An aerobiological perspective in allergy and asthma

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Allergic diseases are amongst the most common chronic disorders worldwide. Today, more than 300 million of the population is known to suffer from one or other allergic ailments affecting the socio-economic quality of life. Major causative agents implicated are pollen grains, fungal spores, dust mites, insect debris, animal epithelia, etc. Several aerobiological studies have been conducted in different parts of the world to ascertain aerial concentration and seasonality of pollen grains and fungi. Especially from clinical point of view, it is important to know the details about the pollen season and pollen load in the atmosphere. The flowering time of higher plants are events that come periodically in each season, but the time of blooming may differ from year to year, in different geographic locations. Based on differences recorded in several years of observations in airborne pollen, pollen calendars are drawn as an aid to allergy diagnosis and management. This review article emphasises on various aerobiological parameters of environmental pollen from different parts of the world with special emphasis from India. The role of aerobiology in the diagnosis and management of allergic diseases is reviewed briefly in this article.

Key words: Aerobiology; Allergy; Allergens; Asthma; Seasonal variations; Pollen

INTRODUCTION

Respiratory allergy is prevalent among all populations with increasing trend all over the world. However, development of civilization often at the expense of the natural environment by pollution or biopollution stimulates the appearance of new health problems, besides increase in allergic diseases. India, with the teeming 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 worldover, there is an increasing interest in the presence and movement of bioparticulate matter

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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, foods, etc [1-8]. 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 [9].

Pollen grains as aeroallergen are well studied from across the world and are important cause of pollinosis. Respiratory system is the direct target organ of airborne pollen 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. Bostock [10] was the first to suspect pollen as the cause of hayfever (allergic rhinitis). Later Blackley [11] established that grasses are important cause of hay fever in UK. After more than 40 years, Schepepegrell [12] from 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 [9, 13-20].

Although pollen 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 air. The fungi that produce spores and get airborne are called ‘aerospores’. These are implicated in the causation of allergic diseases and infections in immunocompromised patients. They are established to cause Type I hypersensitive diseases with IgE mediated response. The common symptoms of hypersensitivity are bronchial asthma, allergic rhinitis and atopic dermatitis.

Source of pollen aeroallergens

The transport of pollen grains by wind or by the insects, from floral anther to recipient stigma is the critical reproductive event among higher plants. The dispersion of replicate units in massive abundance assures the success of wind pollination as well as its human health effects including asthma, rhinitis, atopic dermatitis, etc. Pollen prevalence (grains per cubic meter) at any point reflects (plant) source strength and location as well as the dynamics of the intervening environment conditions such as climatic factors, pollution and degree of exposure. The presence of pollen, profile of species, concentration, etc depends on various climatic factors such as temperature, humidity, wind direction, sunshine, substrate precipitation and other seasonal factors. Because of change in the climatic conditions, the study of variations in the diurnal and seasonal prevalence becomes very important [21].

Monitoring airborne pollen allergens

Knowledge about diurnal, seasonal and annual fluctuations in airborne pollen and fungal spores in any geographical area is essential for effective diagnosis and treatment of allergy. Aerobiological sampling is therefore carried out to achieve this aim through various sampling devices currently used for bioparticulates.

The recognition of aeroallergens is divided into two phases: 1) Collection of material and 2) Sample analysis [22].

Different methods employed to achieve the objectives generally exploit the following basic regimes of collection:
1. Fall out on a fixed surface through gravitational force
2. Impaction on a rapidly moving surface
3. Impaction through suction of air
4. Filtration
5. Immunochemical assays

For analysis of data following procedures are followed:
1. Microscopic enumeration of individual particles
2. Immunochemical assays for bulk reservoirs

Sampling devices

Monitoring of airborne biological particles is carried out by various gravimetric, impaction, and suction sampling devices [23-25]. In addition, new immunochemical techniques are also used for detecting allergenic pollen and measuring of the size of allergen-carrying particles [26].

Gravimetric sampler

This is based on the principle that bioparticulates settle down on a surface due to gravitational force. The Durham gravity-sampling device consists of two horizontal disks with a diameter of 22.1 cm and 8.1 cm [23]. The upper disc protects the slide from rain and sun. 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. However, this sampler is no more used in developed world, but developing countries still report the use of gravity
settlement method for pollen and fungal spores. It does not give diurnal variation and is dependent on wind velocity. Even in still air the number of large particles collected on the surface will be overestimated and smaller particles that have a slower settling velocity will be underestimated.

**Impaction sampler**

Rotorod Sampler: Rotorod Sampler developed by Perkins [25] 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. 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 pollen/spore catch obtained by these three models is almost similar. These three samplers are used to study both pollen and fungal spore types on continuous basis.

**Suction samplers**

The method requires suction of certain volume of air according to a known velocity and for a chosen duration on trapping.

Hirst trap: the Hirst spore trap was 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 quantitative data. The method requires suction of certain volume of air according to a known velocity and for a chosen duration on trapping. It records the atmospheric concentration of pollen grains, fungal spores, and other biological particles as a function of time through morphological identification.

Burkard seven day volumetric sampler

The hirst 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 min. Adhesive coated tape mounted is used in on a rotating drum Burkard continuous seven day sampler. The drum is connected to a timer and rotated at constant speed. 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 most widely used samplers to study diurnal or seasonal trends for pollen grains as well as fungal spores all over the world.

Burkard portable (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. 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 pollen grains/fungal spore count under light microscope.

**Immunochemical assays**

The immunochemical assays for airborne allergens relied on large (e.g. 20 × 25 cm) fibre glass filters exposed for 24 h period in high volume sampler. They are rated for continuous operation and adapted to receive filter support. Hivol devices are developed to study total suspended particles and devices are operated traditionally with filters surface directed upwards. In a study carried out in Arizona, variability of allergen shedding of airborne cat allergen was carried out by Immunochemical assay. Cats were placed in a lucite chamber with an air sampler attached. Radioallergosorbent (RAST) / enzyme-linked immunosorbent assay (ELISA) inhibition type and monoclonal two-site radioimmunosurveys are used to express air concentrations in allergy units [27].

**Analysis of pollen samples**

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

**Direct microscopy (slides)**

The microscopic identification of distinctive particles (pollen/fungi) 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 pollen grains, 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 pollen/fungal spores.

**Immuoassay**

Immunochemical analysis following descending elution, exposed from filters 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.
Rumex and levels. Grass pollen demonstrates a biphasic pattern. Tree pollen present. The most pronounced drop has been in weed pollen area established that pollen levels have declined from 1993 to the

Pollen aerobiology

Aerobiological surveys

Pollen aerobiology

Airborne pollen and their concentration vary in the different seasons depending upon the flowering seasons and climatic factors, which are quite variable in different geographical region of the world.

Durham [23] initiated aerobiological survey in the USA in 1925. Later, the sampler devised by him was adopted by the American Academy of Allergy (1946), as a tool to identify airborne particles. In Colorado pollen patterns were compared between Vail (8,200 feet elevation), Aspen (7,900 feet), and Denver (5,280 feet) from 1984 through 1988, while counts were generally lower in the mountain sites than Denver. Ragweed was essentially absent from Aspen and Vail, and Chenopod-amaranth counts were very low. Cedar, pine, and Aspen frequently pollinated despite active snowfall [28]. In USA, most of the investigators have recorded gramineae, Ambrosia, Quercus, Chenopodiaceae, Amaranthaceae, Pinaceae, Plantaginaceae, Artemisia, Xanthium as important consistuents of the atmosphere [29-31]. The frequently encountered pollen at Canada are Acer, Abies, Artemisia, Populus, Betula, Quercus, Rumex and Salix [32-34]. A study at New Jersey-New York City area established that pollen levels have declined from 1993 to the present. The most pronounced drop has been in weed pollen levels. Grass pollen demonstrates a biphasic pattern. Tree pollen composes most annual pollen measured [35].

Aerobiological survey in UK was initiated at Cardiff by Hyde and Williams [36], which was later extended to several other stations in Great Britain. Based on the data, Hyde published an atlas of airborne pollen grains of the UK [36-38]. The dominant pollen types recorded from the UK are Alnus, Artemisia, Betula, Corylus, Quercus, Fagus, Pinus, gramineae and others [39-41]. Emberlin [41] analyzed the annual variation in grass pollen in London during 1961-1990. In Montreal (Quebec, Canada), the influence of meteorological factors on Ambrosia pollen concentrations was evaluated between 1994 and 2002 and its adequate monitoring was considered critical [42].

In France, studies carried out at Montpellier, Marseilles and Paris, Lyon have shown Chenopodiaceae, Compositae, Cupressaceae, Pinus, Plantain, Poaceae, Alnus, Betula, Quercus as the pollen species encountered in large numbers [43, 44]. Another important center was Switzerland, where Leuschner [16] carried out survey using individual pollen collectors and found Aesculus, Artemisia and Salix as important pollen contributors in the atmosphere.

Survey carried out at Darmstadt, Germany revealed that 70% of the total pollen catch consisted of birch, grasses, nettle, oak and pine [45]. As a result of the five-year survey in the Netherlands, Spiesksma [46] demonstrated that in summer 95% of the pollen catch were of weeds namely Artemisia, Chenopodiaceae, Plantago, Rumex and Urtica.

Sweden is an important centre for aeropalynological studies. The most abundantly encountered pollen types are Pinus, Betula, Urtica, Ulmus, Quercus, Poaceae, Alnus and some others [47-49]. Extensive studies on the airborn pollen and the mode of sampling has been carried out by Käpylä [50] in Finland, with Artemisia, Betula, Pinus, Poaceae and Urtica being the dominant species. In the air of Denmark, the important pollen contributing species are Alnus, Artemisia, Betula, Corylus, Poaceae and Ulmus [51]. In Norway, Faegri [52] did pioneering work concerning pollen deposition in the 1940’s. The dominant species were Oxynia digna, Salix, Betula, Poaceae, Pinus, Castanea, Corylus, Alnus and Artemisia [53, 54]. In western Ligurian coast of Italy, a 10-year survey of pollen counts was performed. Over the period a significant increase in the pollen counts was seen for birch and Compositae (p = 0.001) [55].

Reports from other European countries like Israel, Portugal, Yugoslavia and Spain revealed that the significant pollen contributors to the atmosphere are Alnus, Chenio/Amaranth, Corylus, Cupressus, Morus, Olea, Pinus, Poaceae, Populus, Quercus and Taxus [56-58].

Various airborne herbaceous species of Plantago, Chenopodiaceae–Amaranthaceae, Rumex and Urticaceae were studied using Hirst
type Sampler form 1995-2005 in Spain and Poland [59]. In yet another study pollen counts were studied in Toledo, Spain from 2005-2010. Herbs accounted for 20% of total pollen count with Poaceae contributing largest and being the leading cause of respiratory allergies [60].

In a continuous two year aeropallynological survey of the atmosphere of Bitlis, (Turkey) Gramineae, Urticaceae, Juglans spp., Quercus spp., Umbelliferae, Cupressaceae/Taxaceae, Fraxinus spp., Salix spp., Plantago spp., Pinus spp., Rumex spp., Moraceae and Chenopodiaceae/Amaranthaceae were responsible for the high amounts of pollen in the investigated region. 58.38% of total pollen grains were appeared during May and June [61]. In a one year research program from 2005-2006 at Istanbul, 80% of important airborne pollen were identified from Cupressaceae, Urticaceae, Pistacia sp., Quercus sp., Platanus sp., Fraxinus sp., and Xanthinium sp. [62].

A 10-year volumetric aerobiologic study was conducted in the city of Heraklion, located in the center of the north-shore of the island of Crete, Greece, main allergenic families and genera encountered in descending order of frequency were, Oleaceae, Quercus, Platanaceae, Cupressaceae, Pinaceae, Populus, Moraceae, and Carylaceae [63].

In South Africa, the important pollen types identified in air are Acacia, Compositae, Cupressus, Eucalyptus, Graminae, Ligustrum and Prosopis [64]. Latter studies revealed that the airspora mainly comprises Morus, Cannabis, Poaceae, Celtis, Cynodon, Compositae, Pinus, Asteraceae and Fabaceae [65-67].

Mercer [68] carried out pollen count in Adelaide, Australia. The major pollen contributors to the environment were Casuarina, grasses, Myrtaceae, Pinus, Plantago, Populus, Quercus and few other [69, 70]. Smart and Knox [71] have shown that Lolium and Phalaris are the major sources of atmospheric pollen in Australia. In a recent study at Sydney, of the total airborne pollen, tree pollen comprised 65% of total pollen concentrations, weeds and herbs 11%, grasses 18%, and unidentified pollen (termed other) 6% [72].

In Japan, palynological survey started in the 1960’s. The most important being Japanese cedar followed by pine [73, 74]. Artemesia, Casuarinaceae, Euphorbiaceae, Graminae, Moraceae and Pinaceae are the major contributors to the atmosphere of China [75]. Chen and Huang [76] observed that in Taiwan the tree species contribute 56 percent of the total pollen count. The important pollen types from West Asia are Gramineae, Chenopodiaceae/Amaranthaceae, Cyperaceae, Pinaceae, Plantaginaceae, Acer, Cupressus, Morus, Poaceae, Populus, Pinus and others [77-80]. Date-Palm trees (Phoenix dactylifera L.) are the most abundant crops in the United Arab Emirates. Pollen counts were about 800 counts/m³ within the Date-Palm farms and decreased by about 80% just 100 meters away from the farm area and almost diminished beyond 200 meters [81].

Three allergenic pollen of taxa Cupressus, Pinus and Olea were studied in different parts of North Jordan from 2006-2007 using Volumetric method. The pollen count of Cupressus was higher in 2006, than in 2007 while for Pinus and Olea the opposite was the case. The longest pollen season was observed for Cupressus [82].

In India, first atmospheric survey was initiated in Calcutta by Cunningham [83]. 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, was successfully completed by Singh and his colleagues [84]. 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 in India. Altogether, 43 types of pollen have been recorded from Northern India. The dominant types are: Artemesia, Asteraceae, Cassia, Casuarina, Cedrus, Eucalyptus, Holoptelea, Morus, Pinus, Poaceae, Putranjiva, Quercus and Xanthium are other important contributors in the air. The aeroallergens of India were briefly reviewed by Singh & Kumar [85].

In an aerobiological survey from Delhi, ninety-four pollen types were recorded and the major contributors included Morus, Cannabis, Chenopod/Amaranth, Prosopis, Artemesia, and Eucalyptus [86]. A significant reduction in pollen concentration was observed in subsequent years. The concentration of Morus, Cannabis, Prosopis, and Artemesia pollen decreased considerably. It is suggested that the reduction in pollen numbers from 1990 to 1997 in Delhi is due to massive clearing of vegetation for developmental activities of the city. From Central India, surveys carried out at Bombay, Gwalior, Nagpur, Bhopal and Kolhapur revealed that the dominant pollen types are from the Poaceae, Asteraceae, Apocynaceae, Rosa, Ricinus, Ailanthus, Holoptelea, Cyperus, Cicer, Arameona, Cocos nucifera and Hibiscus.

Recent survey 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 were recorded in high concentration in April-May and November-December.

In West Bengal, 59 types pollen were revealed from air -
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their maximum concentration was recorded in May. Important dominant types are Areca catechu, Asteraceae, Chenopodiaceae, Cocos, Pongamia, Trema orientalis and Xanthium. At Gauhati, Asteraceae, Chenopod/Amaranth, Eucalyptus, Poaceae, Putranjiva and Magnifera are the dominant types of pollen. An aerobiological survey conducted from 2004-2006 with Burkard sampler detected Peltophorum pterocarpum and Delonix regia as dominant pollen in the atmosphere of Kolkata [87].

From Southern India, studies carried out revealed that Casuanna, Chenopod/Amaranth, Cocos, Cyperaceae, Eucalyptus, Parthenium, Peltophorum, Poaceae and Spathodia are dominant pollen types. Pollen calendars are very useful for clinicians as well as allergic patients to establish chronological correlation between the concentration of pollen in air and seasonal allergic symptoms. Based on aerobiological data obtained from India, pollen/flowering calendars have been prepared for Calcutta, Sambalpur, Gulberga, Imphal, Kodaikanal by different workers [88-92]. The Centre for Biochemical Technology now Institute of Genomics & Integrative Biology (Council for Scientific and Industrial Research) had published a book on pollen calendars of 12 different states [99].

Clinically important pollen allergens

Pollen 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.

In a study, three hundred seventy-one allergy patients were tested serologically for hypersensitivity towards prevalent tree pollens in the surrounding New York area over the years 1993-2000. It was concluded that in the New York City area, hypersensitivity to tree pollens most often is manifested with allergy to oak, birch, and maple tree pollens [94]. Latter on, among 158 patients with asthma, rhinitis, or both, 102 had positive skin tests to either Eastern red cedar (Juniperus virginiana) or white cedar (Thuja occidentalis). Among those, 52 patients (51%) had positive skin tests to at least one of the cedar pollens [95]. In Cincinnati, the aeroallergens having a significant impact on asthma hospital visits were ragweed, oak/maple and Pinaeae pollen. Their relative risks on asthma hospital visits with respect to a 100-counts/m³ increase in concentration were in the range of 1.23 to 1.54 [96].

The effect of pollen grains on morbidity from childhood conjunctivitis and rhinitis was studied. An increase of 72 ragweed grains per m³, was associated with an increase of about 10% in visits for conjunctivitis and rhinitis [97]. In a study from the Pacific coast to the Atlantic coast of Canada, changes in outdoor aeroallergens and hospitalizations for asthma was assessed. It was concluded that aeroallergens are an important cause of severe asthma morbidity across Canada, and in some situations there might be a modest synergistic adverse effect of ozone and aeroallergens combined [98].

In Cova da Beira, Portugal, the most representative aeroallergens sensitization were grasses mixture (44.9%), Olea europea (27.5%), Parietaria judaica (23.4%), Artemisia vulgaris (17.6%), Robinia pseudoacacia (12.2%), Platanus acenifolia (11.4%), Tilia cordata (11.4%), Plantago lanceolata (10.6%), and Pinus radiata (7.5%) [8].

In a population-based random sample of 498 adults aged 26-60 years were tested for 15 common aeroallergens with skin prick tests (SPTs) in Finland, 24% was sensitized to at least four allergens. Sensitization to multiple allergens was associated with a high prevalence of asthma, allergic rhinitis or conjunctivitis, and wheeze [99].

In a study carried out in southern Croatia, 46.7% were sensitized to Ambrosia elatior. Thus it is an important cause of seasonal allergic rhinitis and asthma in must be included in the routine diagnostic procedures in southern Croatia [100].

In a comparison of skin tests to aeroallergens in Ankara and Seoul, grass pollens were found to be major allergens more often in Ankara than in Seoul (74.34% vs. 15.87%, p < 0.001). Skin test reactivities in Ankara were significantly lower (p < 0.001) than in Seoul to weed (6.91% vs. 37.50%) and tree pollens (4.61% vs. 39.42%) [26].

On the basis of the results of an aerobiological survey in the Klang Valley of Malaysia, two local extracts of grass pollens i.e. Ischaemum and Enilia were recruited for Skin Prick Test. The SPT reactivity was 14.1% and 5.9% respectively [101]. In another study by SPT using eight aeroallergens in 206 asthmatic patients, sensitization was observed in Bermuda grass (7.9%), Acacia sp. (7.9%) [102].

A cross-sectional retrospective study based on SPT conducted on 226 allergic patients referred to allergic clinic of Karaj city, Tehran showed that the most common aeroallergens were: herbatce II (62%), sycomore (57%), chenopodium (53%), tree mix (50%), herbacee III (47%), grass (43%), ash (40%), herbacee I (37%), cedar (27%) [103].

Based on clinico-immunological evaluation of pollen antigens, important allergenic pollen in India has been identified. The work

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on pollen allergy was initiated in the 1950’s by Shivpuri in Delhi. Subsequently, Kasliwal and his colleagues reported important pollen allergens of Jaipur [104]. Shivpuri and Parkash [105] observed Prosopis juliflora as a major cause of pollinosis with 12% patients showing a positive skin reaction. Later, important pollen allergens were identified for Delhi by Shivpuri and his colleagues. They were: Ageratum, Alnus, Amaranthus, Anogeissus, Artemisia, Cassia, Chenopodium, Cynodon, Ipomoea, Paspalum, and Poa annua [106, 107]. We recorded positive skin reactions in 16.9% patients to Pinus roxburghii from the foothills of Himalayas [107].

In an aeropylonymology study from Kolkata from 2004-2006 revealed dominant and perennial pollen taxa from Trema, Poaceae, Casuarina, Cocos, Azadirachta, Peltophorum, Cyperaceae, Delonix and Areca. Skin prick tests were highest with Poaceae and positive correlation between pollen counts and monthly visits was found [108].

From Northern India, important allergens identified are: Prosopis juliflora, Ricinus communis, Morus, Mallotus, Alnus, Quercus, Cedrus, Argemone, Amaranthus, Chenopodium, Holoptelea, and grasses. From Central India the important pollen allergens are: Argemone, Brassica, Cannabis, Asphodelus, Parthenium, Cassia, Azadirachta, grasses, Alnus, Betula, Mallotus, Trewia nudiflora. From Eastern India, allergenically significant pollen types were found as: Lantana, Cucurbita maxima, Cassia fistula, Cocos nucifera and Calophyllum inophyllum. Recent studies based on clinical and immunologic parameters reported Phoenix, Ricinus communis and Aegle marmelos as causative agents of allergy in this region [84].

From South India Cassia, Ageratum, Salvadoria, Ricinus, Albizia lebbeck and Artemisia scoparia have been reported as clinically important aeroallergens [109, 110]. Rao et al. [111] recorded allergenicity to Parthenium hysterophorus pollen extracts in 34% of allergic rhinitis and 12% bronchial asthma patients from Bangalore. Agashe and Soucenadin [112] recorded high skin reactivity to Casuarina equisetifolia in patients from Bangalore.

Clinical studies undertaken at various medical centres under the All India Coordinated Project on Aeroallergens and Human Health [84] sponsored by the Ministry of Environment and Forest, revealed important allergenic pollen for various regions in India. 35 pollen antigens were tested on atopic population. At Chandigarh, skin sensitivity was highest against Rumex acetosa and Ailanthus excelsa (17.6%), followed by Trewia nudiflora (9.7%), Argemone mexicana (9.5%), and Cedrus deodara (9.3%). In Delhi, 12.6% of the atopic population was positive to Amaranthus spinosus, 8.5% to Populus deltoides and 7.5% to Dodonea viscosa, Bauhinia vareigata. In Calcutta, 28.8% of the patients were sensitive against Solanum sisymbriifolium, 21.1% to Crotalaria juncea and 18.2% each to Ricinus communis and Ipomoea fistulosa. In Trivandrum, maximum skin reactivity was recorded to Mallotus philippensis (12.1%), followed by Prosopis juliflora (6.3%). For the first time, Cedrus deodara (Pinaceae) pollen has been recognized as a new allergen from India in the patients from the Himalayan region, where Cedrus deodara occurs naturally [113]. The common allergenic plants of different seasons in India are mentioned in Table 1.

Major allergens vary from place to place. It is important for clinicians to select only those pollen antigens for skin testing which are prevalent in a particular area in which the patient resides.

Cross-reactive allergens in clinical allergy practice

Allergy is the result of binding between the epitopes on the proteins with the IgE. Because of evolution, certain proteins have remained conserved from the different sources. It is known that allergic patients are frequently co-sensitized against different allergen sources. Progress made in the field of allergen characterization by molecular biological techniques has now revealed that sensitization against different allergen sources can be explained as cross-reactivity of IgE antibodies with structurally and immunologically related components present in these allergen sources. The similarities among allergens may facilitate allergy diagnosis in clinical practice by using a few representative cross-reactive allergens to determine the patient’s IgE reactivity profile.

Cross reactive pollen allergens. Studies carried out across the globe suggest cross-reactivity among different plants. Lolium perenne has bee found to be cross-reactive with Acacia, pineapple, Olea europaea, Dactylis glomerata, Ligustrum vulgare, Cyndon dactylon and Pinus radiata [114-119]. Platanus acerifolia has been found to cross-react with Corylus avellana, Prunus persica, Malus domestica, Arachis hypogaea, Zea mays, Cicer arietinum, Lactuca virosa, Musa spp., and Apium spp. [120, 121]. Pollen from Japanese cypress (Chamaecyparis obtusa) cross reacts with Japanese cedar (Cryptomeria japonica) contributing to prolonged symptoms after the cedar pollen season in March and the following cypress pollen season in April [122].

Ricinus communis, commonly grown in India for its oil and abundantly present in wasteland, cross-reacts with Hevea brasiliensis, Mercurialis annua, Olea europaea, Betula, Zygophyllum fabago, Putranjiva roxburghii, and Ricinus (seed) from geographically distinct locations [123-125].
Areca catechu cross-reacts with Phoenix sylvestris, Cocos nucifera, Borassus flabelifer, as reported from India [126]. Cynodon dactylons (common grass) cross-reacts with Pennisetum clandestinum, Stenotaphrum secundatum, Eragrostis, Brassica napus, Olea europaea, Ligustrum vulgare, and Lolium perenne [126-130].

Holoptelea integrifolia and Parietaria judaica belonging to the family Urticaceae are geographically distantly located. H. integrifolia is an important pollen allergen of India cross reacts with P. judaica, on the other hand, is a very dominant pollen allergen of the Mediterranean region. H. integrifolia and P. judaica pollens share cross-reactive as well as unique epitopes [131]. Some examples of cross-reacting tree pollen allergens are presented in Table 2.

### Molecular approach to allergen 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 inter-company variability. These great differences result in unreliable prick test results, prevents comparison of skin tests world over and in generating a dose response data. Also their use in therapeutics makes it necessary to have an international standard.

The need for standardization of allergenic extracts has been recognized since the advent of immunotherapy [132]. The early attempts towards standardization were based on consistency in crude w/v ratio or estimation of protein nitrogen unit content of the extracts. 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 Northern Society of Allergology and adopted by European investigators [133]. One histamine equivalent prick (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. [134] designated 1 HEP as 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 glycoprotein fractions.

### Future trends in allergen research

Since mid 1980, a fast growth has seen in the information of allergy. A large number of major allergens have been isolated, purified and their amino acid sequencing and epitope mapping have been done. The allergenic epitopes have also been analysed using Human T cells [135].

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 [136]. However, at

| Spring (Feb.–Apr.) | Autumn (Sep.–Oct.) | Winter (Nov.–Jan.) |
|--------------------|--------------------|--------------------|
| **Trees**          |                    |                    |
| Ailanthus excelsa  | Anogeissus pendula | Cassia siamea      |
| Bauhinia variegata  | Carica papaya      | Cedrus deodara     |
| Casuarina equisitifolia | Cocos nucifera | Coccus nucifera Eucalyptus sp |
| Holoptelea integrifolia | Mallotus philippensis | Mallotus philippensis Phoenix sylvestris |
| Mallotus philippensis | Prosopis juliflora | Prosopis juliflora |
| Prosopis juliflora | Quercus incana     | Quercus incana     |
| Putranjiva roxburghii |                |                    |
| **Weeds**          |                    |                    |
| Cannabis sativa    | Amaranthus spinosus | Ageratum conyzoides |
| Chenopodium murale | Artemisia scoparia  | Agemone mexicana   |
| Parthenium hysterophorus | Cassia occidentalis | Asphodelus tenuifolius |
| Plantago major     | Ricinus communis   | Chenopodium album  |
| Sueda fruiticosa   | Xanthium strumarium | Ricinus communis   |
| **Grasses**        |                    |                    |
| Cynodon dactylon   | Bothriochloa pertusa | Aegemon dactylon |
| Dicanthium annulatum | Cenchrus ciliaris | Eragrostis tenella |
| Imperata cylindrica | Heteropogon contortus | Phalaris minor |
| Paspalum distichum | Pennisetum typhoides | Poa annua   |
| Poa annua          | Sorghum vulgare    |                    |
| Polygopus monspeliensis |                |                    |
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 [137].

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.

Pharmacogenetics

Asthma and allergy is a “complex” genetic disorder caused by the interactions of multiple interacting genes. In, addition, these disorders require the presence of appropriate environmental triggers for their expression. Studies have suggested that genes within the cytokine gene cluster on chromosome 5 (including interleukins -3, - 4, - 5, - 9 and -13), chromosome 11 (the beta chain of the high affinity IgE receptor), chromosome 16 (the IL-4 receptor), and chromosome 12 (stem cell factor, interferon gamma, insulin growth factor, and Stat 6 (IL-4 Stat) may contribute to asthma and allergy development. In addition, data support involvement of genes involved in antigen-presentation (MHC class II genes) and T cell responses (the T cell receptor alpha chain). Disease contributing alleles may be present on genes for the beta-andrenergic receptor, 5-lipoxygenase, and leukotriene C4 synthase. Genetic analysis holds the promise of being able to identify specific enzymes that determine the response to asthma medications since for any given asthma medication, a certain percentage of the population fail to have a beneficial clinical response. Thus, gene based therapy holds the promise for effective treatment of asthma and allergy in future.

**Allergen avoidance recommendations**

Important pollen and fungal allergens vary in concentration and prevalence from place to place so it is advisable to measure their concentration and establish relationship with the patient symptoms.

The remedial action depends on the actual problem and conditions prevailing in different occupational sites, however following preventive measures in general are recommended.

**Pollen allergens**

1. All the allergenically significant trees need to be deleted from the list of recommended tree plantation in gazette of India.
2. Ornamentals, insect/bird pollinated and medicinally important trees or others which can help in controlling pollution, should be encouraged in various tree plantation and afforestation programs.
3. The existing allergenically significant trees need to be replaced with non-allergenic trees in a phased manner.
4. On medical ground, citizens should have the right to cut or demand removal of allergy causing trees in close vicinity.
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However, these should be replaced with some non-allergenic trees.
5. A genuine beginning needs to be made by sensitizing tree lovers/horticulturist/foresters/botanist and other associated with tree plantation so that the share of allergenically significant plants could be minimized in the near future. The earlier it is done the better.

Allergy has been known for more than a century now, but the subject has suffered due to lack of knowledge about basic mechanisms involved in allergic diseases and poor diagnostic procedures. The rapid development in the field of immunology and clinical diagnosis in the pool of knowledge, is of, direct significance to allergy practitioners. The detailed information on indigenous pollen and fungal allergens will be very useful in diagnosis and management of allergic patients.

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