospitals, industries, and shopping centers, in Rio de Janeiro city. The industries' results showed more important similarity among fungi and total heterotrophs distributions. All indoor air samples distributions were very similar. The temperature and air humidity had no significant influence on the samples dispersion patterns in indoors.

At Vishakhapatnam, a total of 8909 and 9327 CFU/m³ were recorded in from inside and outside, respectively for the 47 types identified. The dominant fungal types were *Cladosporium*, *Penicillium nigricans*, *Aspergillus versicolor*, and *Aspergillus oryzae*. While in Solan in Shimla sampling conducted in a wet house revealed *Penicillium* as the most dominant types contributing 30.7% followed by *Aspergillus* sp. (15.4 %), *Alternaria* sp (10.5%). In the same place sampling conducted in a mud house revealed *Aspergillus* sp. At 35.1% as the most dominant contributor followed by *Penicillium* (26.9%). The other dominant types were *Alternaria*, *Cladosporium*, *Curvularia* and *Fusarium*.

In West Bengal, the volumetric assessment of airborne culturable and nonculturable fungal spore showed higher frequencies of *Aspergilli* /*Penicilli*, *Cladosporium*, *Alternaria*, and smut spores by Burkard Sampler whereas Andersen Sampler showed the prevalence of *Aspergillus niger*, *Aspergillus flavus* and *Cladosporium cladosporioides* in large rural indoor cattle shed.

In Delhi, an indoor survey of fungi in the homes of asthmatic / allergic children revealed highest fungal load in the month of January while the lowest in June in indoors (Fig. 7). A high viable mold concentration was observed in the homes of asthmatic children in Delhi. The predominant fungal types observed were *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *Alternaria* spp, *Cladosporium* spp, *Penicillium* spp, *Rhizopus* spp, and *Curvularia* spp etc. The houses in Delhi contain rich and varied concentration of fungi, almost parallel to what is encountered just outside the air.

Besides the outdoor environment and indoor, work environment are also greatly influenced by fungi especially occupational sites employing organic raw materials e.g. granary, poultry, flour mills, bakery, sugar factory etc. Survey conducted at working environments by Singh and his students in bakery, poultry, sugar factory and libraries in Delhi revealed, *Aspergilli*-*Penicilli* and smut spores as significant contributors in indoor air.

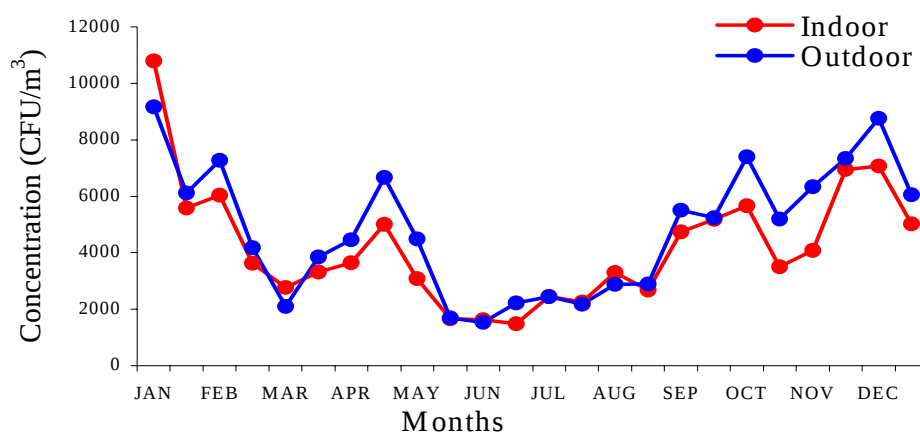


Fig. 7: Seasonal variation in total fungal concentration (CFU / m3) in indoor and outdoor of patients' residences

Poultry

A total of 17 fungal forms were reported from Guwahati out of which *Cladosporium herbarum* was the most dominant followed by *Aspergillus schari* and *Penicillium spp.* In Gwalior 21 fungal types were reported from the poultry. The most dominant being *Aspergillus niger* (24.6 %) followed by *Cladosporium spp* (22.3%), *Penicillium sp.* (10.7 %), *Aspergillus flavus* (9.1 %), *Botrytis*, *Fusarium*, *Aspergillus glaucus*, *Aspergillus fumigatus* and *Curvularia* were the other dominant species. While in Bangalore the *Aspergillus sp.* (531 CFU) were the most dominant contributors the environment followed by *Penicillium spp.* (301 CFU) and *Cladosporium sp.* (169 CFU). *Mucor* and *Rhizopus* were amongst the other dominant types. A total of 14,164 and 12,837 colonies were recorded inside and outside, respectively from a poultry farm in Vishakhapatnam. A total of 54 fungal types were reported out of which majority belonged to *Aspergillus* and *Penicillium* group. The other dominant fungi recorded were *Cladosporium*, *Penicillium nigricans*, *Trichoderma* etc.

Garbage Storage

A total of 11,040 and 10,630 col/m3 were recorded in Vishakhapatnam from inside and outside, respectively. The dominant fungal types were *Aspergillus niger*, *Cladosporium*, *Aspergillus versicolor*, *Aspergillus fumigatus*, *Mucor* etc.

Jaggery Godown

A total of 12,855 and 10,570 col/m3 were recorded in Vishakhapatnam from inside and outside respectively. The dominant fungal types were *Aspergillus niger*, *Cladosporium*, *Aspergillus versicolor*, *Penicillium brefeldianum*, *Curvularia* etc.

Library

A survey conducted in a library in Gwalior, *Cladosporium* species (30.3 %) inside and (38.2 %) outside was reported as the most dominant fungal type followed by *Aspergillus niger*

(20.5%) inside and (15.2 %) outside. Other dominant typed includes *A. teneus* and *Fusarium* sp. Another survey conducted in Bangalore City library also showed *Cladosporium* species (51.6%)

Dairy Farm

A total of 9000 CFU/m³, 8500 CFU/m³, 940 CFU/m³, 10,110 CFU/m³, were recorded from reception, processing area, packaging area., cold storage and outside air, respectively in Vishakhapatnam. The dominant fungal types were *Aspergillus niger*, *Cladosporium*, *Curvularia*, *Aspergillus nigricans* in reception; *Aspergillus niger*, *Cladosporium*, *P. nigricans* in processing area and packaging are; *Aspergillus niger*, *Cladosporium* in outside air. Very few colonies were recorded in the cold storage.

Dust Microflora

In California, the analysis of the surface dust from residential environments revealed that it does not appear reasonable that the frequently quoted total fungi concentration exceeding 10(5) CFU/g is definitive evidence that a residential surface is contaminated with unusual amounts of culturable fungi. The use of settled dust results alone to establish the presence of unusual fungal types or concentrations appears to be inappropriate.

In Sweden, in an effort to relate microbial exposure indoors to well-being and health, an integrated methodology for characterizing the microbiology of indoor environments where specific microbial monomeric constituents in building materials and inhalable house dust particles are determined by using mass spectrometry-based methods utilizing important new capabilities of chemical marker.

In Swiss sawmills, the assessment of wood workers' that process mainly coniferous wood species, exposure to fungi revealed that *Penicillium* sp. were the predominant fungi.

Bioassay of Fungal Allergens

Clinical / Immunological Studies

The biological and immunological assay of fungal allergens is carried out to find out the sensitization in hypersensitive patients for asthma and rhinitis is briefly described here.

Skin Prick Test: Lyophilised extracts from antigen are reconstituted in 1:10 (w/v) concentration in 50% glycerinated buffer. Skin Prick Test is carried out for patients with different exyacts. A drop of each antigen is placed on precleaned volar surface of the arm with the applicator and pricked with 23 G needle at an angle of 45o. The skin is lifted and needle withdrawn after the prick. Excess antigen is wiped off with the tissue paper. Along woith antigen, diluent and histamine (1 mg/ml) are also used as negative and positice control. Based on the wheal diameter skin reactions are graded.

Underneath the lining of the skin are mast cells, which targets at worms and parasites. Mast cells are also armed with proteins called IgE antibodies, which act as remote sensors in the local environment. For e.g. a person allergic to *Aspergillus flavus*, will have IgE antibodies capable of recognizing the shape of the above fungal allergens, in much the same way that a

lock "recognizes" the shape of a key. This triggers the mast cells to release their contents into the tissues, triggering an allergic reaction thereby causing a wheal and flare response

Intradermal: It is another type of skin test in which a small amount of very dilute allergen (0.01 ml) is injected into the upper layers of the skin to make a bleb of 2-3 mm., normally using a diabetic insulin syringe. Phosphate Buffer Saline and Histamine (100 ug/ml) are also injected as negative and positive controls, respectively.

It is a more painful test than skin prick testing. It is more sensitive however more likely to lead to false positive and clinically irrelevant results. For this reason, it is more commonly used for evaluation of patients with sensitivity to antibiotics or insect venom.

Patch Testing: It is useful for the assessment of contact allergic dermatitis which are triggered by nickel metal, cosmetic preservatives or various plants etc. The allergens are mixed with a non-allergic material (base) to a suitable concentration. They are then placed in direct contact with the skin, usually on the upper back, within small aluminium discs. Adhesive tape is used to fix them in place, and the test sites are marked. The patches are left in place for 48 hours, after which the reading is taken.

Provocation Testing: The suspected allergen is introduced directly into the nose, lung or eye to see if they can provoke allergic reaction. In Bronchial provocation testing, hyperresponsive airways is identified and characterized by having the patient inhale an aerosolized broncho-spastic agonist (such as methacholine or histamine). Results of pulmonary function tests (eg, spirometry, specific conductance) performed before and after the inhalation of increasing concentrations of the agonist are used to quantitate response.

This test also helps in allowing testing with vapors and fumes in the same form as that encountered in workplace settings however exposure is to a single high dose of an allergen corresponding to months or years of natural exposure.

Assessment of Allergen Specific IgE

ELISA: It utilizes two antibodies, of which first is specific to the antigen and the second antibody is coupled to an enzyme. This second antibody gives the assay its "enzyme-linked" name, and causes a chromogenic or fluorogenic substrate to produce a signal.

The specific IgE detects antigen-specific IgE antibodies in the patient's serum. They are useful when testing for inhalant allergens (pollens, molds, dust, mites, animal danders), foods, insect stings, and other allergens such as drugs etc. This is often (but not always) raised in people with allergies and in those with internal parasites. Nevertheless, an elevated IgE does not prove that symptoms are due to allergy and a normal IgE level does not exclude allergy. Thus measuring IgE levels has a limited role to play in assessing patients with possible allergic conditions.

RAST (Radio Allergo Sorbent Test): It attempts to correlate the presence of allergy to serum levels of antigen-specific IgE as an index of allergic reactivity. This procedure is very much similar to the above one measuring antigen specific IgE. However, the antigen (allergen) is covalently bound to a cellulose disc. The availability of much more antigen on the disc permits the high sensitivity to bind the small quantities of IgE present in the test

serum. In this the antibody is detected by a radiolabeled ligand, and the radioactivity of the plate is counted on a Gamma counter.

Eosinophil Counts: Eosinophils are specialized white cells that are designed to kill worms and parasites. They also can cause inflammation in the tissues in allergy. High levels are observed in blood samples from people with hay fever, asthma and atopic eczema etc. Nevertheless, an elevated eosinophil count does not prove that symptoms are due to allergy, and a normal eosinophil count does not exclude allergy. Thus measuring eosinophil counts has a limited role to play in assessing patients with possible allergic conditions.

Histamine Release Assay: It is a technique to evaluate the *in vitro* release of histamine from leukocytes (i.e., basophils) in response to exposure to an allergen, and thus is designed to provide an *in vitro* correlation to an *in vivo* allergic response (i.e., skin prick testing). Measurements of histamine release required isolation of leukocytes from whole blood followed by the isolation of the released histamine. Recently, a special type of glass fiber has been developed that binds histamine with high affinity and selectivity to be used as a "solid phase" to absorb the histamine that is released directly into the blood.

Allergy is caused by an exaggerated immune response to the antigenic stimuli. These IgE mediated reactions are caused by the interaction of mast cells (and eosinophils) coated with allergen specific IgE and a cross linking allergen (Fig 8). The clinical outcome is inflammation commonly displayed by urticaria, rhinitis, vomiting and diarrhea, depending on the route of allergen entry. In extreme reactions, anaphylactic shock can result leading to death. Chronic allergic responses most commonly present themselves as asthma and eczema. All these symptoms are the consequence of an imbalanced immune system making an unsuitable response to an environmental or food allergen.

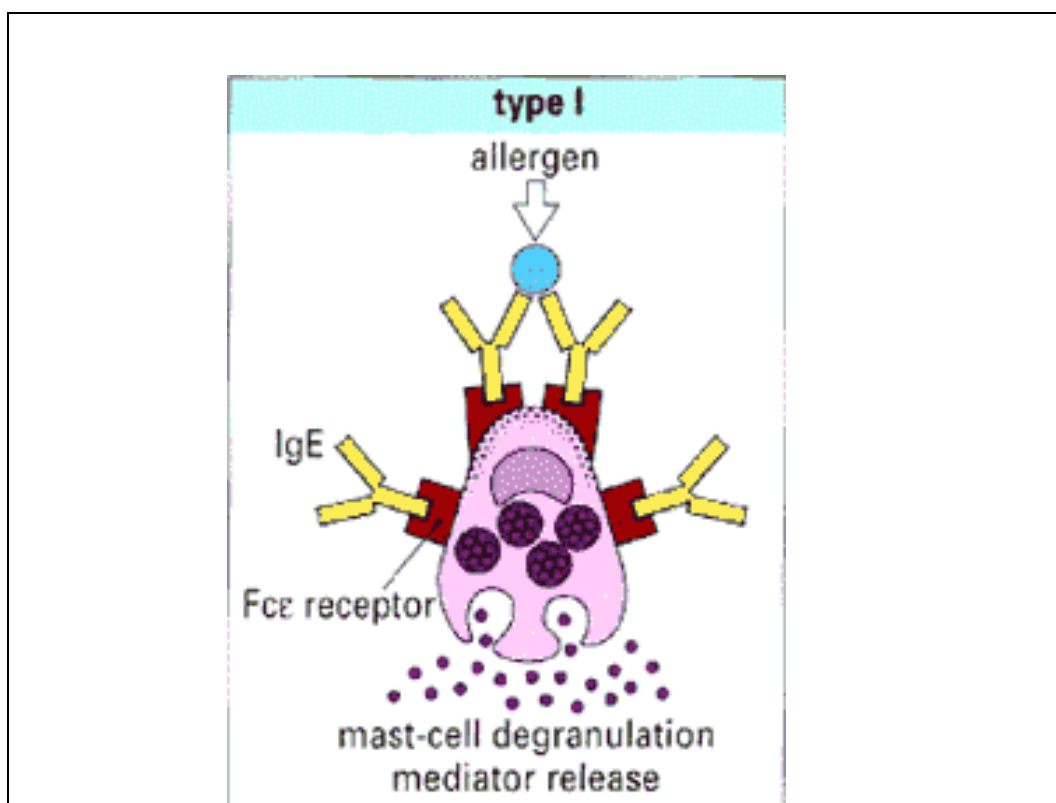


Fig. 8: Release of mediators on secondary encounter with allergen

Fungi which contributes to environmental allergen remain suspended in the environment and on inhalation by humans induces different symptoms of respiratory disorders in hypersensitive individuals. Fungi are cosmopolitan in nature and possess highly evolved spore liberation mechanism. The spores are generally small (3 to 100 μ) and remain suspended in the atmosphere for a long time, from a few hours to several days. While suspended, they are inhaled by humans and induce different symptoms of respiratory disorders in hypersensitive individuals. The spore after inhalation gets deposited through pharynx, larynx and trachea into upper respiratory airway or lower respiratory airway. The aerobiological pathway of the release of spores, dispersion and deposition on plants, animals as well as human system is explained in Fig. 9.

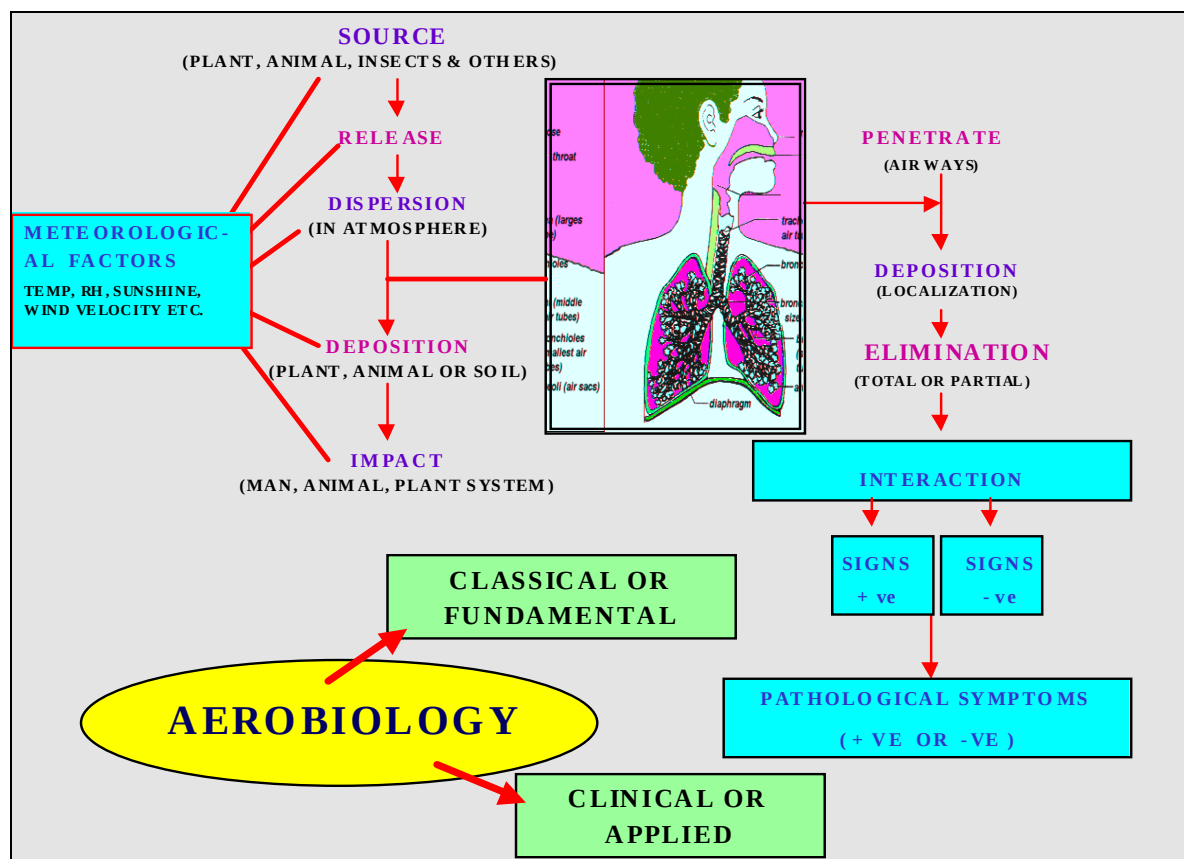


Fig. 9: Aerobiological and clinical pathways involved in Hypersensitivity reaction

The most important group of air-disseminated fungi that causes respiratory allergic diseases in humans is the conidial fungi, which comprise the form class Deuteromycetes. Most of the spores produced by the imperfect fungi vary in shape, size, texture, color, number of cells, thickness of the cell wall, and methods by which they are attached to each other and to their conidiophores. Some of the important fungi associated with allergic diseases in Type I and TYPE III hypersensitivity are:

Type I Hypersensitivity

Type I allergic disease to fungal allergens are typically manifested either as rhinitis (hay fever) or as asthma. Allergic reactions, including respiratory allergy, may occur in two

phases. The early-phase reaction occurs within minutes as a result of the release of preformed mediators. The most common fungi associated with the disease are *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botrytis*, *Cephalosporium*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Gliocladium*, *Helminthosporium*, *Paecilomyces*, *Penicillium*, *Phoma*, *Scopulariopsis*, *Stachybotrys*, *Trichoderma*, *Trichophyton*, *Trichothecium*, *Ulocladium*, *Saccharomyces*, *Candida*, *Epicoccum*, *Stemphylium* and some others.

Type III Hypersensitivity

Type III hypersensitivity is mediated by immune complexes essentially of IgG antibodies with soluble antigens. It involves late-phase responses, which occur 3 to 4 hr after allergen exposure as a result of cellular infiltrates responding to early-phase mediators. A dual reaction involves both early and late phase reactions. It involves circulating antibody that reacts with free antigen. These circulating complexes can then become deposited on tissues. Tissue deposition may lead to reaction with complement, causing tissue damage. This type of hypersensitivity develops as a result of systematic exposure to an antigen and is dependent on i) the type of antigen and antibody and ii) the size of the resulting complex. It has a lot in common with type I except that the antibody involved is IgG and therefore not prebound to mast cells, so that only preformed complexes can bind to the low affinity FcγRIII. IgG mediated allergic disease has been reported from following fungi *Aspergillus fumigatus*, *Aspergillus nidulans*, *Candida spp*, *Penicillium spp*, *Thermoactinomyces*, *Acremonium sp.*, *Alternaria sp.*, *Botrytis sp*, *Cladosporium sp.*, *Trichoderma sp.*

Molecular Characterization of Fungal Allergen

Fungal allergens are major causative agents of atopic disorders. The major problem in diagnosis is lack of standardized and well-characterized fungal extracts. Immunochemical and molecular characterization of fungal allergens has been hampered by the lack of pure proteins and to inherent variation among fungal proteins and in their poor yield. The advents of molecular cloning technology and the development of phage surface display technology for cloning genes have facilitated the isolation of more relevant recombinant allergens. The knowledge of the primary, secondary, and tertiary structures of these allergens, the immunodominant regions of these proteins and their interaction with T and B – cell epitopes, results in better understanding of the molecular mechanisms of allergy and may open new frontiers of immunological intervention to treat patients. Some of the major fungal types characterized on molecular basis are contributed in the Table 1. These protein fractions were purified using techniques like lectin affinity chromatography, gel filtration, electroelution and high-pressure liquid chromatography characterization was carried by immunoblot, ELISA and protease assays.

Prevention and Treatment of Allergy

Incomplete understanding of the roles of both the intrinsic immune system and the environmental causes of allergic disease has necessitated an approach to allergy prevention that starts with the experience while not fully understanding the causes. It is not therefore surprising that both primary and secondary preventative strategies are only partially successful at best.

Table 1: Major Allergenic Protein Fractions of some of the Allergenically important Fungi

S. No.	Fungi	Major allergens (kDa)			
1.	<i>Penicillium notatum</i> .	33	68	32	28
2.	<i>Penicillium citrinum</i> .	39-	33	70	
3.	<i>P. oxalicum</i>	34	30	16	
4.	<i>Penicillium chrysogenum</i>	67	43	32	34
5.	<i>Aspergillus oryzae</i>	34			
6.	<i>Aspergillus flavus</i>	34-			
7.	<i>A. fumigatus</i>	44			
8.	<i>Drechslera monoceras</i>	14.4	36		
9.	<i>Fusarium solani</i>	65	45		
10.	<i>Alternaria alternata</i>	31			
11.	<i>Epicoccum purpurascens</i>	33.5			
12.	<i>Epicoccum nigrum</i>	43	26	17-	
13.	<i>Curvularia lunata</i>	80	37-		
14.	<i>Cladosporium herbarum</i>	63	36-		

1. Avoidance of allergen exposure

Fungal exposure can be reduced by some simple procedures like preventing spore infiltration by closing doors, windows, and using air conditioning, controlling moisture by means of dehumidification, seal water leaks (ventilate bathrooms and kitchens and use air conditioning during the summer months and times of high humidity), and use dehumidifiers. Apart from this clean and remove contaminated materials by applying fungicides (chlorine bleach with detergent or quaternary amine preparations), use high-efficiency air filters, maintain heating ventilation and air-conditioning systems; and use personal protective equipment (particle mask when involved in cleaning contaminated materials). However, avoidance is not possible in case of multiple sensitization or in case e.g. where the offending agent is unknown

2. Pharmacotherapy

The purpose of Pharmacotherapy is to make the patient symptom free. Among the therapies described at the recent annual meeting of the American Academy of Allergy, Asthma, and Immunology were:

Salmeterol: Patients who use salmeterol 42 mcg bid or other asthma therapies such as beta-agonists did not have a higher-than-expected incidence of cardiac arrhythmias.

Fluticasone: Novel dosage forms called the Diskhaler and the Diskus have been found to effectively deliver therapeutic doses of the drug to asthmatic patients.

Triamcinolone: This drug has been reformulated with a non-CFC inhalant propellant and has been found to be effective and well tolerated in pediatric asthmatic patients (Poster 1328).

Pranlukast: This orally active, investigational leukotriene receptor antagonist improved pulmonary function and asthma symptoms; it simultaneously reduced the use of rescue albuterol.

The improvements were noted to occur as quickly as within 1 week and were sustained for the 12 weeks of the study.

3. Allergen Immunotherapy

Allergen Immunotherapy (also called allergy vaccine therapy) involves the administration of gradually increasing quantities of specific allergens to patients with IgE-mediated conditions until a dose is reached that is effective in reducing disease severity from natural exposure. The major objectives of allergen Immunotherapy are to reduce responses to allergic triggers that precipitate symptoms in the short term and to decrease inflammatory response and prevent development of persistent disease in the long term. Allergen Immunotherapy is safe and has been shown to be effective in the treatment of stinging-insect hypersensitivity, allergic rhinitis or conjunctivitis, and allergic asthma. However, it is not effective in the treatment of atopic dermatitis, urticaria, or headaches and is potentially dangerous if used for food or antibiotic allergies. Safe administration of allergen Immunotherapy requires the immediate availability of a health care professional capable of recognizing and treating anaphylaxis.

Patients should not be taking beta-adrenergic blocking agents when receiving Immunotherapy because these drugs may mask early signs and symptoms of anaphylaxis and make the treatment of anaphylaxis more difficult. Unlike antiallergic medication, allergen Immunotherapy has the potential of altering the allergic disease course after three to five years of therapy.

Future Priorities in Fungal Allergy

New developments in Immunotherapy

Recent animal and human studies using fragments of DNA attached to allergen offer the prospect of stimulating a potent anti-allergic immune response without the risk of allergic reactions. These vaccines are currently being trialed in humans, and have shown promising results in animal studies. Such methods offer the possibility of developing preventative allergy "vaccines" that might prevent the onset of disease if administered to children at high risk. From time to time, studies describing more convenient and less frequent treatments have been described, but these are not currently commercially available

The introduction of recombinant allergens has undoubtedly allowed better standardization of allergen extracts and affords the opportunity for individualized treatment, which is tailor made according to individual sensitivities. However, at present, there are no published trials of recombinant allergens for Immunotherapy

The use of short T cell peptides for immunotherapy has the potential to stimulate "protective" Th1 and/or T regulatory responses whilst avoiding systemic side effects associated with cross linking of IgE on mast cells and basophils, which is the risk associated with conventional whole allergen extracts. Initial studies in cat allergy are encouraging, although, again, further studies are required.

TH₂ to TH₁ shift: It is hypothesized that transit through this critical window, with active minimization of allergen exposure and respiratory syncytial virus infection, will reduce the development of persistent disease. The incidence of occupational asthma in adults and acquisition of allergic diseases in immigrants to westernized countries from countries where

protective factors would be expected to operate in infancy. To the extent that allergic responses can develop throughout life, the success of strategies applied in infancy will be limited

The primary reasons are suggested to lie in sensitization to multiple allergens and the ubiquity of opportunities for exposure at different domestic sites coupled with the partial effectiveness of the interventions.

The relentless increase in the prevalence of allergic diseases in the last century in affluent countries, their low prevalence in some populations living “indigenous” or “rural” lifestyles, the 2- to 3-fold differences in prevalence of different population groups within US cities, and the remission in disease in low-allergen environments, such as Antarctica and mountain sanatoria, all point to the powerful and fundamental role of the environment in driving both expression of disease and its symptom severity. The lower allergic prevalence in children in anthroposophic communities or in the general community 50 years ago also suggests that some smaller shifts in lifestyle within a broader allergy-prone environment might prove to be helpful. There is an urgent need to further understand the complexity of the interactions of lifestyle and environment on factors governing susceptibility and protection as it relates to the induction and expression of allergic diseases.

Conclusion

It is thus concluded that there is a rich fungal biodiversity in Indian environment both outdoors and indoors. However, there is an urgent need to organize all the information available in the form of seasonal calendars and all allergic fungal types enlisted should be made readily available for the use of common man and physicians as a diagnostic tool.

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