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CLINICAL ALLERGY

2nd Edition

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Contents

1. **Immune Response in Health and Allergic Diseases: An Overview** 1
   MK Agarwal, Shashi Chawla

2. **Epidemiology of Allergy: Global** 25
   Nikhil Gupta, Rajendra Prasad

3. **Epidemiology of Respiratory Allergy in India** 28
   Raj Kumar, Rajendra Prasad

4. **Aeroallergens in India: Current Status** 31
   AB Singh

5. **Cross-reactivity for the Practicing Clinician** 48
   Harshveer Singh Malhi

6. **Role of Aerobiological Monitoring in the Practice of Allergy** 55
   Shripad N Agashe, Jacob N Abraham

7. **Significance of Pollen Calendar in Management of Allergy** 57
   Asha Khandelwal, Rajendra Prasad

8. **Allergen Preparation and Standardization: An Overview** 76
   AB Singh, Chandni Mathur

9. **Diagnosis of Allergy: Clinical** 82
   Rajendra Prasad, Nikhil Gupta

10. **Diagnosis of Allergic Diseases In Vivo: Skin Tests** 86
    VK Jain, Shubhra Jain, Abhimanyu Garg

11. **Laboratory Diagnosis of Type I Allergic Respiratory Disorders: An Extract-based to Molecular-based Approach** 94
    MK Agarwal, Shashi Chawla

12. **Patch Test** 105
    Mahesh Goyal, Aditya Goyal, Srishti Sareen

13. **Provocation Tests in Diagnosis of Allergy** 111
    Suresh Koolwal, Roopam Sehajpal

14. Diagnosis in Allergy: Serology—Component-resolved Diagnosis 119
PC Kathuria

15. Prevention of Allergy 124
VK Jain, Rajendra Prasad

16. Preventive Measures and Devices in Management of Inhalant Allergy Disorders 131
Komarla Nagendra Prasad

17. Treatment of Allergic Diseases: Antihistaminics 142
RC Saxena, Mona

18. Allergen Immunotherapy: General Guidelines 151
VK Jain, Rajendra Prasad, Shubhra Jain

19. Cluster Allergen Immunotherapy 158
PC Kathuria

20. Future of Allergen-specific Immunotherapy 163
Sagar L Kale, Swati Sharma, Bharti Arora, Naveen Arora

21. Noninjective Allergen-specific Immunotherapy 171
Komarla Nagendra Prasad

22. Sublingual Immunotherapy 179
BV Balachandra, PK Vedanthan

23. Quality of Life in Allergic Diseases 188
VK Jain

24. Allergic Rhinitis 193
Ashok Shah

25. Ocular Surface Immune Response and Allergies 211
Manila Khatri, Swati Priyadarshini, Ankita, Sandeep Saxena

26. Urticaria 218
Ramji Gupta

27. Angioedema 222
PC Kathuria

28. Atopic Dermatitis 229
Mahesh Goyal, Aditya Goyal, Srishti Sareen

29. Drug Allergy 234
Sitesh Roy

30. Epidemiology of Food Allergy: Special Reference to Bronchial Asthma 260
Raj Kumar

31. Food Allergy: Diagnosis and Treatment 263
PC Kathuria

32. Evaluating Potential Allergenicity of Genetically Modified Food Crops 270
Ankita Mishra, Naveen Arora

33. Insects as Source of Inhalant Allergens 277
MK Agarwal, Shashi Chawla

34. Latex Allergy 287
PA Mahesh

35. Human Seminal Plasma Allergy 293
Ashok Shah

36. Aspirin Allergy 297
Kiran Vishnu Narayan

37. Aspirin-exacerbated Respiratory Disease 310
B Vidyasagar, BJ Arun

38. Anticonvulsant Hypersensitivity Syndrome 317
Arun Raja V, Suneetha P, Alladi Mohan

39. Hymenoptera Sensitivity 326
Narmada Ashok, PK Vedanthan

40. Radiocontrast Allergy 338
Richa Gupta

41. Allergic Reactions to Anesthetics 341
PS Shankar

42. Cow's Milk Protein Allergy 345
Major K Nagaraju

43. Pregnancy and Allergy 355
Kiran Vishnu Narayan

44. Allergy March **363**
PA Mahesh, Amrutha D Holla

45. Allergy and Infection **371**
Anura Weerasinghe

46. Allergic Bronchopulmonary Aspergillosis **373**
Rajendra Prasad, VK Jain, Nikhil Gupta

47. Allergic *Aspergillus* Sinusitis **380**
Ashok Shah

48. Pediatric Allergic Disorders and their Management **385**
Shally Awasthi, Shambhavi Mishra

49. Biomarkers in Allergy **404**
Naveen K Bhatraju, Anurag Agrawal

50. In Vitro Testing for Specific IgE **417**
Major K Nagaraju, N Karthik

51. Diet and Allergy **423**
Surya Kant

52. Need of Allergy as a Medical Specialty **428**
Rajendra Prasad

53. Allergen Avoidance in Naso-bronchial Allergy **430**
VK Jain, Shubhra Jain, Girish Jain

54. Guidelines for Practice of Allergen Immunotherapy in India: 2017-An Update **437**
SN Gaur, Raj Kumar, AB Singh, MK Agarwal, Naveen Arora

Index *473*

CHAPTER 4

Aeroallergens in India: Current Status

AB Singh

INTRODUCTION

Man is dependent on air. He breaths about 35 lb of air per day when compared to consumption of 3 lb of water and 1.5 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 number of inorganic and organic contaminants. Airborne bioparticles (biopollutants) such as microorganisms, pollen grains, fungal spores, hyphae of fungi, spores of mosses and ferns, insect debris, animal dander, and mites are known to be the chief causative agents of allergic disorders such as bronchial asthma, allergic rhinitis, and atopic dermatitis.[1-6] Among all these agents, pollen grains and fungal spores are the most predominant allergens in air. A detailed information on their daily, seasonal, and annual variations in the atmosphere is of paramount importance in effective diagnosis and therapeutic management of these ailments.

Pollen grains are among the earliest known allergens and are at present the major source of morbidity among atopic subjects.[7] Respiratory system is the direct target organ of airborne pollens taken in by inhalation. This results in immediate hypersensitivity disorders in genetically predisposed individuals and late hypersensitivity in others, causing clinical manifestations of allergic rhinitis, allergic alveolitis, asthma, atopic dermatitis, etc. John Bostock[8] was the first to suspect pollens as the cause of hay fever (allergic rhinitis). Later, Blackley[9] established that grasses are the important cause of hay fever in the UK. After more than 40 years, Scheppegrell[10] from the USA felt the need for field exploration and aerial surveys to record aeroallergens from the atmosphere. Subsequent studies from all over the world established pollen grains as the major causative agent for respiratory allergic disorders.[5,6,11-17]

Although pollen grains have 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.[18-21] Fienburg[22] 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 molds—indoors and outdoors, all over the world. Since then, several investigators from different geographical locations have investigated the role of fungal spores in allergic diseases.[16,23-29]

SOURCE OF AEROALLERGENS

Pollen Allergens

Plant spores, in a broader sense, are produced in all the divisions of the plant kingdom, but only those formed by the flowering plants, known as pollen grains, are the major component of air spora. Wind-pollinated (anemophilous) taxa are the chief source of inhalant pollen allergens including grasses, weeds/cultigens, and trees. The flowers of these taxa possess a set of adaptive characteristics such as reduction in size and number of floral appendages, lack of nectar, and small, smooth, dry, simple, and buoyant pollen grains. The amount of pollen production is also high in such species.[30]

The airborne allergenic pollen grains are traditionally grouped together as grasses, weeds, or tree pollen.

This is done merely because of convenience as they follow a generalized pattern of flowering in different seasons in the year. However, exceptions to generalized flowering pattern do occur. For example, *Prosopis juliflora* erratically flowers almost throughout the year, but with two peak pollination periods, i.e., in March–April and September–November. However, difference in the flowering pattern of the taxa is not uncommon for many species in India as reported by Singh and coworkers.[31,32]

Fungal Allergens

There are more than 80,000 species of fungi, which have an 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 environments, the sources of fungal spores include cereal crops, decaying vegetables, and organic wastes on which fungi thrive. While in indoor environments, 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 (Deuteromycetes) with little information on allergenicity of the members of other fungal groups. The literature is still insufficient regarding the development of allergic asthma by inhaled fungal spores,[33] and there is very little information available on the characterization of antigenic fractions of common fungal allergens.

MONITORING AIRBORNE ALLERGENS

For effective and efficient diagnosis and treatment of respiratory allergies caused by aeroallergens, information on diurnal, seasonal, and annual variations, and the type and concentration of airborne pollen and fungal spores is a prerequisite. Aerobiological sampling is therefore carried out to achieve this aim through various sampling devices currently used for bioparticulates.[34]

The recognition of aeroallergens is divided into two phases: (1) collection of material and (2) sample analysis.[35]

Different methods employed to achieve the objectives generally exploit the following basic regimes of collection:

- Fall out on a fixed surface through gravitational force
- Impaction on rapidly moving surface
- Impaction through suction of air
- Filtration
- Immunochemical assays.

And for the analysis of data, three procedures are followed:

1. Immunochemical assay for bulk reservoirs
2. Microscopic enumeration of individual particles
3. Tally of colonies produced in culture or semisolid media.

SAMPLING DEVICES

The various sampling devices and their principles of working have been enumerated as follows:

Gravimetric Samplers

This is based on the principle that bioparticulates settle down on a surface due to gravitational force. The Durham's gravity-sampling device consists of two horizontal disks with a diameter of 22.1 cm and 8.1 cm, respectively. The upper disk protects the slide from rain and sun. Nevertheless, this method presents certain disadvantages and has been discontinued in many parts of the world. It does not give diurnal variation and is dependent on wind velocity and favors large pollen grains. In spite of these drawbacks, it is economic, simple, independent of power, and still used in the Indian subcontinent by several workers. The slides are exposed daily, at a fixed hour, coated with adhesive glycerin jelly. After exposure, the slides are mounted in a drop of molten glycerin jelly, for various allergen types trapped. The results are expressed as number/unit area.

Impaction Samplers

In the impaction sampler, particles are sampled passively from air on adhesive-coated stationary or rotating rods.

Rotorod Sampler

The Rotorod sampler developed by Perkins[36] was the first such device to receive wide attention. Adhesive-coated clear lucite rods of 1–3 mm width are used as the collection surface. Collection efficiencies vary with the speed of travel and vary inversely with the width of the sampling area. This is a lightweight, portable, and DC-operated sampler. It has an arm that holds two cubical rods coated with silicone grease. The exposure time can be increased or decreased as required. The rods are mounted on a grooved slide in basic fuschin stain and scanned under microscope. For both, pollen and fungal spores, particles recovered are counted microscopically by transmitted light, and the results are easily expressed as "particles recovered/unit volume of the air sampled."

Suction Samplers

The method requires suction of certain volume of air according to a known velocity and for a chosen duration on a trapping. There are several instruments currently in use. The Hirst spore trap[37] is the most commonly used sampler. Trapping of bioparticles is done on slides coated with glycerin jelly, and slides are replaced each day with fresh slides.

Burkard 7-day Sampler

The Hirst spore trap was modified to Burkard trap in which slides were replaced with a drum, which can rotate and run continuously for 7 days with a definite speed (Fig. 4.1). Burkard continuous 7-day sampler uses a glycerin-coated tape mounted on a rotating drum. The drum is connected to a timer and rotates at a constant speed. The tape is changed every 7 days. Exposed tape is cut in seven strips corresponding to 7 days and mounted on a slide. This is a very good sampler to study diurnal or seasonal trends for fungal and pollen allergens.

Burkard Personal Slide Sampler

Burkard personal slide sampler is compact, battery operated, 10 cm in height, and 8 cm in diameter (Fig. 4.2A). It has a rectangular orifice at the top and a slit on the side to insert the microslide coated with glycerin jelly. The sampler takes in 10 L/minute air, particles got impacted on the slide in the form of a streak. It is mounted in glycerin and scanned. The exposed slides or trap is scanned for different pollen/spore traps and counts are expressed as number per cubic meter.

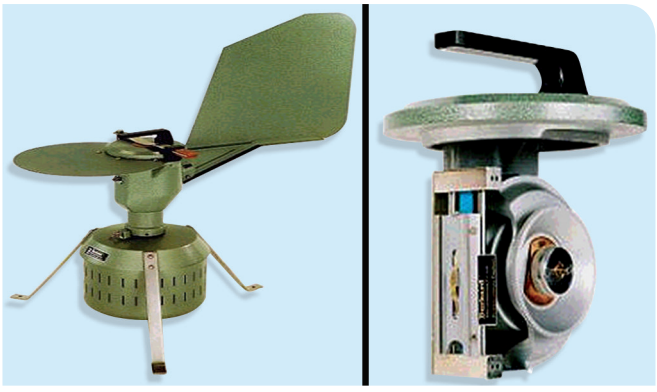


Fig. 4.1: Burkard 7-day volumetric sampler.

Burkard Petri Plate Sampler

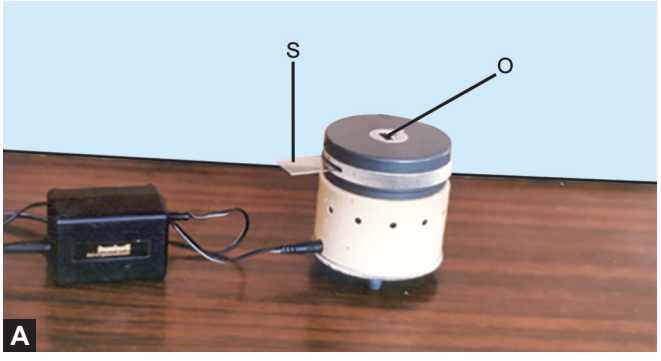
Burkard petri plate sampler is similar to the above sampler except that it has a stage to hold a petri plate containing nutrient agar media instead of slide. These two samplers are appropriate for spot sampling (Fig. 4.2B).

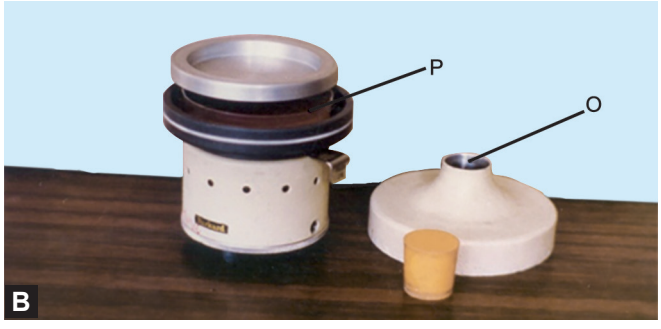
Andersen Sampler

The best device for estimating culturable spores in indoor and outdoor environments is the Andersen six-stage/two-stage sampler. It utilizes petri plates containing nutrient media kept under sieves of different pore sizes, each having 400 pores. Circular orifice takes up air at the rate of 28.3 L/min and passes it through the sieves kept in the order of decreasing pore size. Finally, air passes out after impacting on the last petri plate. Exposed petri plates are incubated at 28–30°C to allow the colonies to grow, and later on the number and types of colonies are counted.

Filtration Devices

Filters of definite pore size offer volumetric potential especially appropriate for smaller aerosol classes and where ambient velocities are low. Bioparticulates are





Figs. 4.2A and B: (A) Burkard single petri plate sampler; (B) Burkard slide sampler.

sucked through a filter and impinge on the media in a holder. Both media and holder are relatively inexpensive. But these are not widely used as in the morning air, as they may not faithfully align with the prevailing air stream.

The cultured samples may be examined microscopically after clearing the filters with immersion oil and other compatible fluids. Specific stains may be applied first. Alternatively, filters exposed with aseptic precautions may be collected on liquid culture media for colony growth, and colony-forming units can be identified.

Immunochemical Assays

Recently described immunochemical assays for airborne allergens relied on large (e.g., 20 cm × 25 cm) fiberglass filters exposed for 24 hour period in a high-volume (hi vol) sampler. They are rated for continuous operation and adapted to receive filter support. High volume devices are developed to study total suspended particles, and devices are operated traditionally with filters' surface directed upwards.

ANALYSIS OF SAMPLES

Regardless of their method of collection, samples of mixed biologic aerosols are analyzed by one of these following techniques.

Direct Microscopy

The microscopic identification of distinctive particles is an approach validated by years of practical applications of both gravimetric and volumetric samplers. A variety of particles, including certain basidiospores, ascospores, and spores of rust, smuts, downy mildew, and primitive green plants, are recognizable, which fail to grow on most laboratory media. Therefore, it is a most dependable way to identify most of the pollen and fungal spores.

Culture Analysis

This includes the tally of colonies produced in culture or semisolid media. This technique of analysis is employed to identify the culturable fungal spores on the basis of their colony characteristics, which otherwise cannot be identified under the microscope.

The culture plates, after exposure, are incubated at appropriate temperature, and impacted spores are allowed to grow for a couple of days till colonies start forming. Each colony represents one spore and considered a colony-forming unit.

Immunoassay

Immunochemical analysis following descending elution offers an analytic approach to dust without potential or defined form (e.g., dander, seed pomace, arthropod effluvia). If micronic aerosols do carry pollen allergens, these fractions are also accessible to immunoassay in bulk samples obtained by filtration. Procedure is based on inhibition by RAST and ELISA.

AEROBIOLOGICAL SURVEYS IN INDIA

Pollen Aerobiology

In India, the first systematic atmospheric survey was started in Calcutta by Cunningham[38] more than a century ago. After a prolonged gap, Kasliwal and his colleagues undertook such studies in Jaipur,[39] however, Shivpuri and his students did extensive studies in Delhi.[13] Since then researchers, all over India, have conducted exhaustive studies on airborne pollen types and their concentration. An "All India Coordinated Project on Aeroallergens and Human Health" sponsored by the Ministry of Environment and Forests, Govt. of India, has been successfully completed by AB Singh and his collaborators.[40] Important pollen and fungal allergens from 18 different places have been identified, quantified, and characterized for their allergenic properties. This provides the most scientific and up-to-date information on aeroallergens of India.

In Delhi, surveys have been conducted from time to time by Singh & Shivpuri,[41] Singh & Babu,[42] and Malik et al.[43] The dominant pollen types recorded are grasses, Cheno/Amaranth, *Ailanthus, Ricinus, Morus, Xanthium, Cannabis, Artemisia*, and *Holoptelea*. Lakhanpal & Nair[44] reported 30 pollen types from the atmosphere of Lucknow, whereas, Mittre & Khandelwal[45] reported 36 types. Under the All India coordinated project on aeroallergens and human health,[40] 43 types of pollen were recorded. The dominant types were *Holoptelea,* Poaceae, *Eucalyptus, Casuarina, Putranjiva*, etc. *Holoptelea* contributed 22.2% pollen to the air from March to May. Poaceae pollen grains were recorded (11.8%) with maximum concentration in April to June followed by Asteraceae. Under the same project at Solan (Himachal Pradesh), out of 22 types of pollen recorded, pollen of the family Poaceae and

Asteraceae were recorded in high concentration. The peak pollen season of Asteraceae was from March to October with maximum concentration in April. Poaceae pollen grains were recorded in high concentration in September. *Cassia*, *Quercus*, *Pinus*, and *Cedrus* were also important contributors to the pollen load.[40]

From Central India, surveys carried out at Bombay, Gwalior, Nagpur, Bhopal, and Kolhapur revealed that the dominant pollen types were from Poaceae, Asteraceae, and Apocynaceae families, *Rosa, Cicer, Ricinus, Ailanthus, Holoptelea,* Cheno/Amaranth, and Cyperus.[46,47] Grass pollen in India has been reviewed by Chaturvedi et al.[48] and has observed that highest percentage was reported from Aurangabad (80.64%) followed by Bhavnagar (70.26%) and Raipur (66.73%), all in Central India. Aerobiological surveys carried out at Mumbai, Pune, and Kolhapur revealed *Cicer, Ricinus communis, Holoptelea*, Cheno/Amaranth*, Argemone, Cocos nucifera*, and *Hibiscus* as the dominant pollen types.[49,50]

A recent survey[40] at Pune revealed *Parthenium* to be the highest contributor to the pollen load with two peak seasons, i.e., from September to November and January to April. *Cocos* and *Cassia* were observed throughout the year. *Cocos* pollen grains were recorded in high concentration in April to May and November to December.

At Aurangabad, *Datura alba* was prevalent in air from August to October with 8.2% annual concentration. *Cleome* contributed 6.8% pollen in June to August. Other important contributors were *Alternanthera, Typha, Bougainvillea*, etc.[40]

In Eastern India, Chanda and his students took up the work in the 1970s to assess the pollen types of Calcutta. Chanda and Sarkar[51] observed that grass pollen grains were the major contributors (39%) to the total pollen load in air. The other dominant pollen types being *Azadirachta, Caesalpinia pulcherrima, Carica papaya, Mangifera indica, Amaranthus, Chenopodium, Xanthium*, and *Argemone*.[52] From the Eastern Himalayas, Gupta and Chanda[16] and Singh and Devi[53] recorded the dominant tree pollen types as *Acer, Alnus nepalensis, Betula, Bucklandia populnea, Eucalyptus*, and *Pinus*. A recent survey[40] in West Bengal revealed, 59 types of pollen in air, and their maximum concentration was recorded in May. *Trema orientalis* pollen was in high concentration from May to July. Pollen types of families Asteraceae and Chenopodiaceae were maximum in June, and other important pollen types were from Asteraceae, *Pongamia, Areca catechu, Xanthium*, and *Cocos*. At Gauhati, Poaceae, Cheno/Amaranth, Asteraceae, *Putranjiva, Mangifera*, and *Eucalyptus* were the dominant types of pollen.[40]

From Southern India, studies carried out earlier at various places such as Visakhapatnam, Bangalore, Trivandrum, Kodaikanal, and Chennai revealed that *Casuarina, Parthenium, Spathodea*, Cheno/Amaranth, *Cocos*, and *Eucalyptus* were dominant pollen types. However, at Visakhapatnam, 24 pollen types were recorded, and Poaceae*, Peltophorum, Cocos, Casuarina,* Cyperaceae, and *Eucalyptus* were the dominant types.[40,54-58] The aerobiological survey at Trivandrum revealed 15 pollen types, and the dominant were Poaceae, Cheno/Amaranth, *Aporosa*, etc.[40] At Chennai, 38 pollen types belonging to 24 genera were identified. Pollen types from Poaceae were the most abundant types and contributed 19.4% to the total pollen load from June to August. Pollen types of *Acalypha* were recorded in highest concentration in the month of August, which is followed by the pollen of *Casuarina* in the month of January and March.[40]

Pollen calendars are very useful for clinicians as well as allergic patients to establish chronologic correlation between the concentration of pollen in air and seasonal allergic symptoms. Keeping this in mind, pollen calendars have been prepared for different geographical locations of the world.[14,28,59-61] Based on the aerobiological data obtained from India, pollen/flowering calendars have been prepared for Calcutta, Sambalpur, Gulbarga (now known as Kalaburagi) Imphal, and Kodaikanal by different workers.[53,57,62,63] The Center for Biochemical Technology (Council for Scientific and Industrial Research) now Institute of Genomics and Integrative Biology, had published a book on pollen calendars of 12 different states of India and is still very popular among allergists and clinical practitioners.[31] Important pollen seasons for grasses, weeds, and trees prevalent in India are provided in the book. A recent book by Singh and Khandelwal[32] with plant pictures, their local and scientific names, concentration, and season and pollen morphology, etc., provides a good tool for the selection of appropriate allergens for testing and immunotherapy.

Fungal Aerobiology

Fungi are ubiquitous in nature and are reported to be prevalent from different parts of the world, both in the indoor and outdoor environments. Airborne survey of fungal allergens has been reported from different parts of India.

Outdoor Environments

From Delhi and Dehradun, *Alternaria* is reported to be very high followed by *Cladosporium* in the studies concluded using the gravitational method.[64,65] At Solan and Lucknow, 17 and 40 types of fungal spores, respectively, were recorded from ambient air. Aspergilli-Penicilli followed by *Cladosporium, Helminthosporium, Epicoccum*, and *Drechslera* are the dominant types.[40]

Studies carried out at Gaya, Gauhati, Calcutta, Gorakhpur, and Imphal[40] revealed Aspergilli-Penicilli, *Cladosporium,* ascospores, rust and smuts spores, *Nigrospora, Periconia, Ganoderma*, and *Rhizopus* as the major types from East India.

From Western India, Deuteromycetes contributed 70% of the total fungal air spora. A recent survey has reported 18 spore types at Aurangabad and 22 at Pune. *Cladosporium,* Aspergilli-Penicilli*, Curvularia, Rhizopus*, and *Helminthosporium* were the abundant types.[40]

The dominant forms in Visakhapatnam, among 34 types, were Aspergilli-Penicilli, *Cladosporium, Curvularia,* Basidiospores, and Uredospores. In Chennai, 50 types were identified and, apart from the common genus*, Perconia, Curvularia*, and *Ganoderma* are the dominant types. In Trivandrum, *Tetraploa* was another dominant fungal spore prevalent in the air among other common types.[40]

In central India, surveys were conducted at Gwalior, Aurangabad, and Nagpur, and 15, 18, and 25 fungal types were recorded, respectively. Prominent types were Aspergilli-Penicilli, *Cladosporium, Helminthosporium, Curvularia, Drechslera, Pyricularia, Syncephalastrum*, and *Uredospores*.[40]

Indoor Environment

Besides the outdoor environment, indoor and work environments are also greatly influenced by fungi, especially occupational sites employing organic raw materials, e.g., granary, poultry, flour mills, bakery, and sugar factory.[2,66,67] A survey conducted at working environments of bakery, poultry, sugar factory, and libraries in Delhi revealed Aspergilli-Penicilli and smuts spores as significant contributors in indoor air.[68-70] Fungal spora and their seasonality at some of these work environments have been worked out in detail by our group and is described briefly here.[71]

Bakery

Altogether, 74 types belonging to 33 genera were isolated from the bakery environment in Delhi by Singh and Singh.[34] *Aspergillus flavus* was the predominant type in the storage (34.9%) and packing sections (20.2%) of the bakery. Highest concentration was recorded during summer months from June to September. *Cladosporium* concentration was less inside than just outside the bakery. It was significantly high in concentration from October to February. In the case of spore catch, Aepergilli/Penicilli were the dominant (69.2%) type with maximum concentration in October. Smut spores were the second highest contributor in the packing section (28.5%) from February to April.

Poultry

In the poultry environment, 130 fungal forms were isolated from the air survey. Predominant fungi observed particularly in the shed area and in the poultry air are *Candida albicans, Scopulariopsis brevicaulis, Penicillium nigricans*, and *Penicillium frequentans*. In the hatchery section, Aspergilli/Penicilli spores were abundant. As far as the seasonality of these fungi is concerned, *Candida* did not have a definite season. *Cladosporium* showed a seasonal prevalence from November to February. *A. flavus* had seasonal exacerbation from July to October.

Granary

Aspergillus contributed a maximum of 68.9% followed by *Cladosporium* in granary. Colony forming units of *A. flavus* had two distinct seasons from September to November and May to August, whereas, *Cladosporium* was prevalent in winter months. Other important contributors to the granary environment were *Rhizopus, Curvularia, Aspergillus versicolor, Aspergillus fumigatus*, and *Epicoccum nigrum*. *Aspergillus* alone contributed 74.7% inside the grain storage. The spores of *Alternaria* were observed in each month with seasonal exacerbation in summer from March to June.

Sugar Industry

Cladosporium was recorded in high concentration (60%) in both the cane cutting and bagasse storage regions of the sugar industry, almost parallel to outside concentration and was prevalent from November to March. This period also coincides with the main crushing season of the sugar industry. Other major contributors were *A. fumigatus, Epicoccum, Saccharomyces*, and smut spores. *Epicoccum* was characteristic for the month of November to May. Other fungi like *Aspergillus niger, A. versicolor, and A. flavus* did not show a definite seasonality.

Several epidemiological studies have correlated symptoms of allergic patients to fungal spores concentration in their houses (Roby and Sneller, 1979; Anderson and Korsgaard, 1986; Martin et al., 1987; Waegemaekers et al., 1989). Epidemiological survey conducted by Singh and coworkers (Singh, 1994) revealed 40% workers having respiratory problems in bakery, 59% in poultry, 40.5% in granary and 42.5% in sugar industry. The prevalence is 4–5 times higher in Delhi than what was estimated about 34 decades ago for general population.[72]

CLINICALLY IMPORTANT ALLERGENS

Pollen Allergens

Pollen types causing allergy are quite variable in different ecozones, which makes it very important to identify pollinosis-causing species from every region and prepare extracts from them for diagnosis and immunotherapy for the benefit of allergy sufferers.

Based on clinico-immunological studies with pollen antigens, important allergenic pollen of India have been identified. The work on pollen allergy was initiated in the 1950s by Shivpuri in Delhi. Subsequently, Kasliwal and his colleagues reported important pollen allergens of Jaipur.[73] Shivpuri & Parkash[74] observed *P. juliflora* as a major cause of pollinosis with 12% of patients showing a positive skin reaction. Later, important pollen types causing allergy were identified for Delhi by Shivpuri and his colleagues, some of them are *Ageratum, Ailanthus, Amaranthus, Anogeissus pendula, Artemisia, Cassia siamea, Cenchrus, Chenopodium, Cynodon, Ipomoea fistulosa, Paspalum distichum*, and *Poa annua*.[75] Singh et al.[76] recorded a positive skin reaction in 16.9% of patients to *Pinus roxburghii* from the foothills of Himalayas.

From Northern India, the important allergens identified are *P. juliflora, R. communis, Morus, Mallotus, Alnus, Quercus, Cedrus, Argemone*, etc. from studies carried out by Agnihotri & Singh[6,17,77] Reported, *Amaranthus*, *Chenopodium, Holoptelea*, and grasses are important offending allergens from this region (Table 4.1).

Tiwari[78] and Chaubal & Gadve[79] identified the important pollen types causing allergy from Central India. They are *Argemone, Brassica, Cannabis, Asphodelus, Parthenium, Cassia, Azadirachta*, and grasses. Under AICP project, *Morus* and *Trewia nudiflora* are recently added to the list of allergens.[40]

From Eastern India, Chanda et al.[80] carried out studies in Calcutta and reported that allergenically significant pollen types were *Lantana, Cucurbita maxima*, *Cassia fistula*, *C. nucifera*, and *Calophyllum inophyllum*. High sensitivity to *C. nucifera* has also been recorded by Karmakar et al.[81] Recent studies based on clinical and immunologic parameters reported *Phoenix*, *R. communis*, and *Aegle marmelos* as causative agents of allergy in this region.[40]

Acharya et al.[82] and Agashe & Anand[83] have reported *Cassia*, *Ageratum, Salvadora, Ricinus, Albizia lebbeck*, and *Artemisia scoparia* as important aeroallergens of South India. Subbarao et al.[84] recorded allergenicity to *Parthenium hysterophorus* pollen extracts in 34% of allergic rhinitis and 12% of bronchial asthma patients from Bangalore. Agashe & Soucenadin[85] recorded high skin reactivity to *Casuarina equisetifolia* in patients from Bangalore.

Clinical studies undertaken recently by various medical centers under AICP on Aeroallergens and Human Health[40] revealed the important allergenic pollen and fungi for various regions in India. Skin test reactivity from different centers is presented in Table 4.2. Altogether, 35 pollen and 30 fungal antigens were tested on atopic population. In Chandigarh, skin sensitivity was maximum against *Rumex acetosa* and *Ailanthus excelsa* (17.6%) followed by *T. nudiflora* (9.7%) and *Argemone mexicana* (9.5%), and 9.3% of the atopic persons showed sensitivity against *Cedrus deodara*. In Delhi, 12.6% of the atopic population were positive against *Amaranthus spinosus*, 8.46% to *Populus deltoides*, and 7.46% against *Dodonaea viscosa* and *Bauhinia variegata*. In Calcutta, 28.8% of the patients were sensitive against *Solanum sisymbriifolium* while, *Crotalaria juncea* (21.1%) and 18.18% each against *R. communis* and *I. fistulosa*. In Trivandrum, maximum skin reactivity was recorded against *Mallotus philippensis* (12.1%) followed by *P. juliflora* (6.3%).

Fungal Allergens

Like pollinosis, fungal allergy is also well established. The significant fungal types causing allergy have been identified on the basis of skin tests performed on symptomatic patients.

Agarwal and Shivpuri[86] performed intradermal skin tests on 1292 patients in Delhi using 27 fungal extracts. They found 19 fungal extracts to be of allergenic

Table 4.1: Common allergenic plants of different seasons in India.

Spring	*Autumn*	*Winter*
February–April	*September–October*	*November–January*
Grasses		
Cynodon dactylon	*Bothriochloa pertusa*	*Cynodon dactylon*
Dichanthium annulatum	*Cenchrus ciliaris*	*Eragrostis tenella*
Imperata cylindrica	*Heteropogon contortus*	*Phalaris minor*
Paspalum distichum	*Pennisetum typhoides*	*Poa annua*
Poa annua	*Sorghum vulgare*	–
Polypogon monspeliensis	–	–
Weeds		
Cannabis sativa	*Amaranthus spinosus*	*Ageratum conyzoides*
Chenopodium murale	*Artemisia scoparia*	*Argemone mexicana*
Parthenium -	*Cassia occidentalis*	*Chenopodium album*
-hysterophorus	*Ricinus communis*	*Asphodelus tenuifolius*
Plantago major	*Xanthium strumarium*	*Ricinus communis*
Suaeda fruticosa	–	–
Trees		
Ailanthus excelsa	*Anogeissus pendula*	*Cassia siamea*
Holoptelea integrifolia	*Eucalyptus* sp.	*Salvadora persica*
Prosopis juliflora	*P. juliflora*	*Mallotus philippensis*
Putranjiva roxburghii	*Cedrus deodara*	*Cedrus deodara*

Table 4.2: Skin test results from different medical centers in India.

Name of allergen	*Kolkata*	*Chandigarh*	*Delhi*	*Trivandrum*
Pollen Antigen				
Aegle marmelos	15.6	–	2.98	–
Ailanthus excelsa	–	17.6	–	–
Alnus nitida	–	4.8	3.5	–
Amaranthus spinosus	15.4	3.2	–	–
Argemone mexicana	–	9.5	4.26	4.8
Bauhinia variegata	17.8	–	7.46	–
Betula utilis	–	–	4.98	–
Borassus flabellifer	9.7	–	–	–
Carica papaya	7.07	–	4.48	–
Caryota urens	4.11	1.5	1.5	–
Cassia fistula	5.88	4.8	–	3.0
Cassia glauca	–	5.9	1.75	
Cocos nucifera	11.5	–	6.98	3.2
Casuarina equisetifolia	9.2	–	6.47	–
Callitris		4.8	2.3	–
Cedrus deodara	8.0	9.3	7.1	4.8
Celtis australis	–	–	3.48	–
Crotalaria juncea	21.05	6.4	–	–
Cycas circinalis	15.4	–	–	–
Dodonaea viscosa	4.34	4.3	7.46	1.5

(Contd...)

(Contd...)

Name of allergen	*Kolkata*	*Chandigarh*	*Delhi*	*Trivandrum*
Delonix regia	10.78	1.5	1.16	3.1
Holoptelea integrifolia	–	2.4	6.59	–
Ipomoea fistulosa	21.1	–	4.97	–
Mallotus philippensis	8.25	5.9	1.39	12.1
Phoenix sylvestris	8.0	–	–	3.1
Plantago ovata	10.8	3.2	4.48	–
Podocarpus	1.96	5.9	–	–
Populus deltoides	3.4	3.2	8.46	
Prosopis juliflora	15.7	4.3	6.98	6.3
Quercus incana	–	4.8	5.81	–
Quercus dilatata	–	–	4.48	–
Ricinus communis	18.18	3.6	5.26	4.8
Rumex acetosa	–	17.6	–	–
Solanum sisymbriifolium	28.8	–	6.96	–
Trewia nudiflora	12.9	9.7	1.75	–
Fungal Antigens				
Alternaria alternata	–	3.5	1.1	–
A. candidus	4.9	–	5.4	–
A. clavatus	–	–	4.48	–
A. flavus	5.46	5.22	1.75	–
A. fumigatus	4.9	2.61	–	–
Aspergillus japonicus	3.12	4.76	3.51	1.6
A. nidulans	9.8	3.22	–	–
A. ochraceous	4.0	1.2	2.2	–
A. oryzae	4.9	–	5.47	–
A. sydowii	6.08	3.57	–	–
A. terreus	7.69	5.95	3.48	–
A. versicolor	10.99	–	2.2	–
Cladosporium cladosporioides	5.1	4.76	–	1.6
Cladosporium herbarum	3.65	–	4.48	–
Chaetomium	–		2.0	–
Curvularia pallescens	8.0	1.74	–	–
Epicoccum nigrum	10.0	3.57	–	–
Fomes pectinatus	7.14	4.76	–	–
Fusarium roseum	6.86	1.74	1.75	–
Ganoderma lucidum	6.86	2.6	6.2	1.6
Neurospora implicata	10.5	3.22	3.51	–
Neurospora sitophila	3.1	2.6	3.51	–
Paecilomyces variotii	9.1	3.22	4.17	–
Sphacelotheca cruenta	14.3	3.22	6.94	–
Sporotrichum pruinosum	6.67	6.45	5.55	–
Rhizopus nigricans	5.88	1.74	–	–
Uromyces aloes	1.23	6.45	5.56	–
Ustilago cynodontis	4.87	3.2	–	–
Ustilago scitaminea	2.7	9.68	2.8	–
Ustilago tritici	1.98	–	2.98	–

significance. Some of these are *Alternaria brassicae, Curvularia, Candida, Monilia, Phoma, Acrothecium, Helminthosporium, Mucor*, and *Aspergillus tamarii.* Shivpuri[29] further performed skin tests on 300 patients and observed besides the abovementioned fungi, *Cladosporium herbarum, A. niger*, and *A. fumigatus* were also important allergens.

Acharya[82] investigated on 300 nasobronchial allergy patients in Andhra Pradesh, important allergenic fungi were *A. flavus*, *Helminthosporium*, *Neurospora*, *Candida*, *Cladosporium*, etc. From Bangalore, *Mucor mucedo, Fusarium solani, Curvularia, Nigrospora*, etc., are observed to be important allergens.[83]

Clinical studies at various places in India under AICP on aeroallergens and human health[40] reported *A. ochraceus, A. japonicus, Cladosporium, Alternaria alternata, A. versicolor, Uromyces*, and *Ustilago* are major allergenic fungi (Table 4.2). Information on *A. ochraceus, A. japonicus, Uromyces, Ustilag*o, *Neurospora sitophila*, and *Sporotrichum* has been obtained for the first time in India with respect to their allergenic properties and constitute important fungal allergens of India.

MOLECULAR APPROACH TO ALLERGENS CHARACTERIZATION

Pollen extracts are complex mixtures of several proteins, lectins, complex carbohydrates, lipids, nucleic acids, enzymes, etc. Every time an extract is prepared, there is considerable variation in their content within a company, besides intercompany variability. These great differences result in unreliable prick test results, prevent comparison of skin tests world over, and in generating dose–response data. Also, their use in therapeutics makes them necessary to have an international standard.

The need for standardization of allergenic extracts has been recognized since the advent of immunotherapy.[87] The early attempts toward standardization was based on consistency in crude w/v ratio or estimation of Protein Nitrogen Unit content of the extract. But it was found to be a poor indicator of allergen content.

The standardization of allergenic extracts can be done by biological standardization. The concept of biologic equivalence test was established by the Northern Society of Allergology and adopted by European investigators.[88] One HEP unit was defined as a positive skin prick test, which gave a wheal diameter equivalent to that given by a prick with 1 mg/mL concentration of histamine hydrochloride. Later on, Dirksen et al.[89] designated 1 HEP as the concentration of extract that gives a wheal diameter equivalent to 1 mg/mL of histamine hydrochloride.

Pollen and fungal extracts are also studied for their immunochemical properties, mainly protein and glycoproteinic fractions.

Pollen Allergens

Several pollen allergens are characterized for their protein profile. A number of fractions were identified in these antigens and most of the fractions are found to be acidic. Further allergenic fractions are also identified by RAST or Western blotting. Some of the well-characterized antigens *are P. juliflora*,[90] *R. communis, Holoptelea integrifolia*,[91,92] *Phoenix sylvestris*,[93] *I. fistulosa*,[94] *Madhuca indica*,[95] and *C. deodara*.[17]

Many pollen allergens are characterized under AICP on aeroallergens and human health.[40] They are *Mallotus philippensis, Quercus incana, C. nucifera, Celtis, Betula*, etc. (Table 4.3). All these pollen antigens are identified for the first time and are characterized for their antigenic and allergenic properties.

The protein profile as fractionated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) showed considerable heterogeneity with the number of bands varying from 8 in *Broussonetia papyrifera* to 23 in *Caryota urens*. All 38 pollen antigenic extracts and 21 fungal extracts were characterized (Table 4.3) by using SDS-PAGE and isoelectric focusing (IEF). The profile of some of the antigens is described later.

Aegle marmelos

A total of 15 fractions was fractionated on SDS-PAGE. Their molecular weight ranged from 14.3 to 116 kD. Of these, most distinct protein bands were of 14.3, 21, 25.7, 37, 60, and 85 kD. While by electrofocusing, 11 fractions were detected in the acidic region (3.5–6.2 pI).

Alnus nitida

There were 21 proteins fractionated by SDS-PAGE. Their molecular weights ranged from 12.5 to 11.6 kD. Sixteen bands were identified by IEF within the pI range of 3.5–6.0. Among these 4.2, 5.2, and 5.3 pI bands were prominent.

Table 4.3: SDS-PAGE and isoelectric focusing (IEF) characterization of pollen antigens.

	SDS-PAGE		*IEF*	
Antigen	*Total No. of bands*	*MW range*	*Total No. of bands*	*pI range*
Aegle marmelos	15	14.3–116.0	11	3.5–6.2
Ailanthus excelsa	14	14.5–128.0	9	3.5–5.6
Alnus nitida	21	12.5–116.5	16	3.65–6.0
Argemone mexicana	17	12.2–125.0		
Amaranthus spinosus	14	12.2–124.0	17	3.5–8.95
Betula utilis	17	11.7–109.0	22	3.1–8.5
Borassus flabellifer	10	14.3–90.0	9	3.5–5.85
Broussonetia papyrifera	8	11.8–118.0	16	3.2–8.15
Carica papaya	6	17.0–43.0	6	3.75–4.9
Cassia fistula	13	12.2–134.0	9	3.6–5.3
Cassia sophera	10	13.0–131.0	12	3.7–6.2
Casuarina equisetifolia	13	13.5–126.5	16	3.9–8.2
Cedrus deodara	17	12.3–116.0	20	4.3–9.4
Ceiba pentandra	15	12.4–128.0	8	3.5–5.65
Celtis australis	11	14.3–95.0	12	3.45–9.4
Cocos nucifera	22	10.2–135.0	12	3.55–5.9
Dodonaea viscosa	18	14.0–111.0	13	3.2–6.1
Elaeis guineensis	7	11.7–140.0	12	3.7–5.85
Ipomoea biloba	9	12.2–60.0	19	3.95–8.3
Ipomoea fistulosa	19	14.3–116.0	12	3.57–8.55
Juglans regia	15	12.0–126.0	19	4.75–9.12
Mallotus philippensis	25	11.9–140.0	28	3.6–9.3
Pecan nut	17	11.7–140.0	17	4.1–9.3
Peltophorum pterocarpum	10	13.2–138.0	15	3.5–8.8
Phoenix sylvestris	10	14.2–125.5	13	4.1–9.3
Pinus patula	12	12.2–125.0	5	4.4–8.25
Populus deltoides	24	13.0–120.0	13	3.8–6.6
Plantago ovata	4	12.2–118.0	8	3.7–4.9
Punica granatum	10	16.5–112.0	21	3.9–9.3
Quercus dilatata	17	14.3–120.0	17	3.9–8.2
Quercus incana	16	14.3–120.0	22	3.9–9.35
Rumex setorum	22	14.3–118.0	16	3.95–6.7
Syzygium cumini	15	11.6–113.0	15	3.7–5.85
Solanum sisymbriifolium	8	13.5–115.0	19	3.57–7.3
Trewia nudiflora	10	11.5–119.0	16	3.5–5.95

Amaranthus spinosus

A total of 14 proteins were detected in the molecular weight range of 12.2–124 kD. Proteins of 113 and 122 kD were most distinct. On IEF, 17 proteins were fractionated in the pI range of 3.5–8.95. Two proteins were localized in the alkaline region (7.4 and 8.95). Rest of the proteins were with acidic pI. Proteins of 4.3, 4.65, 5.1, 5.2, and 9.85 pI were prominent among all.

Betula utilis

Antigenic extract fractionated on SDS-PAGE revealed 17 protein fractions ranging in molecular weights of 11.7 to

109 kD. Protein bands of 17.8 and 14 kD were the most distinct bands. Twenty-two proteins were identified by IEF in the pI range of 3.1–8.1. Two of the proteins were in the basic region.

Borassus flabellifer

Borassus antigen was fractionated in 10 protein bands between 14.3 and 90 kD. Among these 90.81, 56.8, 41, and 43.2 kD bands were the more prominent than others. While there were nine protein fractions identified in the acidic pI region (3.5–5.85), 3.5, 5.25, 5.3, 5.75 pI proteins are prominent among all.

Carica papaya

Antigen revealed only six protein bands by SDS-PAGE as well as IEF. Molecular weights of the proteins were between 17.0 and 43 kD, and pI was 3.75–4.9.

Ceiba pentandra

There were 15 bands between 10.4 and 128 kD molecular weight, when antigens was fractionated on SDS-PAGE. A band of 29 kD was the most intensely stained band. Only eight *protein* bands were identified on the IEF fractionation in the pI range of 3.5–5.65.

Ipomea biloba

Antigen was fractionated on SDS-PAGE and nine bands were detected. The molecular weights of these fractions varied from 12.2 to 60 kD, and 14.3, 23, 50, and 60 kD bands were distinct. As much as nine proteins were fractionated by IEF in the pI range of 3.95–8.3. Two of the proteins were localized in the alkaline region.

Plantago ovata

Plantago antigen was fractionated in only four bands on SDS-PAGE (12.2–118 kD) with 12.2, 22, and 33.5 kD the prominent ones. On IEF eight bands were detected in the low-pI range (pI 3.7–4.9) and most of the bands were clear and well stained, though 3.7, 3.95, 4.1, 4.4, and 4.6 pI fractions were prominent than others.

Populus deltoides

Twenty-four proteins were fractionated on SDS-PAGE with molecular weights ranging from 13.0 to 120 kD. Proteins of 15.2 and 56.0 kD were more prominent than others. By IEF, 13 proteins were identified in the pI range of 3.8–5.6. No bands were in the basic region. Proteins of 5.3 and 5.7 pI were prominent.

Quercus incana

Sixteen protein fractions were detected on SDS-PAGE. These proteins had the molecular weights ranging from 14.3 to 120 kD. A total of 22 protein bands were detected in the pI range of 3.9–9.35.

Solanum sisymbriifolium

Antigenic extracts of *S. sisymbriifolium* when fractionated on SDS-PAGE revealed eight bands in the molecular weights ranging from 13.5 to 115 kD; while on IEF 11 proteins could be detected in the acidic region (4.0–5.9).

Fungal Allergens

The most common fungal components that become airborne are conidia/spores. The fungal types that have been found dominant, all over the world, have a large species and strain. Besides, fungi have the tendency to undergo mutation under slight stress conditions. Some of them are so prone to change that it is impossible to maintain similar culture, that may yield similar antigenic components.[96] It has been reported by many workers that the prevalence of shared antigenic and allergenic components among closely related or even unrelated genera of fungi exists.[97–99] Due to these reasons, a very few fungi are studied in detail with a view to characterize the allergen content. The difficulty also arises because of the high number of allergenic components in the crude extracts.[33] Some of the allergens studied for molecular heterogeneity are *Cladosporium*, *Aspergillus*, *Drechslera Candida*, and Basidiomycetes.[10,20,96,100-105]

Many fungal allergens have been recently characterized in India. They are *Aspergillus ochraceus, Fomes, Ganoderma*, *Epicoccum*, *Fusarium*, *A. flavus*, and some others.[7,105-110] Further, under the All India coordinated project (AICP) many fungal allergens were characterized, such as *Paecilomyces, Uromyces, Ustilago tritici*, etc. IgE-binding fractions of some of these are identified and described in Table 4.4.

Aspergillus clavatus

Thirteen bands could be detected in the antigenic extract of *A. clavatus*, which lied in between 11.5 and 176 kD

Table 4.4: SDS-PAGE and IEF characterization of fungal antigens.

Antigen	*SDS-PAGE*		*IEF*	
	Total no. of bands	MW range	Total no. of bands	pI range
Alternaria alternata	8	13.8–145.5	–	–
Aspergillus fumigatus	22	24.0–134.0	11	3.9–6.0
A. japonicus	–	–	11	3.73–7.9
A. ochraceus	9	13.8–125.0	–	–
A. versicolor	4	20.0–130.0	2	4.55–4.76
A. sydowii	22	12.2–138.0	15	3.5–6.6
A. clavatus	13	11.5–176.0	8	2.1–6.7
A. candidus	9	12.4–166.0	2	4.7–5.1
C. herbarum	17	13.5–135.0	–	–
Curvularia lunata	–	–	4	3.8–4.8
Epicoccum nigrum	9	16.4–185.0	–	–
Fusarium roseum	14	13.5–139.0	–	–
Helminthosporium oryzae	19	12.2–138.0	7	3.9–5.9
Neurospora implicata	–	–	10	3.65–6.25
Paecilomyces variotii	7	12.0–138.0	14	3.7–6.2
Sphacelotheca cruenta	6	12.2–121.0	9	4.1–5.85
Sporotrichum pruinosum	20	12.5–128.0	–	–
Uromyces aloe	4	14.0–114.0	16	3.05–6.95
Ustilago cynodontis	10	12.2–130.5	9	4.1–6.35
Ustilago tritici	5	14.2–172.0	12	4.3–6.35
Ustilago scitaminea	13	12.2–126.5	15	3.65–6.4

molecular weights. A protein of 20 kD was most prominent. Only eight proteins were detected on IEF. All were in the acidic pI range (2.1–6.7).

Helminthosporium

Nineteen protein fractions were detected in the molecular weight range of 12.2–138 kD. Only seven fractions were detected on IEF. They were in the acidic pI range from 3.9 to 5.09. A protein of 3.9 pI was most distinct.

Sphacelotheca cruenta

Only six proteins were identified in the crude extract with molecular weights ranging from 12.2 to 128 KD. Bands of 12.2 and 121 kD were distinct among all the proteins.

Sporotrichum pruinosum

A total of 20 protein fractions could be detected in SDS-PAGE. Their molecular weights ranged from 12.5 to 128 KD.

Ustilago tritici

Antigenic extract was fractionated on SDS-PAGE and only five proteins were detected in the molecular weight range of 14.2–172 kD. On IEF, six proteins were detected in the pI range of 4.1–6.7. All the proteins were acidic.

Ustilago cynodontis

Only two proteins were fractionated on SDS-PAGE. Their molecular weights were 17 and 170 kD. A band of 17 kD was more prominent. Whereas, eight protein fractions

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CLINICAL ALLERGY

Salient Features

- The book is written with the aim of defining a practical approach to every aspect of allergy
- Chapters practically cover all the aspects of allergy ranging from epidemiology, varying presentation of allergy, diagnosis, and practical approach to the treatment of various allergic diseases
- It also covers practical approach to prevention of allergy and pediatric issues in management of allergic diseases
- Chapters include allergic rhinitis, ocular allergy, urticaria, angioedema, atopic dermatitis, drug allergy, and food allergy
- Special chapters on latex allergy, human seminal plasma allergy, aspirin allergy, anticonvulsants hypersensitivity syndrome, hymenoptera allergy, radio-contrast allergy, local and general anesthetic allergy, cow-milk allergy, sublingual immunotherapy, cluster immunotherapy, and future of immunotherapy are also included in the book
- This book also contains chapter on pollen calendar with the use of photographs of allergenic plants mainly found in India
- The contributors of this book were chosen for their specific expertise and interest in respective areas. Chapters have been written in the background of current literature and practical experience gained from day-to-day dealing with different patients suffering from allergy
- General practitioners, specialists, undergraduate and postgraduate medical students will find this book as a practical guide.

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