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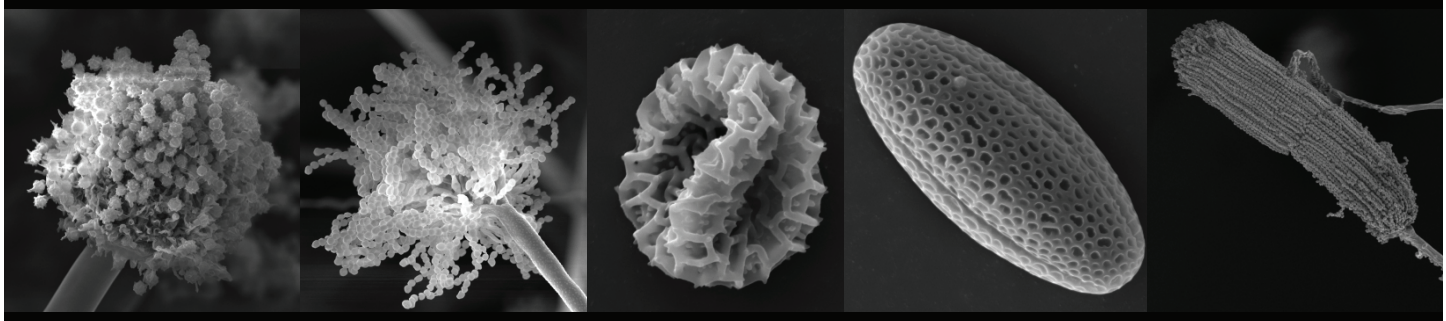

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Review Article

AN OVERVIEW OF FIFTY YEARS OF AEROBIOLOGICAL AND IMMUNO-CHEMICAL RESEARCH ON FUNGAL ALLERGENS IN WEST BENGAL, INDIA

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Air plays a crucial role in transporting pollen grains and fungal spores, which can trigger allergic reactions in humans. The link between these particles and allergies was not recognized until the 19th century, with John Bostock first identifying pollen grains as a cause of hay fever in 1819, and Charles Blackley confirming this in 1873. Today, allergic diseases such as allergic rhinitis, bronchial asthma, and atopic dermatitis affect 20-30% of the global population, especially children and the elderly.

Bioparticles from sources like pollen, fungal spores, mites, insect parts, and other organic materials are the primary allergens in the air. Understanding the prevalence, seasonal variations, and annual changes in aeroallergens is vital for diagnosing and treating allergies. In India, where the climate and flora vary widely, studying spore distribution across regions is particularly important.

Over the past 50 years, aerobiological studies in West Bengal, including research from institutions like Bose Institute, Institute of Child Health, B. R. Singh Hospital in Kolkata, and Visva-Bharati University in Santiniketan, have focused on airborne fungal spore concentrations and seasonal patterns. Common fungal allergens in both indoor and outdoor environments include *Mucor* sp., *Rhizopus* sp., *Syncephalastrum* sp., *Chaetomium* sp., *Aspergillus* spp., *Penicillium* spp., *Cunninghamella* sp., *Pleospora* sp., *Alternaria* spp., *Dreschlera* sp., *Curvularia* sp., *Cladosporium* sp., *Fusarium* sp., and *Ganoderma* sp. Clinically significant allergens include *Aspergillus* spp., *Alternaria* spp., *Cladosporium* sp., *Ganoderma* sp., *Mucor* sp., *Rhizopus* sp., *Fusarium* sp., and *Curvularia* sp. Climatic factors heavily influence spore distribution, with concentrations peaking during the rainy (July-October) and winter (November-February) seasons, and being lowest in summer (March-June). Several fungal allergens have been purified, characterized, and used in immunotherapy.

Key Words: Fungal spores, Aerobiology, Allergy, Allergens, Asthma, West Bengal, India.

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INTRODUCTION

Aerobiological studies have gained immense importance in recent years. Pollen and fungal spores are predominant aeromicrobial components, which have significant importance due to their phytopathogenic and allergic effects[1]. Recent surveys carried out in India show that about 20-30% of the population suffer from allergic rhinitis and 15% from asthma[2,3]. Thus, from a clinical point of view, it is important to determine the details about the occurrence of allergenic fungal spores in the atmosphere, their chemical composition and molecular characterization[4].

West Bengal, located in Eastern India on the Bay of Bengal, is situated between 22°34' North latitude to 88°22' East longitude[5]. It is blessed with very rich flora, extends from the high Himalayan foot hills in northern region to the islands of Sundarbans at the southern part and from the eastern great Gangetic plane to the western laterite plateau. Various trees, shrubs and herbs can be observed along the latitudinal and longitudinal gradients. Hence, the state provides considerable variation in the quantity and quality of airborne pollen and fungal spores in different geographic regions of West Bengal.

Although many studies on the allergic properties of airborne spores of various species have been carried out by several workers in India as well as in West Bengal,[6,7,8,9] but comprehensive documentation on fungal spore allergens of West Bengal has not been made during the past 50 years. It is India's fourth most populous state with over 91 million inhabitants (2011 census) and a major agricultural producer[5]. It is a vast and geographically diverse state. So, in the present study, the state has been sub-divided into five principle zones based on the

plant and land diversity. These include: (i) Darjeeling Himalayan hills and Terai region, comprises the districts of Darjeeling, Jalpaiguri and Cooch Behar, (ii) North Bengal plains, contains district of North Dinajpur, South Dinajpur and Malda, (iii) Gangetic plains which comprise Murshidabad, Nadia, East Burdwan, Hooghly, Howrah, Kolkata, North 24 Parganas and some parts of South 24 Parganas, (iv) Western laterite plateau (Rarh) in which Birbhum, West Burdwan, Bankura, Purulia, and West Midnapur are included, (v) the Southern Coastal Plains and mangrove area contain South 24 Parganas and some parts of East and West Midnapur[10] (Table 1.1). These areas show some differences in the vegetation types and in climatic conditions. For that, although some fungal spores are common, there are some differences in the prevalence of allergic fungal spores in the different parts of West Bengal.

An attempt has been made in this review to analyze important aeromycological surveys which carried out in different parts of West Bengal with special reference to allergenic significance during the last fifty years.

RESULTS AND DISCUSSION

In 1873, British physician D. D. Cunningham conducted the first survey of atmospheric biopollutants at Alipore Central Jail, Calcutta, then capital of India[11]. His findings illustrated the presence of pollen, fungi, algae, and fragments in the air (Fig. 1.1), linking these airborne

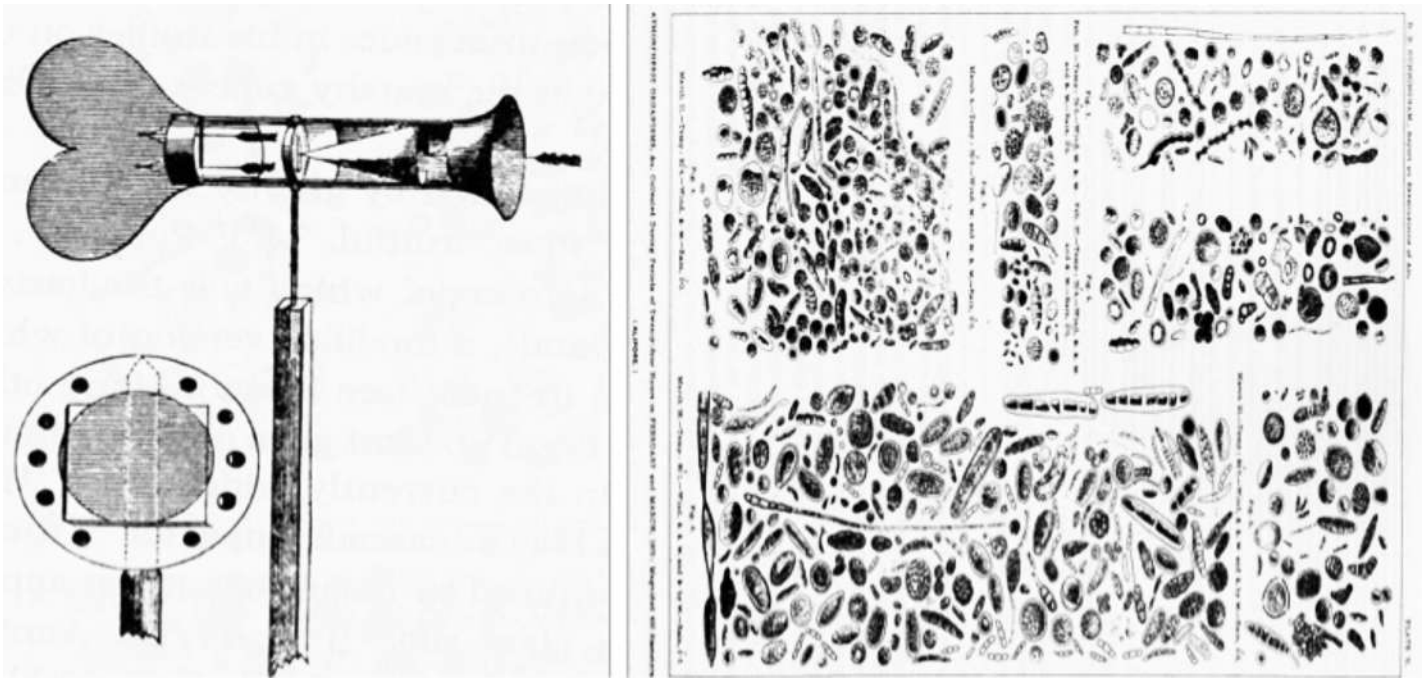

Fig. 1.1: Cunningham's aerobiological sampler (A) and observed particles (B).

Table 1.1: Aerobiological work carried out in major geographical regions of West Bengal

Sl. No.	Geographical Regions	Districts Cover
1.	Himalayan hills and Terai region	Darjeeling, Jalpaiguri, Cooch Behar.
2.	North Bengal plains	North Dinajpur, South Dinajpur, Malda.
3.	Gangetic plains	Murshidabad, Nadia, East Burdwan, Hooghly, Howrah, Kolkata, North 24 Parganas and some parts of South 24 Parganas.
4.	Western laterite plateau (Rarh)	Birbhum, West Burdwan, Bankura, Purulia, and West Midnapur.
5.	Southam Coastal Plains and Mangrove area	South 24 Parganas and some parts of East and West Midnapur.

organisms to "Zymotic diseases". After this pioneering work, Indian aerobiological research saw a century-long hiatus until a resurgence during the 4th International Palynological Conference in Lucknow (1977). Subsequently, Indian aerobiologists, botanists, and environmentalists established the Indian Aerobiological Society (IAS) in Calcutta in 1980, during a workshop jointly organized by the British Council and Bose Institute. Aerobiological studies in Kolkata and West Bengal were initiated at Bose Institute under the guidance of Professor S. Chanda and his students. They developed spore calendars for Kolkata, Falta, and Kalyani. In Eastern India, Chanda and his Kolkata team, alongside Singh from Imphal, conducted extensive surveys to identify fungal spore types in West Bengal and Manipur, respectively[11,12,13].

Airborne Fungal Survey

Fungal spores are very diverse in origin and morphology. There are more than 80,000 species of fungi and they have evolved their mechanisms of spore dispersal[14,15]. Different types of spores are spread out in different phylogenetic groups, viz. Myxomycotina, Mastigomycotina, Zygomycotina, Ascomycotina[16]. Gregory (1966) classified those spores which are dispersed from the place of origin as Xenospores and Memnospores, those remaining in the place of origin[17]. Spores can be found both in outdoor air as well as in indoor environments such as store houses, hospitals, libraries, residential buildings, etc. Due to their small size, spores remain suspended in the atmosphere for a long time. When inhaled by susceptible individuals, they cause respiratory disorders[14].

In India, the aeromycological studies were initiated in 1959 by Sreeramulu at Visakhapatnam[18]. Subsequently, such studies were conducted in other parts of India, including West Bengal[19]. In West Bengal, aeromycoflora study was initiated by Dr. S. Chanda and his students. Aeromycological studies in West Bengal were conducted in two different ways. These include (a) study of airborne fungal spores in the atmosphere of outdoor environments of different places, (b) study of fungal spores present in indoor environments.

a. Outdoor aeromycoflora

Aerobiological surveys carried out in Eastern India revealed Aspergilli/Penicilli, *Cladosporium* sp., ascospores, rust and smut spores, *Nigrospora* sp., *Periconia* sp., *Ganoderma* sp. and *Rhizopus* sp. as major fungal types[2] (Table 1.2).

Table 1.2: Dominate fungal spores in different regions of West Bengal

Sl. No.	Fungal species	Himalayan Hill stations and Terai regions	Gangetic plains	Laterite plateau regions	Southern Coastal Plains and Mangrove areas
1.	*Aspergillus sulphureus*	+	–	–	–
2.	*Aspergillius parasiticus*	+	+	+	–
3.	*Aspergillus flavus*	–	+	+	–
4.	*Aspergillus nidulans*	–	–	+	+
5.	*Aspergillus niger*	–	–	+	+
6.	*Aspergillus terreus*	–	–	+	+
7.	*Aspergillus tamari*	–	+	–	–
8.	*Aspergillus fumigatus*	–	+	–	+
9.	*Aspergillus japonicus*	–	+	–	–
10.	*Aspergillus alternata*	–	+	–	+
11.	*Aureobasidium pullulans*	+	–	–	–
12.	*Alternaria* sp.	–	+	+	+
13.	*Bispora* sp.	–	–	+	–

Contd.

Contd. Table 1.2

14.	*Cercospora* sp.	–	–	+	–
15.	*Cephalosporium curtipes*	–	–	+	+
16.	*Cunninghamella* sp.	–	–	+	–
17.	*Candida* sp.	–	–	+	–
18.	*Curvularia* sp.	+	+	+	+
19.	*Cercospora* sp.	+	–	–	–
20.	*Cladosporium* sp.	+	+	+	+
21.	*Chaetomium* sp.	–	+	+	–
22.	*Coprinus* sp.	–	+	–	–
23.	*Dreschlera* sp.	+	+	+	–
24.	*Epicoccum* sp.	+	+	–	–
25.	*Eupenicilliurn javanicum*	–	+	–	–
26.	*Fusarium* sp.	+	+	+	+
27.	*Gliocladium roseum*	–	+	–	–
28.	*Ganoderma* sp.	+	+	+	–
29.	*Helminthosporium* sp.	–	+	+	–
30.	*Mucor mucedo*	–	+	+	–
31.	*Mycelia sterilia*	–	+	–	–
32.	*Nigrospora* sp.	+	+	–	–
33.	*Papularia* sp.	–	–	+	–
34.	*Pestalotia* sp.	–	–	–	+
35.	*Pleospora* sp.	–	–	+	–
36.	*Pithomyces* sp.	+	+	–	–
37.	*Penicillium* spp.	+	+	+	+
38.	*Phoma nebulosa*	–	+	–	–
39.	*Periconia* sp.	+	+	–	–
40.	*Rhizoctonia* sp.	+	–	–	–
41.	*Rhizopus oryzae*	–	+	+	+
42.	*Spegazzinia* sp.	–	–	+	–
43.	*Spicaria elegans*	–	–	+	–
44.	*Sporidesmium* sp.	–	–	+	–
45.	*Syncephalastrum* sp.	–	–	+	–
46.	*Torula* sp.	+	–	–	–
47.	*Trichoderma pseudokoningii*	–	+	–	+
48.	*Trichothecium* sp.	–	–	+	–
49.	*Tharninidium* sp.	–	–	–	+

Study was conducted in Himalayan hill stations and Terai region, especially in tea garden by using liquid vegetable waste, deproteinized leaf juice instead of usual media. Several fungal spores of *Pithomyces nigrospora, Curvularia senegalensis, Penicilliurn citrinum, Penicillium purpurogenum, Penicilliurn griseofulvum, Aureobasidium pullulans, Torula* sp., *Aspergillus sulphureus, Drechslera oryzae, Rhizoctonia* sp. were reported. It was also reported that the town area showed a much more abundance of fungal spores than the tea garden area[20]. Later such study was substantiated by Majumdar and Bhattacharya[21]. They studied the foothills of the Eastern Himalaya and identified a total of 18 fungal spore types. Some predominant types were *Alternaria* sp., *Aspergillus* spp., *Cercospora* sp., *Cladosporium* sp., *Curvularia* sp., *Dreschlera* sp., *Epicoccum* sp., *Fusarium* sp.

The first aeromycoflora study in Gangetic plain was carried out by Barat and Das[22] in Calcutta by exposing plates of malt agar for 5 minutes at 4 feet above the surface. Colony counts were high from January to May, *Cladosporium* sp. being dominant in the earlier and *Aspergillus* sp. in the latter part respectively. *Curvularia* sp. and *Penicillium* sp. were common throughout the year, while *Alternaria* sp. occurred in considerable numbers only in February to April. A survey work was also done by Chakraverty and Sinha[23] in 5 outdoor places of Kolkata (Residential area, City park, City street, River side and Lake area) for five months. This revealed a high concentration of *Aspergillius parasiticus* spores in the air. City park, showed a comparatively higher concentration and residential areas or work places showed significantly low concentration of fungal species. In another aeromycoflora study, over a paddy field in West Bengal during rabi season, was initiated through culture plate exposure technique for two consecutive crop seasons near Barrackpore (50 km north of Kolkata) by Uddin[24]. The study revealed the dominant species of *Cladosporium* sp., *Aspergillus* sp., and Epicoccum-purpurascens. Dominance of *Cladosporium* sp. and Epicoccum purpurascens in winter was gradually diminished towards summer, while *Penicillium* sp. showed a reversed picture. *Curvularia* sp. showed no seasonal variation. *Alternaria* sp., *Fusarium* sp., *Helminthosporium* sp. and *Nigrospora* sp. were the phytopathogenic fungi recorded from air; of which *Alternaria* sp. was the dominant[24]. The study of air spores in residential area and market place in Calcutta was carried by Chanda *et al*.[20] A five-year (1994–1999) continuous survey of aeromycoflora was carried out in an agricultural farm at a suburban area of Greater Calcutta by Chakraborti *et al*.[25] A total of 26 fungal spore types were identified. The most abundant types were Basidiospores followed by *Cladosporium* sp., *Periconia* sp., *Nigrospora* sp., Aspergilli group, Ascospores. The seasonal periodicity of the major dominant types was maximum in autumn, during the month of October. This coincided with the harvesting period of the rice crop during the rainy season. A slight gradual increase in fungal spore concentration was also observed in May at the time of summer rice harvesting period. In Petriplate exposure of nutrient media, different species of aspergilli group, *Alternaria* sp., *Cladosporium* sp., *Curvularia* sp., *Nigrospora* sp., and other taxa were identified[25]. Two-year study (October, 1996 to September, 1998) of aerospores in five different rural places showed prevalence of viable fungi, such as *Aspergillus flavus, Aspergillus japonicus, Aspergillus fumigatus, Alternaria alternata, Cladosporium cladosporioides, Curvularia pallescens, Fusarium roseum, Rizopus* sp. etc.[26] A study was conducted using rotorod samplers mounted at different heights in an agricultural field near Kolkata at weekly intervals for two consecutive years (November 1997–October 1999) by Chakraborty *et al*.[27] Some major and perennial fungal spore types were Aspergilli group, *Cladosporium* sp., *Nigrospora* sp., etc. The smaller spores were dominant at greater heights and larger spores and conidia were more prevalent at lower levels. The total spore count was higher just after the rainy season during winter. Among fungal spores, *Drechslera oryzae* – the pathogen causing brown spot of rice was also found to be a potent allergen[27]. A total of 37 aerospora was recorded in two consecutive sampling years (2002-2004) in Madhyamgram, a rural place near Kolkata. Among the aerospora recorded the predominant ones (mean annual contribution >1.0%) were originated from *Alternaria* spp., Aspergilli/Penicilli, *Chaetomium* sp., *Cladosporium* sp., *Coprinus* sp. *Curvularia* sp., *Drechslera* sp., *Ganoderma* sp., *Nigrospora* sp., *Periconia* sp., *Pithomyces* sp., ascospores and basidiospores[28]. The composition and variability of airborne fungal spores were also studied by Das and Gupta-Bhattacharya[29], using two complementary sampling methods in an outdoor environment in

Kolkata suburbs for 2 years, from November 2002 to October 2004. They identified 37 fungal spore types in the air samples with dominant species of *Cladosporium* sp., Aspergilli/Penicilli, *Nigrospora* sp., *Periconia* sp., *Chaetomium* sp., *Drechslera* sp., *Alternaria* sp., *Coprinus* sp., *Ganoderma* sp., *Pithomyces* sp., and rust spores. In general, a higher spore count was recorded in winter. The highest fungal species variability was observed in the early monsoon (June). Relative humidity could significantly predict the seasonal periodicity of the maximum number of airborne spores. *Cladosporium cladosporioides* was recorded beyond the proposed threshold limit value in January 2003 and March 2004; *Aspergillus fumigatus* and *Aspergillus nidulansin* in winter which might have posed considerable health risk to sensitized individuals[29]. A systematic survey of aerofungal load in two outdoor environments in Farakka, West Bengal, India was carried out for a period of 2 years (October, 2013 to September, 2015) by Roy *et al*.[30] A total of 27 culturable fungal types were recorded from two study sites, of which *Cladosporium* sp. and *Aspergillus* sp. showed a higher percentage of occurrence. Other predominant fungal types were *Alternaria* sp., *Curvularia* sp., *Fusarium* sp., *Penicillium* sp., Mycelia sterilia, *Fusarium* spp., *Mucor mucedo, Nigrospora sphaerica, Rhizopus nigricans* and *Trichoderma* sp. In both the investigating sites, winter season showed maximum CFU (colony forming unit) concentration. The high concentration of spores in winter season was perhaps due to the accumulation of large number of vegetables and other substrata in and around study sites[30].

In Western laterite Plateau region of West Bengal, a comparative survey of airborne fungal spores in five indoor and five outdoor environments in Burdwan, was carried out for a period of two years (March 1991 to February 1993) using rotorod samplers and sedimentation plates[31]. A total of 29 types of spores were identified, categorized into Phycomycetous (*Mucor* sp., *Rhizopus* sp., *Syncephalastrum* sp.), Ascomycetous (*Chaetomium* sp.), Basidiomycetous (*Ganoderma* sp.), and Fungi Imperfecti. The study revealed the lowest spore counts during summer and the highest during the rainy season. *Aspergillus* sp. was notably abundant across all surveyed environments. The prevalence of *Aspergillus* sp., *Curvularia* sp., *Alternaria* sp., *Cladosporium* sp., *Drechslera* sp., and *Fusarium* sp. in these environments was attributed to their adaptability to various substrates. The presence of *Cladosporium* sp. during winter months indicated sensitivity to higher temperatures. Earlier work by Raha and Bhattacharya documented 24 fungal spores using a Rotorod sampler in Santiniketan (160 km northwest of Kolkata), identifying Aspergilli, *Cunninghamella* sp., *Rhizopus* sp., *Syncephalastrum* sp., *Chaetomium* sp., *Pleospora* sp., and *Ganoderma* sp. *Aspergillus* sp. contributed most significantly to the total aerospora, followed by *Curvularia* sp., etc. with concentrations lowest in summer and peaking during the monsoon and post-monsoon periods.

The high concentration of spores in winter was due to a large number of saprophytic forms, of which Cladosporium was the major component[32,55]. A distinct seasonal periodicity can be seen in their annual distribution of the occurrence of aerospora, perhaps due to differences in meteorological conditions. The concentrations of spores were lowest in summer and highest during the monsoon and post monsoon periods. The high concentration of spores in winter was due to a large number of saprophytic forms, of which Cladosporium was the major component. The airspora of an outdoor environment at Santiniketan was trapped for one year (September 2013 to August 2014) by Karak and Bhattachraya[33]. A total of 27 airborne fungal spores were identified, of which Aspergilli/Penicilli, *Cladosporium* sp., Basidiospores and Ascospores contributed a significant portion of airborne fungal load. *Cladosporium* sp. (dry spore) occurred during the winter season, showing a peak of 53.0%, while Ascospores and Basidiospores (wet spores) were the major contributors in the monsoon and post-monsoon seasons. The antigenic extracts of four fungal species namely, *Cladosporium cladosporioides, Aspergillus fumigatus, Aspergillus niger and Alternaria alternata* evoked moderate skin reactions in susceptible subjects.

West Bengal features an extensive coastline along the districts of South 24 Parganas, East Midnapore, and West Midnapore. Although there is limited local research, neighboring Odisha has been the focus of studies by various researchers[34]. These studies provide a foundation to potentially identify similarities in airborne fungal presence, such as *Pestalotia* sp., *Curvularia* sp., *Alternaria* sp., *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., and *Cephalosporium* sp., along the coastal regions of West Bengal. Chanda *et al*.[20] con-

ducted a study in April-May 1994, using deproteinized leaf juice (DLJ) and liquid vegetable waste to isolate aeromicroflora in ecologically distinct zones, deviating from conventional media.

In a study conducted in the sandy coastal zone of Midnapore district in Southern Bengal, fungal spores were identified, including *Tharninidium* sp., *Rhizopus oryzae, Penicillium oxalicum, Penicillium digitatum, Alternaria alternata, Fusarium pallidoroseum,* and *Trichoderma pseudokoningii*[20]. Additionally, small patches of Casuarina equisitifolia were found on sandy beaches in East and West Midnapur district, where fungal species such as *Pestalotia* sp., *Cladosporium cladosporioides, Alternaria alternata, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Aspergillus terreus, Curvularia lunata,* and *Fusarium* sp. were predominant in the humid shady forests[35].

b. Indoor aeromycoflora

Indoor fungal spores are frequently reported by researchers, and since humans spend a significant amount of time indoors, the indoor mycoflora plays a critical role in immunological issues such as asthma and allergies. Unfortunately, studies on indoor air mycoflora in West Bengal are limited compared to those conducted on outdoor environments. Various indoor environments in West Bengal have been studied extensively by researchers, including chira mills, granaries, paper-related industries, storage areas, libraries, bakeries, domestic houses, cattle sheds, markets, hospitals, lecture halls, and school rooms. These studies have observed some similarities in the occurrence of allergic fungal spores across different environments.

(i) **Residential houses:** A study by Chakravarty and Sinha[23] revealed that in Kolkata, *Aspergillus parasiticus* count is low in residential areas. Ten fungal species were identified from the residential place of a semi urban area (Burdwan), in which *Fusarium* sp., *Drechslera* sp., *Nigrospora* sp., *Periconia* sp., *Pithomyces* sp. were found along with *Aspergillus* sp., *Cladosporium* sp. and *Alternaria* sp.[31]. Twelve fungal species were recorded from the survey of rural residential houses, in which dominated species were *Aspergillus* spp. and *Curvularia* sp.[36].

(ii) **Hospital:** A study by Chakravarty and Sinha[23] revealed that in Kolkata, *Aspergillus parasiticus* count was relatively low in hospital ward than the market or storage house. Eight different spore types were recorded from a hospital word in Burdwan where dominated species were *Aspergillus* sp., *Cladosporium* sp. and *Alternaria* sp.[31] Out of 12 fungal species recorded from the survey of a rural hospital ward, *Aspergillus* sp. was found to be a dominant one. Some other genera like, *Rhizopus* sp., *Alternaria* sp., *Cladosporium* sp., *Curvularia* sp. were also reported[36].

(iii) **Markets:** A study in market place at Kolkata revealed *Aspergillus parasiticus* count in a relatively higher value than the school room, storage house or residential areas[23]. Eleven fungal species identified from the indoor market of Burdwan where *Aspergillus* sp., *Cladosporium* sp. and *Curvularia* sp. were predominated taxa[31]. A rural indoor market was surveyed where a total of 12 fungal species were found. The dominated taxa were *Aspergillus* sp., *Alternaria* sp. and *Cladosporium* sp.[36].

(iv) **Library:** University library of Visva-Bharati, Santiniketan was studied where a total 14 fungal spore types were recorded, of which *Cladosporium* sp. was dominant followed by *Aspergillus* spp.[36].

(v) **Lecture hall:** A study by Chakravarty and Sinha[23] revealed that in Kolkata, a lecture hall of metropolitan area show higher concentration *Aspergillus parasiticus* than the school room, hospital ward and residential areas.

(vi) **School rooms:** A study by Chakravarty and Sinha[23] revealed relatively lower *Aspergillus parasiticus* count in school room than that of market storage house or lecture room.

(vii) **Storage places:** 10 fungal species were recorded from the storage place of Burdwan, a semi urban area, in which *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Periconia* sp. were the major constituents[31].

(viii) **Cattle sheds:** One suburban and one rural cow-shed areas were surveyed and a total of 29 fungal spore types were recorded from rural and 24 types from suburban cow sheds. In both the places, a relatively high frequency of *Clado-*

sporium sp., *Aspergillus* spp., *Penicillium* sp, *Periconia* sp., *Nigrospora* sp. and ascospores and basidiospores were recorded[37].

(ix) **Paper related industries:** Four selected sites associated with the paper trade in the Calcutta metropolis, like paper storehouse, printing press, a book bindery and a retail book store, were surveyed by Andersen sampler[38]. A total of 26 fungal types from all the survey sites were recorded during the period, of which the most dominant were *Aspergillus niger* (36.1%), *Cladosporium cladosporioides* (30.2%) and *Fusarium solani* (11.62%). Some other important allergic species like *Curvularia lanata* (18.8%), *Aspergillus fumigatus* (22.6%), are predominant under these conditions.

(x) **Bakery:** Three different sections of rural bakerys were surveyed and, on average, 36 fungal species (of which 21 belonging to Deuteromycotina) were recorded. Some dominated species are *Aspergillus* spp., *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp., *Nigrospora* sp., *Periconia* sp., *Drechsler* sp. and *Torula* sp.[39].

(xi) **Granary:** A Granary in Kolkata metropolitan city revealed dominance of Aspergillus parasiticus[33]. A total of 11 Fungal species were recorded from a rural granary, in which *Aspergillus* spp. were dominant followed by *Cladosporium* sp., *Alternaria* spp.[36].

(xii) **Chira mill:** Nine fungal species were recorded from a Chira mill in Burdwan in which major fungal genera were *Aspergilli* sp., *Cladosporium* sp., *Curvularia* sp., *Drechslera* sp., *Tetraploa* sp. and *Fusarium* sp.[31].

Immunochemical studies of major aeroallergens:

Fungal spores are major elements for the various immunochemical diseases[40]. Some major clinically important fungal allergens recorded from West Bengal are discussed below:

Immono-clinically important fungal allergens:

Many fungal species have been shown as potential allergens causing respiratory diseases both in indoor and outdoor conditions. Some fungal species cause severe respiratory and cutaneous allergic diseases. Some species cause plant diseases also. Several investigators identified various allergenic fungal species. The results of various skin prick tests revealed that various fungal groups showed various stages of allergy.

Rhizopus sp., *Alternaria* spp., *Aspergillus* spp., *Curvularia* sp., *Fusarium* sp. were the major outdoor fungal species which showed maximum sensitivity in skin prick test when studied in outdoor environments in rural areas[31,32]. *Alternaria* spp., *Aspergillus* spp., *Helminthosporium* sp. and *Nigrospora* sp. were the major pathogenic fungus in rice field[24]. *Aspergillus japonicus* was found to be the strongest allergen, evoking 74.07% positive reactions to sensitive subjects[27]. In Santiniketan (a rural area) *Cladosporium cladosporioides* showed highest positive reaction[33]. In urban area, major fungal ailments are *Cladosporium* sp., *Alternaria alternata*, *Curvularia* sp. and *Penicillium* sp.[22] and the most important species, *Aspergillus parasiticus* showed the maximum positive reaction in SPT[23, 29].

Indoor environments, including urban and rural houses, as well as suburban areas, were surveyed epidemiologically for respiratory diseases associated with pathogenic fungi. *Aspergillus parasiticus* was notably prevalent in both urban and rural homes[23]. In urban settings, *Aspergillus niger* (27.50%), *Aspergillus fumigatus, Fusarium roseum* (22.22%), *Curvularia pallescens* (20.83%), and *Cladosporium cladosporoides* (19.40%) were prominent[36]. In suburban areas, *Aspergillus niger* (32.22%), *Cladosporium cladosporioides* (18.05%), *Curvularia lunata* (16.07%), Alternaria alternata (10.00%), and *Rhizopus nigricans* (7.27%) were dominant[31]. These fungi were also prevalent in indoor environments such as libraries, storage areas, and markets.

Studies conducted in bakeries, chira mills, and granaries indicated that 40-59% of workers suffered from respiratory diseases[31]. Sensitivity patterns in bakery workers revealed high levels of IgE antibodies to several species of *Aspergillus, Alternaria, Fusarium,* and *Rhizopus*[39]. In the paper industry, skin prick tests were positive for *Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium,* and *Rhizophus* species, with *Aspergillus niger* showing the highest positivity[38]. Chira mills reported *Aspergillus, Alternaria,* and *Cladosporium* as major fungal issues[31]. Cow sheds in rural and sub-

urban areas showed dominance of *Aspergillus fumigatus and Rhizopus nigricans*, with positive skin prick results also for *Cladosporium cladosporioides, Curvularia pallescens*, and *Fusarium roseum*[37].

Molecular Allergology

Recent advancements in molecular biology have significantly advanced the field of allergen biology, giving rise to a new discipline known as 'Molecular Allergology.' This branch has become pivotal in tackling the increasing prevalence of asthma and other allergic diseases by focusing on developing tools and strategies for better allergy management. One promising development is the utilization of recombinant purified allergens, which hold potential for enhancing therapeutic approaches in the foreseeable future.

To date, over 100 molecular allergens have been meticulously characterized across the Western world and are already integrated into clinical practices as diagnostic antigens[41,42]. In contrast, progress in the Indian subcontinent has been limited, with only a few such studies reported. Allergists in this region continue to rely on outdated and less standardized allergen extracts.

Molecular allergology employs a systematic approach involving sero-detection, identification, purification, and the comprehensive characterization of individual allergen molecules. Although substantial progress has been made globally, particularly in the West, the reports from West Bengal indicate a nascent but growing interest in advancing molecular allergology studies in this region.

I. Serological detection and purification of Fungal Allergens from natural sources:

In West Bengal, the humid climate has been considered as ideal for the growth of fungi. Hence, fungal allergy related hospitalization with typical indoor symptoms have been recurrently recorded[43]. Till now, 112 fungal genera are thought to be the source of allergens. So far, 107 allergens from 28 different genera have been approved and named by the IUIS, and some of them are from West Bengal. A detail proteomic study identified 14 novel spore-mycelial allergens from *Rhizopus oryzae* through mass spectrometry[44]. A major allergen from this species, designated as Rhi o 1, was characterized as a 44 kDa aspartic protease with an N-terminal 20 amino acid long signal peptide. Rhi o 1 was purified from this mold and characterized for its immunological properties[45]. Another fungal species, *Curvularia pallescens*, has been reported as an important respiratory sensitizer from eastern India. An extensive proteomic investigation[46] was carried out that led to the identification of certain novel allergens from this species such as Brn-1, vacuolar protease, and fructose-bisphosphate aldolase. Several allergend have been identified from different fresh water and brackish water fishes[47,48].

II. Recombinant allergens:

As already mentioned earlier, the reports on recombinant allergens from India are really very sparse as compared to European and US countries. Still, only a few important respiratory allergens have been successfully cloned to produce recombinant versions from West Bengal as well as India. From eastern India, West Bengal in particular, Rhi o 1 was reported as the most prevalent fungal allergen from *Rhizopus oryzae*. The full-length gene along with the signal peptide was cloned and expressed in a bacterial system[45]. rRhi o 1 also displayed similar immunological and biological properties as well as a similar folding pattern like native Rhi o 1. The rRhi o 1 showed a certain degree of cross reactivity with the German cockroach allergen rBla g 2. A fungal cyclophilin Rhi o 2 has also been enlisted in IUIS allergen database[53], and this recombinant cyclophilin displayed basophil activation as evident from RBL degranulation and CD203c upregulation. Another 18 kDa cyclophilin allergen, Cat r 1 was reported from periwinkle (*Catharanthus roseus*) pollen, which was highly cross reactive with other cyclophilins such as rAsp f 11 and rMala s 6[49]. The pollen cyclophilin Cat r 1 is the only allergen from India till now, whose structure has been solved by NMR spectroscopy[50]. Cat r 1 structure showed the characteristic cyclophilin fold consisting of an internal beta barrel with alpha helices on its either side. A Bet v 1 homolog was discovered from *Catharanthus roseus*. The name of the homolog is T1 protein, a naturally occurring non-allergenic member of Bet v 1 family, which, in spite of having 35–85% sequence similarity, did not show IgE-mediated cross-reactivity with other members of the Bet v 1 family. The overall fold of the T1 model exhibited resembled that of the Bet v 1 group. The absence of cross-IgE reactivity between Bet v 1 and T1 resulted in the lack of surface conservation leading to conformational changes as well as electrostatic properties[51]. The importance of compu-

tational tools for the accurate prediction of epitopes has been well established in certain important allergens reported from India. Homology modeling of a fungal alcohol dehydrogenase allergen revealed the presence of GroES like catalytic domain connected with Rossmann superfamily domain via an alpha helix. A similar approach was taken for Cur l 3, in which certain B-cell and T-cell epitopes were predicted[52]. All the predicted B-cell epitopes exhibited IgE-binding, while the T-cell epitopes displayed strong lymphoproliferative response. The fungal aspartic protease Rhi o 1 displayed a certain degree of cross reactivity with Bla g 2. It was observed that the anti-Bla g 2 mAb 4C3 resulted in partial inhibition of IgE-binding to Rhi o 2[45].

Allergens for Specific Immunotherapy and next-generation allergy vaccine:

The methodology of allergen specific immunotherapy (SIT) has witnessed a slow transition from the use of crude extract to the use of purified natural and recombinant allergens for advancing immunotherapeutic trials. Conventional (SIT) involves the repeated administration of escalating doses of crude allergen extracts over a prolonged period of two to three years or even longer. The purified allergen was preferred over the unreliable extracts in which marker allergens are less abundant. In West Bengal till now no such fungal immunotherapy was done by the researchers.

Next-generation allergy vaccines refer to allergen-derived attenuated molecules that can boost allergen blocking IgG response by directing IgG antibodies are specifically toward the IgE epitope of allergens which interfere in allergen-IgE interaction[54]. Very little study has done in that field from the West Bengal.

CONCLUSION

This study clearly highlights the exponential progress made in the field of airborne allergic fungal spores over the past fifty years in West Bengal. While it is not feasible to examine every paper published, an effort has been made to review key peer-reviewed publications from renowned international journals, as well as significant societal journals from our country. The rapid advancements in aerobiology and allergology have greatly contributed to a better understanding of the prevalence of various fungal allergens across different regions of West Bengal. Additionally, these developments have enhanced our knowledge of the immunochemical nature of different fungal allergens in the area. Based on this comprehensive analysis, the following conclusions can be drawn:

- The vertical profile analysis of atmospheric fungal spores reveals that height is a key factor influencing the abundance of certain fungal species. Additionally, the size, shape, and diameter of the spores play a significant role in their distribution at various heights.
- Common fungal spores found in both indoor and outdoor environments include *Mucor* sp., *Rhizopus* sp., *Syncephalastrum* sp., *Chaetomium* sp., *Aspergillus* spp., *Penicillium* sp., *Cunninghamella* sp., *Pleospora* sp., *Alternaria* spp., *Dreschlera* sp., *Curvularia* sp., *Cladosporium* sp., *Fusarium* sp., and *Ganoderma* sp.
- Among these, species such as *Aspergillus* spp., *Alternaria* spp., *Cladosporium* sp., *Ganoderma* sp., *Fusarium* sp., and *Curvularia* sp. are clinically significant due to their role as fungal allergens. Of these, *Aspergillus parasiticus* has been identified as a major allergen, with the highest rate of positive skin prick test results.
- Climatic factors, particularly the post-rainy season (July to October) and winter months (November to February), significantly affect the concentration of fungal spores in the air. Conversely, spore levels are lowest during the summer months (March to June).
- A few key respiratory fungal allergens have been successfully cloned for the production of recombinant allergens.
- In the field of fungal allergy research, a candidate molecule for vaccines has been developed through epitope mapping of Rhi o 1 and the creation of a hypoallergenic variant.

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Research Article

REDISCOVERY AND LECTOTYPIFICATION OF *PSIADIA CEYLANICA* VAR. *BEDDOMEI* (ASTERACEAE), A POORLY KNOWN SPECIES FROM WESTERN GHATS, TAMIL NADU, INDIA

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Psiadia ceylanica var. *beddomei* (Gamble) Chandras (Family Asteraceae) has recently been rediscovered in the southern region of the Western Ghats, after a lapse of over a century. This remarkable finding is significant, as the plant had not been observed or recorded in the area for an extended period, leading to concerns regarding its existence in the wild. In light of this rediscovery, a comprehensive and detailed description of the species has been compiled. This includes not only a thorough taxonomic evaluation but also high-quality photographs that document its morphological features in the field. Furthermore, a critical process of lectotypification has been carried out to stabilize the nomenclature of the species. The taxonomic notes provided offer an in-depth analysis of the plant's classification. This documentation serves as a valuable resource for future studies and conservation efforts, particularly in the context of the rapidly changing environment of the Western Ghats.

Key Words: Rediscovery, Lectotypification, *Psiadia ceylanica* var. *beddomei* (Gamble) Chandras, Asteraceae,Western Ghats, India

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INTRODUCTION

The genus *Psiadia* Jacq. ex. Willd. comprises 64 taxa and mainly distributed in subtropical regions, especially well represented in Madagascar and the Mascarene Islands, India and Sri Lanka[1]. In India, the genus represented by only two taxa, which are exclusively distributed in the southern Western Ghats[1,2,3]. *Psiadia ceylanica* var. *beddomei* is narrowly distributed in Tamil Nadu2,4,5,6. Extensive literature Review and consultation of herbarium specimens at MH, CALI, CAL, K and others have yielded no records found on this species since Gamble's collection in 1916. Hence, the present collection serves as a rediscovery after laps over a century.

Microglossa zeylanica var. *beddomei* (Gamble) is a basionym of *Psiadia ceylanica* var. *beddomei* (Gamble) Chandras. This variety was first collected by Beddome in 1873 from the Mahendragiri hills of Travancore (Western Ghats) and noted as *Vernonia* sp., his specimen served as the Holotype of this taxa for Gambles nomenclature (MH! 00002272). Later some specimens were also collected by Gamble in 1916 from Sengeltheri, Tirunelveli district of Tamil Nadu, mentioned in the "Flora of the Presidency of Madras"[2]. When publishing the new variety, Gamble did not cite any specimen or an illustration in the protologue. Later, Chandrashekaran transferred the genus from *Microglossa* to *Psiadia*, validated the verity *Psiadia ceylanica* var. *beddomei* in Flora of Tamil Nadu, India[4].

During recent floristic exploration of Megamalai Wildlife Sanctuary (now in Srivilliputhur–Megamalai Tiger Reserve), southern Western Ghats of Tamil Nadu, India, the authors collected interesting specimens of the genus Psiadia from the savanna woodland forest range above 1000 m MSL. The collected plant material was processed for herbarium by the standard prescribed method[7] and herbarium specimens were deposited at SGH. Morphological characters were studied and micromorphological characters analysed under the stereomicroscope for preparation of illustrations. After a critical examination of the collected specimens and the pertinent literature[2,3,7,8,9] and a comparison to the type specimens available at the relevant herbaria (CAL,

CALI, K and MH), it was found that the newly collected material *Psiadia ceylanica* var. *beddomei*, which is reported as rediscovery after laps of century.

TAXONOMIC TREATMENT

Psiadia ceylanica* var. *beddomei (Gamble) Chandras., (1987: 46) (Fig. 2.1)

Microglossa zeylanica var. *beddomei* Gamble (1923: 680)

Lectotype (designated here): INDIA. Mahendragiri hills of Travancore, 1873, Beddome (MH [barcode: bottom left specimen MH! 00002272) (Fig. 2.2).

Shrub up to 1.5 m tall, stem branched, matured branches woody, cylindrical, young stem densely pilose. Leaves linear lanceolate, 6–10 × 1.5–2 cm, base attenuate, petiole short, 2–3 mm, flattened, hairy, grooved; lamina whitish hairy both sides; lateral veins up to 6, densely hairy; margin entire or distantly dentate, apex acute, rarely acuminate. Capitula in corymbose, peduncle densely pilose, cylindrical, much branched. Phyllaries 5 × 1 mm, linear, hairy, green. Head 8–1 cm across, many, crowded on branches of spreading corymbs. Involucral bracts 2–3 × 1 mm, elliptic-oblong, glabrous inside, hairy outside, margin ciliate, apex acute. Ray florets numerous, ligules 5–6 mm long, white, entire, apex minutely lobed, achenes 2 mm long, 3 ribbed, hairy throughout; pappus white, 3 mm long, bristled, corolla tube 3 mm long, lobe 0.8 mm long, style 3.2 mm long, stigma 0.8 mm, exserted. Disc florets few, achenes 2 mm long, 3 ribbed, hairy throughout; pappus white, 3 mm long, bristled; corolla tube 3.5 mm long, slightly dialated above, lobes 1 mm long, tip reflexed; stamens 3 mm long, inserted; filament 2 mm long, white; anther 1 mm, near above, syngenesious.

Habitat: Savanna woodlands, at elevation 850 m above

Distribution and Ecology: So far, only known from Mahendragiri hills of Kanyakumari, Sengelteri hills of Tirunelveli district and Megamalai Wildlife Sanctuary, Theni district, Tamil Nadu, India. *Psiadia ceylanica* var. *beddomei* is growing along with grasses such as *Cymbopogon flexuosus* (Nees ex Steud.) Will. Watson, *Heteropogon contortus* (L.) P. Beauv. ex Roem. and

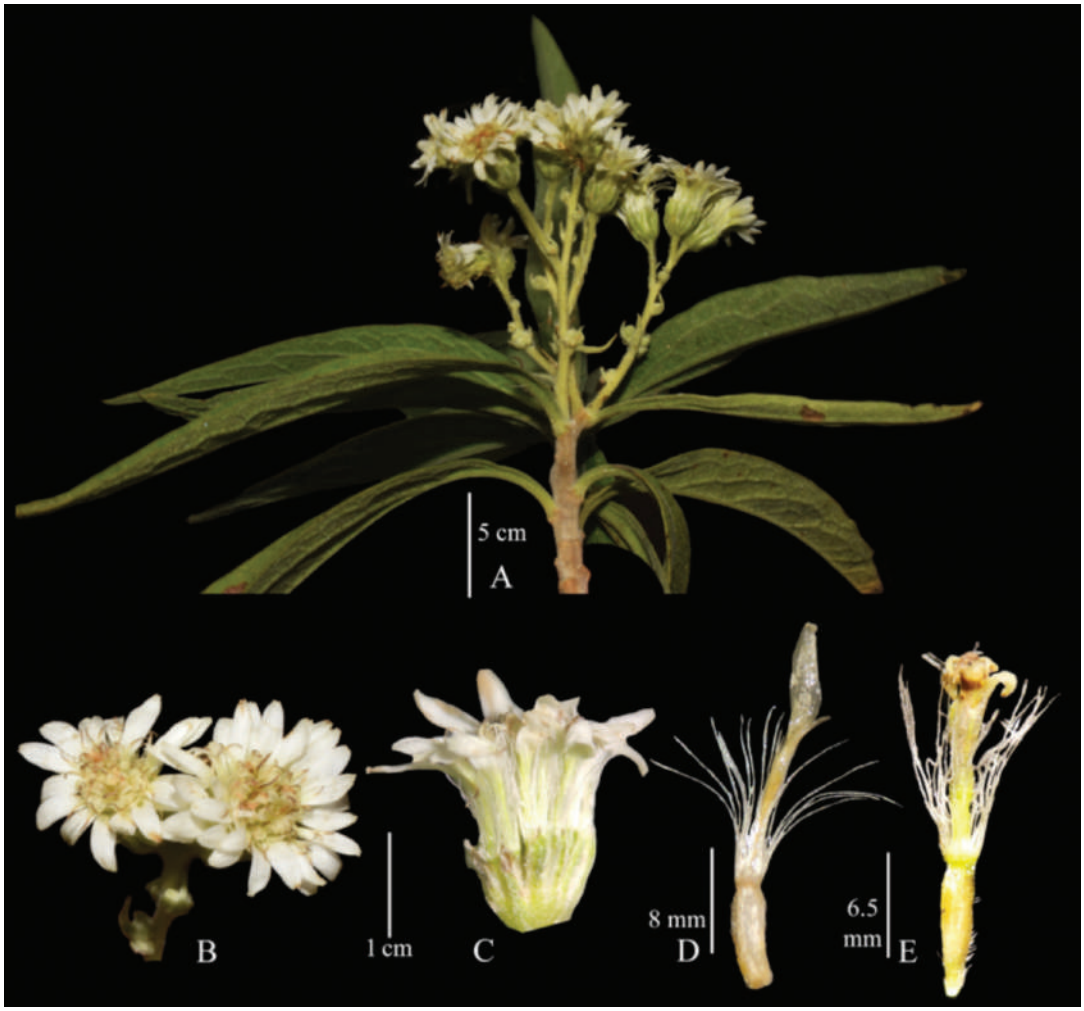

Fig. 2.1: *Psiadia ceylonica* var. *beddomei*: A. Habit; B. Heads top view; C. Head side view including involucral bracts; D. Ray floret; E. Disk floret

Fig. 2.2: Selected lectotype for *Psiadia celonica* var. *beddomei* (MH: R.A. Beddome,; MH 00002272).

Table 2.1: Comparison table between *Psiadia ceylanica* var. *beddomei* and *Psiadia ceylanica* var. *ceylanica*

Characters	Psiadia ceylanica var. beddomei	Psiadia ceylanica var. ceylanica
Petiole	2–3 mm	7–8 mm
Heads	8–10 mm across	3 mm across
Involucral bracts	Elliptic-oblong	Broadly linear
Ligule	White	Yellow or yellowish white
Pappus hair	White	Red
Achenes	3 ribbed, 2 mm	4–5 ribbed, 1 mm

Schult., *Melinis repens* (Willd.) Zizka, *Chlorophytum laxum* R. Br.; herbs such as *Justicia glauca* Rottler, *Bidens pilosa* L., *Biophytum sensitivum* (L.) DC., *Justicia diffusa* var. *prostrata* (Roxb. ex C.B. Clarke) J.L. Ellis, *Eschenbachia japonica* (Thunb.) J. Kost., *Polygala javana* DC., *Rhynchosia cana* (Willd.) DC., *Stylosanthes hamata* (L.) Taub.; stragglers *Elaeagnus indica* Servett., *Ziziphus rugosa* Lam.; shrubs *Indigofera cassioides* Rottler ex DC., *Tarenna asiatica* (L.) Kuntze and trees of *Bridelia retusa* (L.) A. Juss., *Phyllanthus emblica* L., *Pittosporum napaulense* (DC.) Rehder & E.H. Wilson and *Pterocarpus marsupium* Roxb.

Phenology: Flowering and fruiting were observed from July–October.

Additional specimen examined: India. Tamil Nadu, Megamalai Wildlife Sanctuary, on the way to Megamalai (30 July 2023, *Karuppusamy & P. Bharath Simha Yadav* 3580 SGH!)

Note: *Psiadia ceylanica* var. *beddomei* is similar to the *Psiadia ceylanica* Arn., but differed by small petiole 2–3 mm (vs. 6–8 mm), heads 8–10 mm across (3 mm), Involucral bracts elliptic-oblong (vs. broadly linear), ligule white (vs. yellow or yellowish white), pappus hair white (vs. red), achenes 3 ribbed, 2 mm (4–5 ribbed, 1 mm) (Table. 2.1).

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Research Article

DEVELOPMENT OF ENZYME LINKED IMMUNOSORBANT ASSAY FOR THE DETECTION OF VIP3A PROTEIN IN TRANSGENIC *PIGEON PEA*

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Cultivation of genetically modified crops with the help of different Bt genes is ever-increasing at an enormous rate globally. The Bt technology liberates possible contaminants that would adversely influence in environment, henceforth a high-quality proficient procedure intended for detecting the activity of "Bt" toxin is a precondition. Emphasis has been taken on present research for Enzyme-linked immunosorbent assay (ELISA) standardization to accomplish an efficient technique for the qualitative and quantitative determination of specific protein mixtures in transgenic plant tissue. The protein sample resembling antigen immobilized on a microtiter plate is a present analysis using specific antibodies. The alleged technique is based on immunology, simplified and universally used in therapeutic research as well as the agriculture sector.

The main objective of this research was to develop sandwich ELISA for the finding of Vip3a protein in transgenic (Bt) pigeon pea plant leaf tissue. Purified Vip3a protein derrived from a clone of M15 *E. coli* harboring expression construct of Vip3a gene and inoculated in New Zealand female rabbits and were allowed to produce antibodies unambiguously. Secondary antibodies were conjugated in horse redox peroxidase (HRP) substrate. The limit of detection was determined in favor of Vip3a protein be 0.5 ng/ml. Transgenic pigeon pea plant leaf proteins were extracted and were used as antigens for the evaluation of the ELISA assay. PBST buffer was used for the protein extraction from plant tissue and the limit of absorbance for detection was standardized 0.100 to 1.500 at 450 nm wavelength. The optimistic consequences transmitted on PVip3a six lines showed resistant transgenic against pod borer and the four Non-Bt sample demonstrates negative for Vip3a antigen. The PCR technique was used to validate the developed assay and originate comparable results approximate ELISA. The developed immunoassay is considered reliable for the recognition of Bt Vip3a protein from the protein mixture of pigeon pea samples.

Key Words: Antibody, Antigen, ELISA, Vip3a protein, Bt – *Bacillus thuringensis*, PVip3a – Pigeon pea transgenic event.

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INTRODUCTION

Genetically modified plant cultivation is progressively increasing worldwide leading to the development of agricultural biotechnology. The most important gene in this development is derived from *Bacillus thuringiensis*. It is a gram-positive soil bacterium containing a gene that encodes for insecticidal proteins effective against insect pests. Over the last two decades, more than 100 variable polypeptides identified and applied in insect management programs[1].

At present, transgenic plants e.g. pigeon pea, tobacco, maize, potato, cotton, tomato, and rice expressing insecticidal Bt proteins have been observed to control certain chewing insect pests[2]. Thus; targeted protein is expressed in plant tissues and may be present in food ingredients to know the presence in food, the determination of GMO needs analytical techniques to determine introduced protein in crops enhanced through modern biotechnology, achieving the goal of an immunoassay methodology for agricultural biotechnology were applied[3].

Presently GMO detection method[4] practiced in laboratories for these Bt crops is through amplification of DNA-specific PCR followed by agarose gel electrophoresis, southern blot hybridization, western blot hybridization, etc. Immunoassay is being used extensively for the identification of genetically modified crops. Keeping a high rate with the development of newer transgene crops, detection tests are also formatted for growing GM crops and products[5]. However, PCR-based methods require a well-resourced laboratory with a support system consuming a lot of time to achieve the results. Immunoassay provides an alternative means for

the detection of GMOs based on the determination of the protein product for the foreign gene. Polyclonal antibodies raised either in rabbits or goats have been used[6].

Immunoassay instead of detecting DNA in a test sample measures the levels of proteins expressed by DNA sequences inserted by genetic modification[7]. The tests, using an enzyme-mediated immunoassay, have been widely used to detect GM protein in transgenic plants. Because, of its sensitivity, ruggedness, and inexpensive characteristics, it is a choice of breeders for testing of unapproved events, and determining GM content ensuring compliance with non-GM labeling requirements[8]. The current study was carried out to standardize sandwich ELISA for the determination of Vip3a protein in transgenic pigeon pea tissue. The developed ELISA assay for Vip3a was applied to detect transgenic and non-transgenic pigeon pea genotype expressing Vip3a protein, the reliability of the standardized method was validated by PCR method[3].

MATERIALS AND METHODS

Extraction of Vip3a standard Protein from *E. coli*

Purified Vip3a insecticidal protein was isolated from *E. coli* M15-strain and expression vector PQE30 containing the Vip3a cloned gene was used. A culture medium containing terrific broth (12% tryptone, 2.4% yeast extract, 0.04% glycerol, 0.17 M KH_2PO_4, 0.72 M K_2HPO_4) was used for isolation of desired strain. The medium was incubated overnight at 37°C containing antibiotic kanamycin (50 mg/lit) and ampicillin (100 mg/lit). A secondary culture using 1% inoculums was set and incubated at 37°C for 2 hr till reaching optical density 0.6 nm. It was followed by adding IPTG to the grown culture for induction and incubated at 37°C for 4 to 5 hours. It was afterward centrifuged at 8000 rpm for 20 min at 4°C and was re-suspended in a native buffer. Then it was added with a protease inhibitor cocktail for the sonication by giving a shock of 3 seconds.

The culture was then transferred through a Ni-NTA resin pre packed chromatography column and again the culture was centrifuged for 10 min at 10000 rpm. The supernatant was transferred to the followed by washing the protein with native buffer and 30 mM imidazole solution, it was followed by elute insecticidal Vip3a protein in 0.25 M NaCl. Protein fraction was checked with SDS-PAGE and dialysis using a tube by keeping the tube in dialysis buffer overnight was done to collect the protein in a fresh falcon tube. The protein was collected and transferred for concentration by lyo-

Table 3.1: Serial dilution of the Vip3a protein standard concentration (ng/ml) at 450 nm for titration study

Standard code	Standard used for dilution	Standard volume (µL)	Diluent volume (µL)	Final concentration ng/mL
1 ng/mL	Dilute the Original stock to 100 ng/mL			100
	From 100 ng/mL	20	180	10
S7	From 10 ng/mL	100	100	5
S6	S7	100	100	2.5
S5	S6	100	100	1.25
S4	S5	100	100	0.625
S3	S4	100	100	0.312
S2	S3	100	100	0.156
S1	S2	100	100	0.078
Blank	—	—	100	0

[**N.B.**: Performs the ELSIA test with this type of experiment, keep uniform time, temperature and known concentration of protein. Generate the data on reader or cytometer to identify the positive and negative population. Analyze the results for finalizing titration value to be used for setting up qualitative and quantitative ELISA assay development and used for ELISA development[9]].

philization. Lyophilized protein was used for polyclonal Vip3a antiserum production. The concentration was determined based on Beer-Lambert law using Nanodrop (Nanodrop Tech., USA) the Vip3a protein was lyophilized in aliquots and stored at –20°C for further analysis)[4].

Antibodies Production

Polyclonal antibody production using the protein immunization in female New Zealand white rabbits with a dose of 1.5 mg lyophilized Vip3a protein per rabbit immunized intradermally and subcutaneously was done at Avesthagen Ltd. Bangalore. First immunization using Immunogen containing 0.250 mg of Vip3a protein with complete adjuvant were used and for the second, third, and fourth immunization dose containing 0.125 mg protein with incomplete adjuvant were used or 30 days. A post-immunized blood sample (3 ml) was collected within one week of each booster injection to evaluate serum titer and was purified using protein A-Sepharose 4B column by affinity chromatography. Titration of the antiserum against Vip3a protein was carried out by reducing loss of sensitivity, ELISA reader or flow cytometer was used for antibody titration experiment using the antigen of interest and the transgenic plant leaf protein. The experiment was performed in serial dilutions of the standard protein antibody concentration, for ex. 100 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml, 0.156 ng/ml. 0.078 ng/ml and 0.00 ng/ml as a blank (Table 3.1) etc.

Conjugation of the Antibodies

The conjugation of antibodies was performed by the periodate method. Horseradish peroxidase (Sigma) 4 mg was dissolved in 0.5 ml of Milli Q water in amber color bottle and sodium periodate (Merck) was mixed into the HRP solution followed by incubation at room temperature on a magnetic stirrer for 20 min. HRP solution was dialyzed against acetate buffer (0.1 mM, pH 4.4) at 4°C for 24 hours, after incubation 10 µl of carbonate-bicarbonate buffer (0.2 M, pH 9.5) was added. To carbonate-bicarbonate buffer (10 mM, pH 9.5) mix 8 mg of purified Vip3a specific IgG in 1 ml of active enzyme mix antibody solution on the stirrer, afterword 150 µl of fresh sodium borohydrate solution (Merck) added to the above solution and incubated at 4°C for 90 minutes on the stirrer. The product was then dialyzed using PBS for 24 hours at 4°C using 1% BSA (Sigma) and 0.01% sodium mithiolate (Merck), at last BSA was added into the conjugated solution at 5 mg/ml, and conjugated solution was store in fractions at –20°C for further use[10].

Extraction of Bt protein from pigeon pea leaves

Leaf tissues of ten pigeon pea lines (PPVip-1 to PPVip-10), were crushed in a hand-held tissue lyser thoroughly with the extraction buffers PBST (Phosphate buffer saline tween 20 (0.05%). The mixtures were centrifuged at 1000 rpm for 5 minutes to separate cellular debris and antigens of interest present in the supernatant. The supernatant was used for the determination of Bt Vip3a protein through ELISA[11].

Sandwich ELISA Assay

Vip3a sandwich ELISA was developed for governing Bt protein. A 96-well maxisorb microtiter plate (Nunc, Denmark) was used for coating 100 ul per well of the purified polyclonal capture antibody specific to Vip3a IgG (10 ug/ml) diluted in 0.1 M carbonate buffer (pH 9.5 ± 0.2). The plate was incubated overnight at room temperature on a bench top. Wash the plates three times with PBST (Phosphate Buffer Saline Tween 20 (0.05% v/v)), blot dry the plate, and unbound sites were blocked with 250 μl of blocking buffer (2% BSA (sigma) in 1x PBS buffer for 4 hours at room temperature. Then the plates were washed thrice with PBST buffer and air dry for 10-15 minutes. For the sandwich assay, 100 μl samples as antigen were added into each well along with the negative control, standard protein of Vip3a (100ng/ml), The blank well was kept by adding sample diluents, and the plate was covered with a lid for incubation at room temperature for 60 minutes on a bench top. The content was decanted in the sink and the plate was washed four times with washing PBST buffer and Blot dry on a clean tissue paper towel. 100μL of Rabbit anti-Vip3A-IgG-HRP conjugate (secondary antibody diluted 1:10000 times in 1× PBST diluents buffer) was pipetted out and dispensed into each well in the plate[12].

The plates were incubated for 60 minutes at room temperature on an orbital shaker. The plates were washed five times with wash buffer, followed by drying the ELISA plate using patting on tissue paper, followed by dispensing 100 μL of freshly prepared TMB/H_2O_2 (Bangalore Genie) substrate solution into each well and incubating at dark for 10 minutes. The reactions were stopped by adding 100 ul of 0.5N sulfuric acid[7]. The ab-

sorbance was recorded and developed throughout the micro plate using a reader at 450nm wavelength keeping 620 nm as the reference filter (Biorad,California). Three samples for each concentration were analyzed, and PBST or extraction buffer was used as a blank control and analyze results using microplate reader software.

Quantitative ELISA of Vip3a plants

Quantitative measurement of the amount of Vip3a protein was obtained through developed ELISA methodology. Immunoaffinity-purified polyclonal goat and rabbit anti-Vip3A antibodies were used to determine the amount of Vip3a protein per milligram of soluble protein from putative leaf extracts. The sensitivity of the double sandwich ELISA is 1.00-5.00 ng of Vip3a per mg of soluble protein when 50 mg of total protein per ELISA microtiter dish well was used as per the known Vip3a protein standard concentration undertaken for assay development (Table 3.2). The Vip3a protein content in transgenic plants was further quantified through ELISA in leaf tissue at different time of intervals, measurable amount of Vip3a protein observed in the range of 0.15-1.86 ng/gm fresh weight of tissue. Expression levels of Bt-toxins in transgenic plants are shown as ng/gm FW of leaf and were quantified with pure Bt-Vip3A protein (Fig. 3.2).

Extraction of DNA

Young leaf from 10 different pigeon pea cultivars line (PPVip-1 to PPVip-10) was crushed in a mortar and pestle with liquid nitrogen. CTAB (cetytril methyl ammonium bromide) based Doyle, J. modified protocol was used for the genomic DNA isolation from pigeon pea for the PCR reaction[13]. Eluted DNA was quantified using gel electrophoresis (1.0%) and spectrophotometric analysis by Nanodrop, and stored DNA until use at –20°C[8].

Polymerase Chain reaction

The complete PCR assay was programmed on an epi-gradient master cycler (Eppendorf, Germany) and reagents for standard single PCR assays were procured from Bangalore Genei, India. The PCR comprised 2.5 µl of 1× PCR buffer containing 10 mM Tris hydroxy methyl aminomethane (Tris) HCl pH 9.0, 50 mM KCl, and 1.5 mM $MgCl_2$. A typical optimal assay consists of 0.10 µM each primer, 100 µM dNTP, 50 ng/μl template DNA, and 1.0 U Taq DNA polymerase. The standard PCR cycle conditions were initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation step at 94°C for 45 sec, annealing at 54°C for 45 sec and extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min[11]. The primer pairs that were used for amplification by PCR are the gene-specific primers (Forward - 5'GAAGGATGGCTCTCCTGCAGAC3' and Reverse - 5'GAGTTTCTAATCCTAACGTTAGCATC3'). The products were analyzed with electrophoresis on agarose gel (1.5%, w/v) and made visible by alpha digidoc (Alpha Innotech Corp., USA) gel documentation system.

RESULTS AND DISCUSSION

Extraction of Insecticidal Vip3a Protein for immunization to rabbit from Bt (M15-strain) strain, during its

Table 3.2: Vip3a protein standard concentration (ng/ml) at 450 nm

Sl. No.	Standard name	Optical density at 450 nm				Std. Conc. (ng/ml)
		R1	R2	R3	Average	
1.	Standard 1	2.399	2.439	2.383	2.407	10
2.	Standard 2	2.108	1.561	1.986	1.885	5
3.	Standard 3	1.285	1.285	1.269	1.28	2.5
4.	Standard 4	0.774	0.782	0.772	0.776	1.25
5.	Standard 5	0.472	0.446	0.46	0.459	0.625
6.	Standard 6	0.286	0.254	0.272	0.271	0.312
7.	NC	0.2	0.168	0.164	0.177	0.156

sporulation at the stationary phase from Bacillus thuringiensis cell division was done. The acquirable condition for optimal growth such as media composition, pH, incubation temperature, and stirring and oxygen requirements was standardized utilizing differing medium composition and shaking conditions. The Vip3a protein purification results on SDS-PAGE gel show that the purity of protein obtained by SDS 12% gel is better than that of 10%. A strong dense Vip3a protein band noticeable (Fig. 3.1) representing a purity of Vip3a greater than 98% and this was considered adequate to be used directly as an immunogenic for producing antibodies. Synthesizing polyclonal antibodies and their important role in agriculture, pharmaceutical, and medical research evidences many advances in this field of immunological detection. Anti-Vip3a antibodies and their conjugate with horse radish peroxidase (HRP) were used to analyze insecticidal protein which is present in transgenic pigeon pea developed line by ELISA test[10].

The current study of the production, purification, and horse radish peroxidase (HRP) conjugation of polyclonal antibodies against the Vip3a gene in rabbits gives a greater response. According to the standard antibody production, immunization time should be extended to achieve higher-titer antibody-found proteins in gel (Fig. 3.1). The concentrated antibodies with higher titer and sensitivity, and antibodies from animals immunized by proteins in solutions were chosen for ELISA. Purified labeled antibody to HRP binds specifically and develops a reaction with a substrate for the development of a blue color. The purity of the IgG antibody was about 98%, and the optimum dilution of prepared HRP

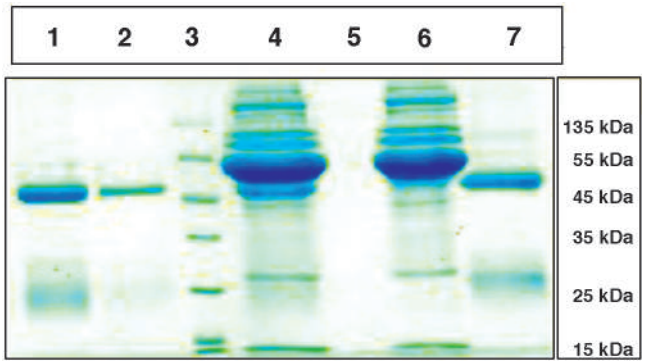

Fig. 3.1: Vip3a antibody purity profile on 12% SDS-PAGE Gel: **Lane 1**: Protein A purified Vip3a antibody; **Lane 2**: Protein A purified Vip3a antibody (post bound elute); **Lane 3**: Protein marker; **Lane 4**: Unpurified Vip3a rabbit serum, **Lane 5**: blank; **Lane 6**: Vip3a rabbit after purification on protein A matrix (post bound) and **Lane 7**: Internal control rabbit IgG purified on protein A matrix from rabbit serum.

conjugated IgG was 1:10000. This conjugated IgG has no cross-reactivity with another insecticidal antigen[14].

Sandwich Vip3a ELISA analysis shows that ELISA development using a concentration of the capturing antibody and the secondary antibody was measured for the polyclonal antibody-based attempt. The standardization results of this consideration are presented in (Fig. 3.2) explaining the limit of detection for Bt Vip3a protein to be 0.5 ng/ml, which was calculated as an average of the blank control values ± 3 standard deviations of the blank control values and there were no cross-reactions between the positive control and other toxins.

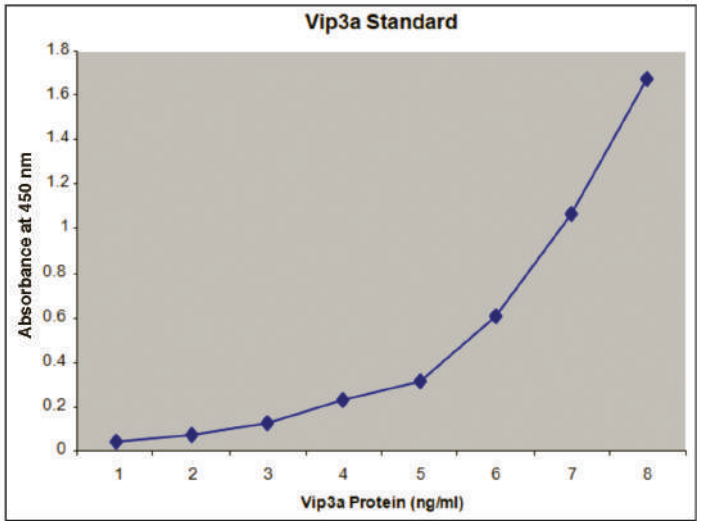

Fig. 3.2: Determination of different concentrations of the Vip3a standard protein using the developed ELISA.

Extraction of Bt proteins from transgenic pigeon pea leaf section for ELISA was optimized to determine the suitability of the developed sandwich assay. The result was predicted after altering the different concentrations of tween-20 in exaction buffer like 0.05 to 5% and found that the absorbance limit of extraction buffers (Phosphate buffer saline and Tween 20) was 0.120 to 0.150. From the varied tween 20 concentrations, it is shown that increasing concentration effect on decreasing OD of protein hence prefers 0.05%. The transgenic sample showed above optical density than blank OD then the sample was considered positive for Vip3a therefore, using assay conditions mentioned in the methods the leaf sample of pigeon pea screened and found PPVip 2, 3, 4, 7, 8 & 10 are positive and 1, 5, 6 & 9 was the negative sample (Table 3.3).

In ELISA assay procedure for mVip3A pure Vip3a protein isolated from *E.coli* having molecular weight 60-65 KD and the HRP conjugated IgG was used in 1:10000

Table 3.3: Qualitative ELISA analysis of Vip3a protein in leaf tissue of Pigeon pea

Sl. No.	Sample Code	Optical density	Results
1.	Blank	0.075	Negative
2.	Negative control	0.125	Negative
3.	Positive control	1.985	Positive
4.	PP-Vip 1	0.175	Negative
5.	PP-Vip 2	1.258	Positive
6.	PP-Vip 3	1.335	Positive
7.	PP-Vip 4	1.425	Positive
8.	PP-Vip 5	0.201	Negative
9.	PP-Vip 6	0.290	Negative
10.	PP-Vip 7	1.358	Positive
11.	PP-Vip 8	1.024	Positive
12.	PP-Vip 9	0.295	Negative
13.	PP-Vip 10	1.586	Positive

dilutions. Absorption of transgenic event containing mVip3A gene in leaf tissue showed 0.40-1.7 at 450 nm absorption, however, on transgenic plant showing 0.089-0.129 optical densities. The absorbance limit of extraction buffers (PBST) was 0.120-0.195, respectively, if the absorbance of the samples was higher than that of the blank, the samples were positive. Wu *et al*.[15] reported Vip3a ELISA quantification in cotton to quantify Vip3a expression in the four homo-zygous transgenic lines. The amount of Vip3a protein was calculated after subtracting the OD value of NT leaf extract from the transgenic lines. The transgenic cotton lines with Vip3a gene demonstrated about a three-fold higher level of Vip3a expression 6.12 and 5.95 ng/gm of total soluble protein. Sandwich developed Vip3a ELISA verification comparing DNA-based PCR test in the present study and shows that detection of transgenic pigeon pea obtained with ELISA compared with Polymerase Chain Reaction. The ELISA tests showed that PVip 2, 3, 4, 7, 8 & 10 was the positive samples and the 1, 5, 6 & 9 samples were the negative. The same conclusions were obtained from tests using the PCR method as demonstrated (Fig 3.3). PCR positive sample shown bands were all detected in the 10 pigeon pea samples and five were negative. These results indicated that the developed immunoassay method was reliable for the detection of Bt Vip3a protein in pigeon pea.

CONCLUSION

Immunological sandwich ELISA assay identifies GMO plants expressing protein from *the Bacillus thuringiensis* gene incorporated in transgenic pigeon peas, which is crucial for the accurate determination of transgenic events in agriculture. New Zealand female white rabbits were used to inoculate Vip3a purified protein from *E. coli* M15 to produce a specific antibody and secondary antibody conjugated with horse redox peroxidase enzyme which is particularly Vip3a specific IgG.

The limit of detection of Vip3a protein in transgenic plants is 0.5 mg/ml and the Vip3a positive standard has not shown any cross-reaction with other Bt toxin. Evaluation of the pigeon pea leaves by extracting protein as antigen through developed assay. PBST Buffer was considered for the extraction of 10 different pigeon pea leaves. It is observed that the results of PPVip3a among 10 lines show six positive and four were negative.

This method is confirmed by DNA-based polymerase chain reaction mainly focusing specific primer pair against Vip3a were selected to determine the presence

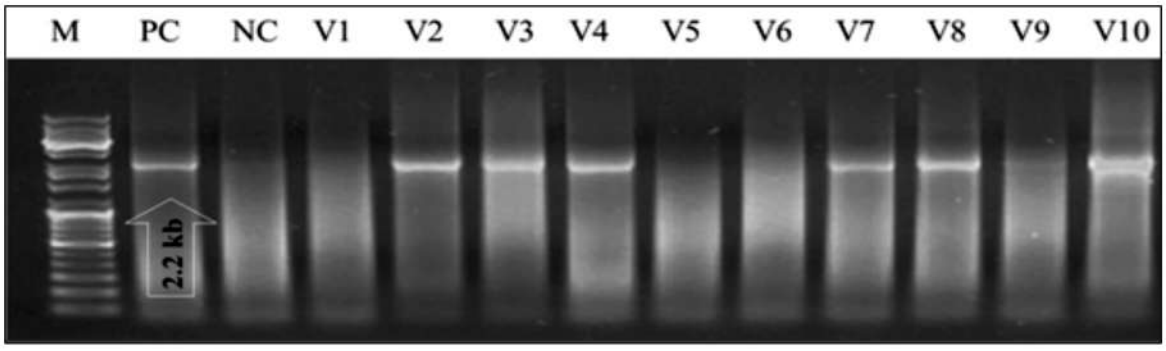

Fig. 3.3: Molecular transgene analysis of the Vip3a gene using PCR method. **Lane 1**: M 3Kb Marker; **Lane 2**: Plastid DNA (PC); **Lane 3**: Negative control (NC); **Lane 4-10** (V1-V10): Putative plant carrying Vip3a gene

of gene for the accurate determination of samples and results similar to that of ELISA assay. Considering similar results obtained through ELISA and PCR method to sample determination ELISA technique require basic equipment and a lesser amount of time and is cost effective henceforth for pigeon pea GMO detection developed immunoassay is appropriate.

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Research Article

ANALYSIS OF ALLERGENIC POLLEN AND FUNGAL SPORES IN SPIDER WEB SAMPLES FROM HISTORICAL MONUMENTS OF HYDERABAD, TELANGANA, INDIA

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This study investigates the presence and distribution of allergenic pollen grains and fungal spores trapped in spiderweb samples from five historical monuments in Hyderabad, India: Arts College, Charminar, Golconda Fort, Qutub Shahi Tombs, and the University College of Science. Spiderwebs, acting as natural traps, provide valuable insights into local airborne pollen and spore types. Findings reveals the prevalence of allergenic pollen from herbaceous and arboreal sources, including Poaceae, *Ageratum conyzoides, Holoptelea integrifolia*, and *Azadirachta indica,* and fungal spores like *Alternaria, Nigrospora,* and *Curvularia lunata*. The study identifies pollen from trees as predominant in Charminar, Golconda, and University College of Science, while herbaceous pollen is more concentrated in Arts College. This research offers a significant understanding of allergen distribution around monuments, presenting public health implications due to the potential for pollen-induced respiratory and skin disorders among local visitors and tourists.

Key Words: Allergenic pollen, fungal spores, spiderweb sampling, respiratory disorders, Hyderabad monuments, public health.

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INTRODUCTION

Aeropalynology is an applied branch of Science that deals with the of spore, pollen and fungal spores present in the atmosphere, which transports to different places through air. Gregory[1] proposed the term " Air-spora" to describe airborne pollen grains and fungal spores, the composition of which varies from place to place. Spider webs are known to be potential natural traps[2] for air borne spores and pollen grains, fungal spores, small leaves, flower and insect parts of a particular geographic area[3,4]. Pollen grains found in the atmosphere are widely known to cause allergenic diseases like hay-fever, asthma and eczema[5,6]. The allergic diseases are severe problem for the community as well as for the national economy[7,8]. ICPA (India Coordinated Project on Aero-allergens) carried out a survey in India reported that about 20-30% of population suffer from allergic rhinitis and 15% of asthma[9]. Pollen grains and fungal spores, which are released into the atmosphere, are major source of airborne allergens and allerginic diseases[10,11]. Due to the prevalence of allergens in ambient air, there has been significant interest in recent decades in developing innovative methods to monitor bio-particles in urban environments. Given the impact of pollen as predominant allergens, particularly on respiratory health, continuous monitoring of these particles in densely populated regions like Hyderabad is essential for understanding their implications for public health[10].

The study site, Hyderabad is the capital of Telangana and the sixth-largest metropolitan city in India. Hyderabad is located in central Telangana on Deccan Pateau at 17°36' N longitude and 78°47' E latitude on the banks of river Moosi with an elevation of about 536 m above the sea level. Hyderabad has a unique combination of a tropical wet and dry climate with annual rain fall ranges from 500-800 mm and temperature is around 21° to 45°C.

Hyderabad city hosts several notable monuments like Golconda Fort, Charminar, and Qutub Shahi Tombs, which are not only culturally significant but also ideal locations for pollen and spore analysis due to their open exposure to ambient environmental conditions. This

urban area is densely populated and industrialized, making it a prime location for studying allergenic bioparticles and their distribution across historical and public sites. Hence the present study is to monitor the air quality regarding the biopollutants and to know the qualitative and quantitative dispersal of pollen and fungal spores in the atmosphere with the help of spider webs. The present study also reflects the distinct characteristics of the regional flora growing around the study sites in particular the allergenic flora.

MATERIALS AND METHODS

The spider webs from the five historical monuments of Hyderabad viz. Charminar, Golconda Fort, Qutub Shahi Tombs, Arts College, and Science College of Osmania University were collected by rolling the end of the stick. The sampling was carried out from January 2023 to December 2023 to ensure the maximum representation of plant taxa in the pollen rain of the area under study and to understand the annual pollen influx.

For the extraction of pollen and spores, the spider-web samples were treated with con. Hydrochloric acid (HCl) to dissolve the meshes instantly. The superfluous materials like small twigs, leaves, fruits and insect body parts were removed with the help of 150 sized sieve mesh, then samples were washed several times with distilled water to remove the acid content by centrifuging and decanting. The filtered material was treated with 10 ml of conc. Hydroflouric acid (HF) in polythene tubes and kept for two days to dissolve silica, then the samples were washed with distilled water by centrifuging to remove the silica content.

The residue was treated with acetolysis mixture[12,13] i.e. acetic anhydride and conc.sulphuric acid (H_2SO_4) in the ratio of 9:1. Then the solution was placed in a boiling water bath at 100°C for 15-20 mins. After centrifugation and decanting with distilled water, the samples were prepared in 50% glycerine solution for observation under the microscope. Identification of different pollen and fungal spores was carried out through consultation of published literates[1,14,15]. The reference slides were stored in the Palynology and Plant Systematics Laboratory, Deaprtment of Botany, Osmania University, Hyderabad (Plate: 4.1).

Depending upon the productivity of the samples, number of spores and pollen were counted for each sample. The encountered spore and pollen have been shown the data of trees, herbs and shrubs and fungal spores.

RESULTS AND DISCUSSIONS

In the present study 69 pollen types were recorded and they represent herbs, shrubs, trees and climbers, 6 types of identified fungal spores and unidentified spores.

Spiderweb Sample 1 (Arts college):

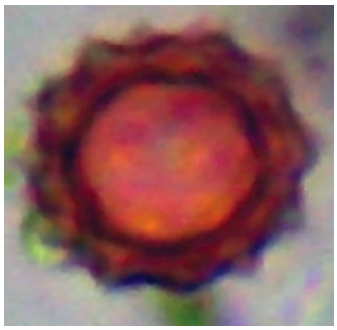
This sample shows pollen of Poaceae (14%) and *Holoptelea integrifolia* (10%) are dominanting taxa which are recovered in good frequencies. *Ageratum conyzoides* (8%), *Azadirachta indica* (8%), *Leucaena leucocephala* (7%), *Peltophorum pterocarpum* (7%), *Capparis grandis* (7%), *Cyperus rotundus* (7%), *Terminalia catappa* (5%), *Tinospora cordifolia* (5%), *Brassica juncea* (3%), Asteraceae type (3%) are reported moderately. Whereas *Eucalyptus globulus* (2%), *Cyanthillium cinnereum* (2%), *Chlorophytum tuberosum* (2%), *Neltuma juliflora* (2%), *Melilotus albus* (2%), *Portulaca oleraceae* (2%), and *Woodfordia fruticosa* (2%) are recovered in low values with some unidentified pollen (Fig. 4.1).

The fungal spores such as *Alternaria* sp. (45%), *Ascospores* (18%), *Curvularia lunata* (8%), Rust spore (7%), *Nigrospora* (5%) and other unidentified fungal spores (17%) were recorded (Fig. 4.1).

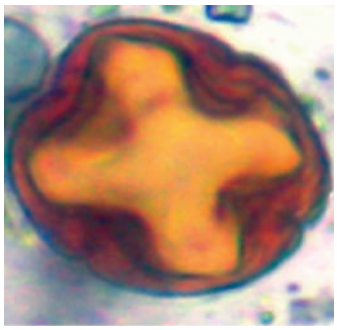
Spiderweb sample 2 (Charminar)

This sample also shows the pollen of Poaceae (14%) and *Ageratum conyzoides* (10%) are dominant. *Azadirachta indica* (8%), *Leucaena leucocephala* (8%), *Cyperus rotundus* (8%), *Peltophorum pterocarpum* (7%), *Brassica juncea* (7%), *Citrus aurantifolia* (5%), *Combretum albidum* (3%), *Punica granatum* (3%), *Terminalia arjuna* (3%), *Trifolium* sp. (3%) are encountered moderately. *Vahlia digyna* (2%), *Bombax ceiba* (2%), *Dillenia pentagyna* (2%), *Butea monosperma* (2%), *Borassus flabellifer* (2%), *Neltuma juliflora* (2%), *Mitragyna parviflora* (2%), *Melilotus albus* (2%), *Woodfordia fruticosa* (2%) are represented in extremely low (Fig. 4.2).

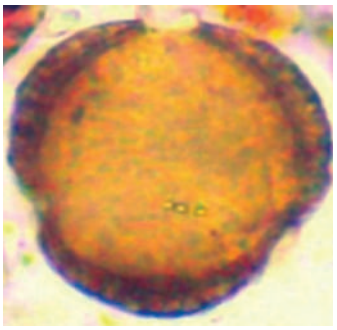
Fungal spores such as *Alternaria* sp. (64%), *Nigrospora* sp. (25%) and unknown fungal spores (11%) were recorded (Fig. 4.2).



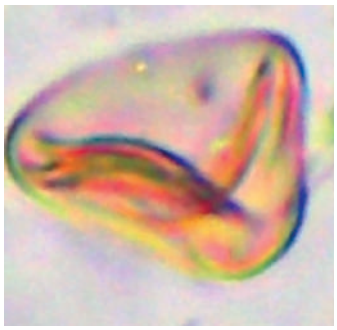
Ageratum conyzoides



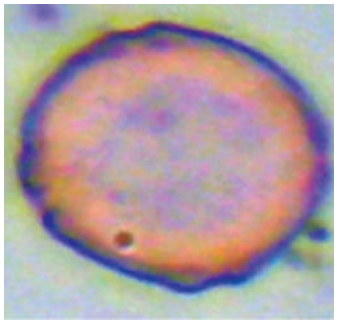
Azadirachta indica



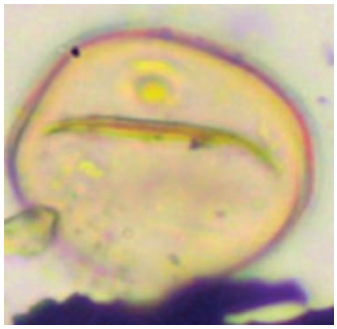
Brassica juncea



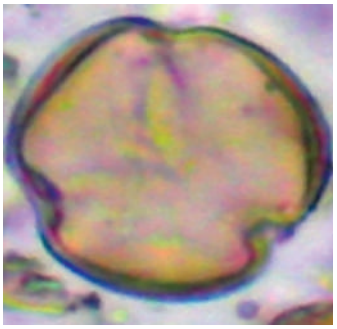
Cyperus rotundus



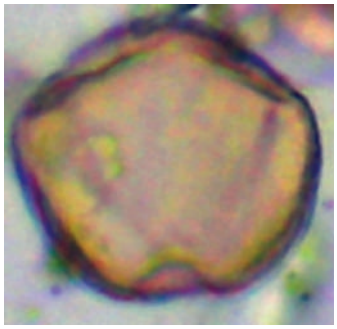
Holoptelea integrifolia



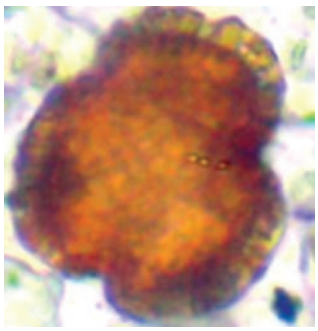
Poaceae



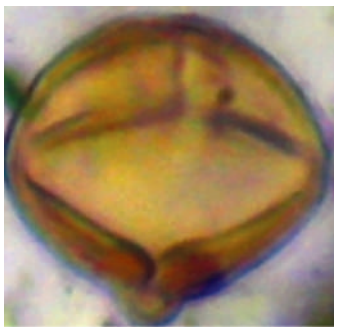
Prosopis julifera



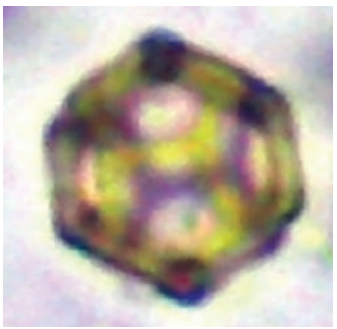
Leucaena leucocephala



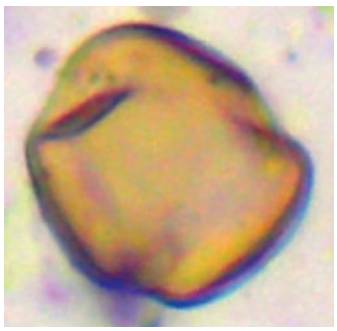
Delonix regia



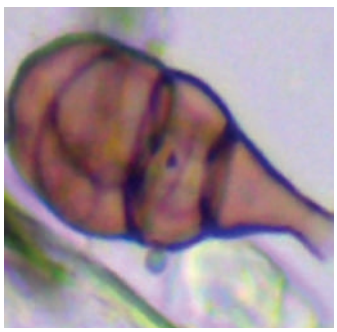
Vahlia digyna



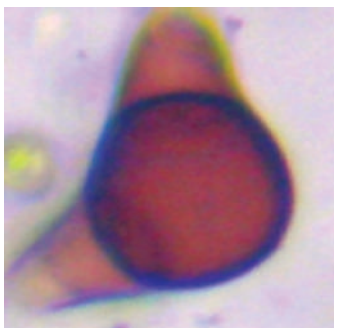
Alternanthera sessilis



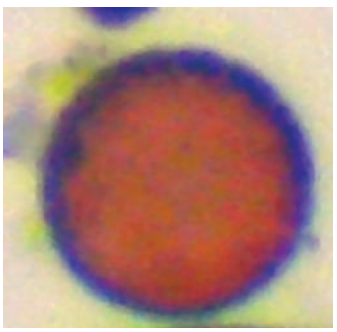
Senna occidentalis



Alternaria sp.



Curvularia lunata



Nigrospora sp.

Drechslera sp.

Plate 4.1: Showing important Pollen photomicrographs recorded from Spider webs

Spiderweb sample – 3 (Golconda)

This sample reveals the pollen of Ageratum conyzoides (14%) and Poaceae (11%) are dominant as non arboreal species. *Leucaena leucocephala, Peltophorum pterocarpum* (7%), *Alternanthera sessilis* (5%), *Azadiracta indica* (5%), *Brassica* sp. (5%), *Celosia argentea* (4%), *Punica granatum* (4%), *Ricinus communis* (4%), *Sapindus trifoliatus* (4%), *Citrus aurantifolia* (3%), *Erythrina indica* (3%) are recorded in moderate values. *Kalanchoe pinnata* (1%), *Luffa acutangula* (1%), Oleaceae (1%), *Monoon longifolium* (1%), *Neltuma juliflora* (1%), *Senna auriculata* (1%), *Senna occidentalis* (1%), *Senna siamea* (1%), *Tamarix dioica* (1%), *Terminalia bellirica* (1%), *Vahlia digyna* (1%), *Woodfordia fruticosa* (1%) are present in low values in the pollen spectra.

The Fungal spores such as *Alternaria* sp. (38%), Nigrospore (15%), Ascospore (6%) and some Unidentified fungal spores (41%) were recorded (Fig. 4.3).

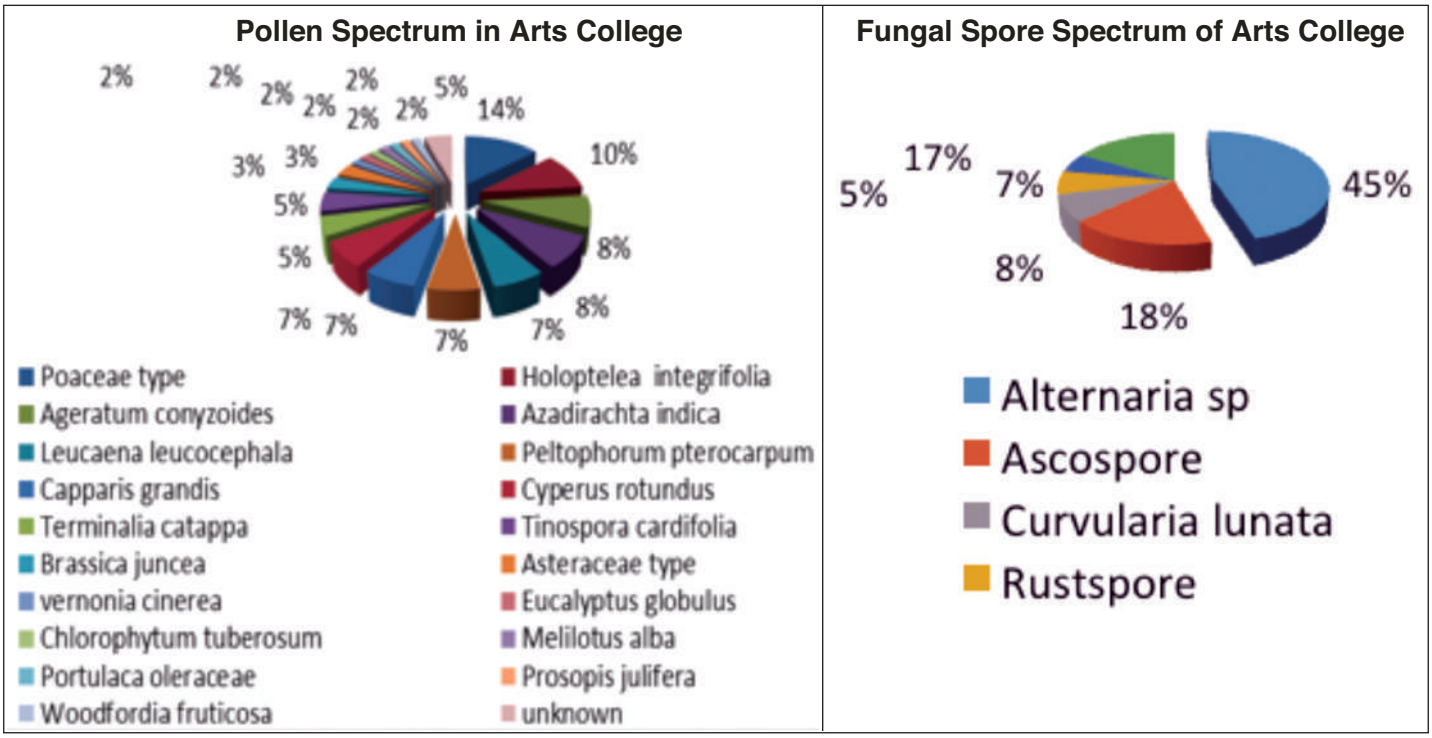


Fig. 4.1: Pollen and fungal spores trapped from Spiderweb Sample 1 (Arts college)

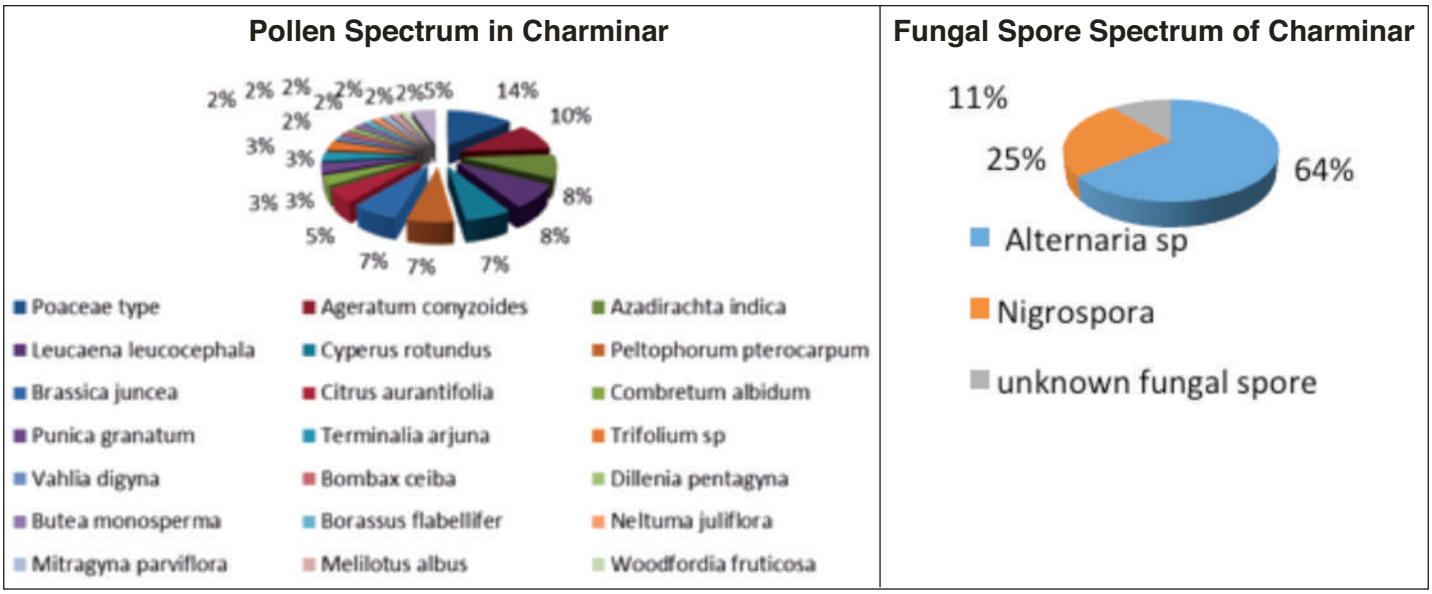


Fig. 4.2: Pollen and fungal spores trapped from Spiderweb Sample 2 (Charminar)

Spiderweb samples – 4 (Qutub shahi tombs)

This sample also depicts the dominance of *Ageratum conyzoides* (23)%, *Peltophorum pterocarpum* (7%), *Brassica juncea* (7%), *Azadiracta indica* (7%), *Gloriosa superba* (5%), *Alternanthera sessilis* (5%), *Tectona grandis* (5%), *Celosia argentea* (5%), *Eucalyptus globulus* (5%), *Olax scandense* (5%) were encountered moderately. *Achyranthes aspera* (2%), *Albizia lebbeck* (2%), *Coriandum sativum* (2%), *Ehretia laevis* (2%), *Gymnosporia emarginata* (2%), *Macaranga denticulate* (2%), *Melilotus albus* (2%), *Punica granatum* (2%), *Vahlia digyna* (2%), *Xanthium strumarium* (2%), *Zizyphus mauritania* (2%), *Senna occidentalis* (2%) are poorly represented in the pollen spectra. The Fungal spores such as Nigrospore (31%), *Drechslera* sp. (12%) and some unidentified fungal spores (57%) were encountered (Fig. 4.4).

Spiderweb samples – 5 (University College of Science)

The recovered palyno assemblage demonstrates the Poaceae pollen (25%) is dominant. *Ageratum conyzoides* (8%), *Azadirachta indica* (7%), *Sapindus trifoliatus* (5%), *Melilotus albus* (5%), *Leucaena leucocephala* (5%), *Ailanthus excelsa* (3%), *Alternanthera sessilis* (3%), *Hibiscus rosasinensis* (3%), *Neltuma juliflora* (3%), *Punica granatum* (3%), *Ocimum basilicum* (3%) pollen are encountered moderately. Whereas

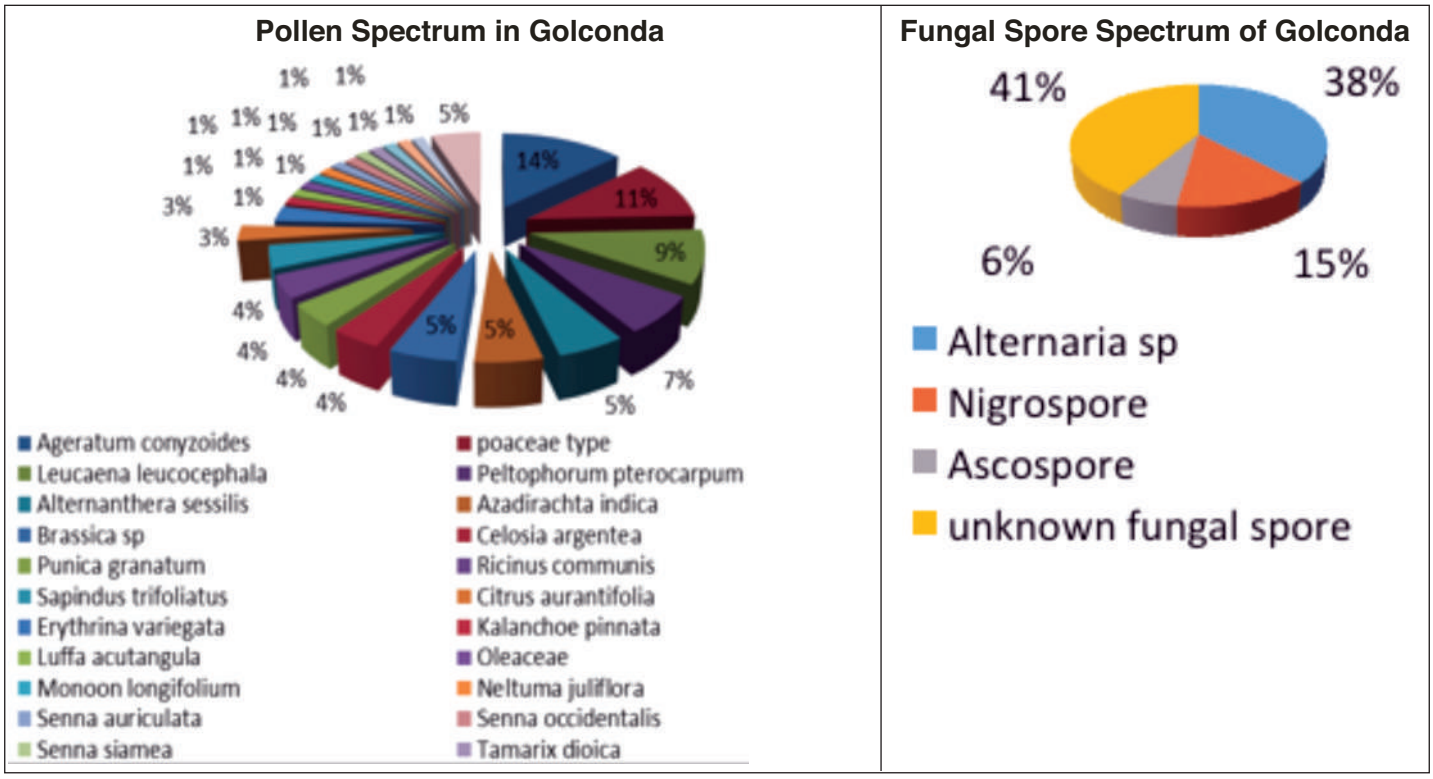


Fig. 4.3: Pollen and fungal spores trapped from Spiderweb Sample 3 (Golconda)

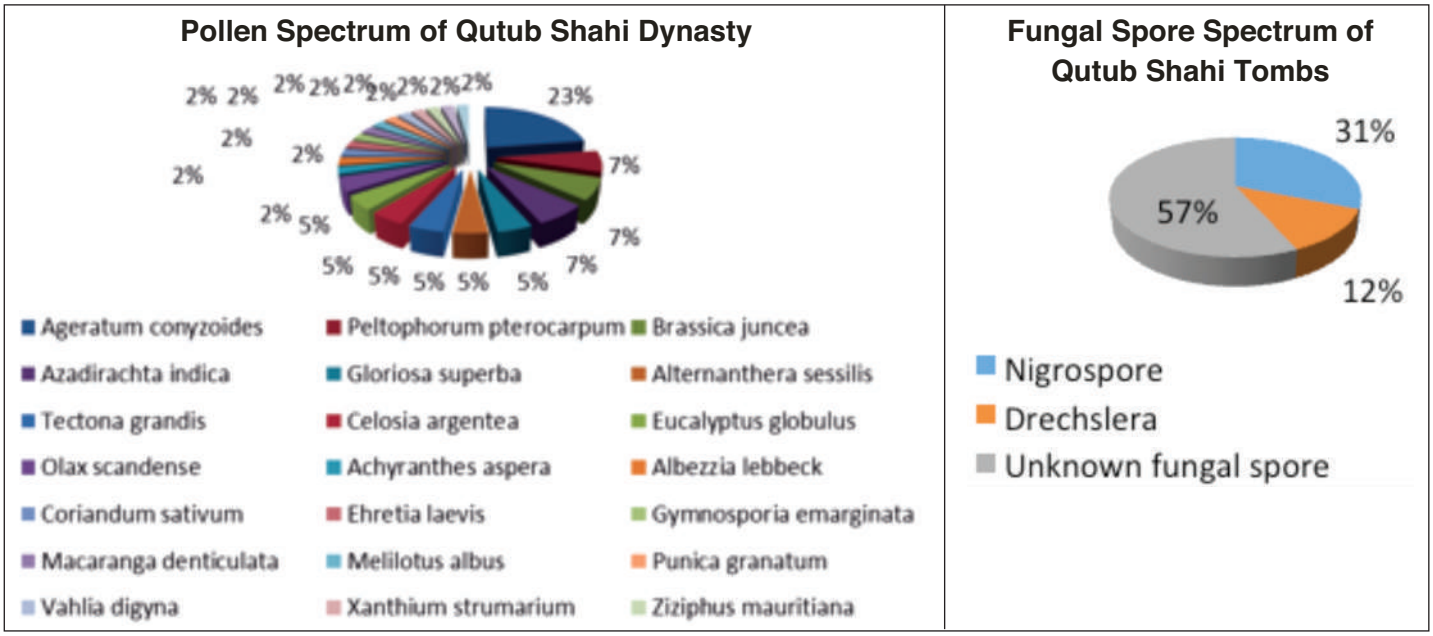


Fig. 4.4: Pollen and fungal spores trapped from Spiderweb Sample 4 (Qutub shahi tombs)

Capsicum (Solanaceae) (2%), *Senna occidentalis* (2%), *Terminalia catappa* (2%), *Ventillago denticulate* (2%), *Mutingia calabura* (2%), *Vachellia nilotica* (2%), *Vitex negundo* (2%), *Zizyphus mauritania* (2%) are recovered in low values. The fungal spores such as *Alternaria* sp. (40%), Ascospore (20%), *Nigrospora* (10%) and other unidentified fungal spores are recorded in low values (Fig. 4.5).

DISCUSSION

The study clearly indicates the identification of allergenic pollen and spores from Hyderabad monuments. Besides fungal spores, quantitatively 69 pollen types of herbs, shrubs, trees and climbers were recorded from these monuments (Fig. 4.6).

Pollen analysis of spiderweb samples from Hyderabad monuments revealed that herbaceous plant pollen was most abundant at the Arts College, followed by the Qutub Shahi Tombs, Golconda Fort, Charminar, and University College of Science. Tree pollen was dominant at the University College of Science, Charminar, Golconda Fort, Qutub Shahi Tombs, and Arts College.

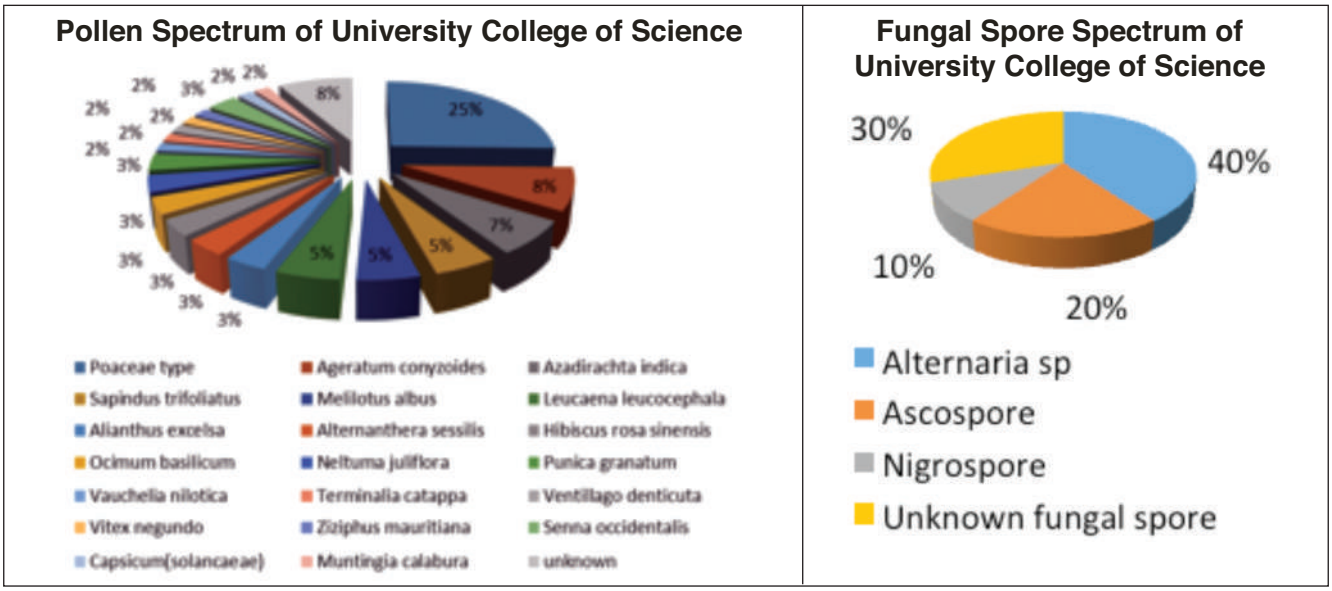


Fig. 4.5: Pollen and fungal spores trapped from Spiderweb Sample 5 (University College of Science)

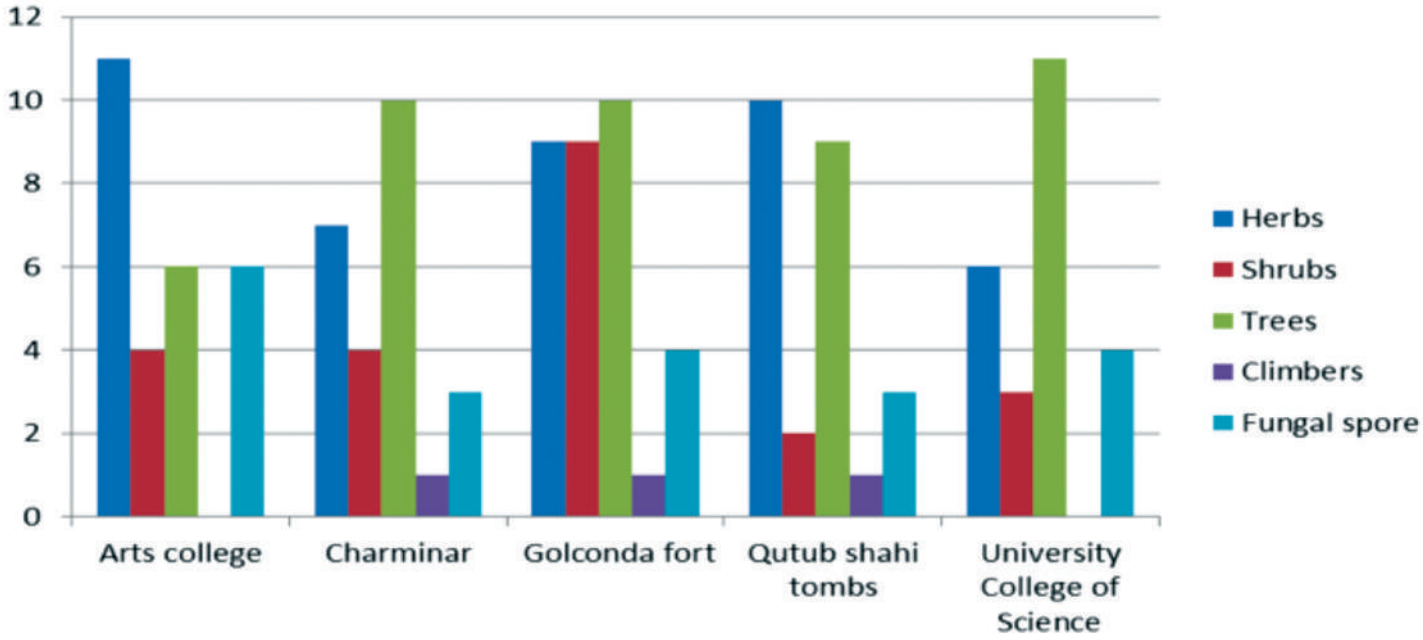


Fig. 4.6: Showing monuments wise plant types representation

A total of 23 herb species, 31 tree species, 12 shrubs, and 3 climbers were identified across all monuments.

The study identified allergenic pollen and spores in spiderwebs, which serve as natural traps for these particles. These allergens can lead to respiratory issues such as asthma, hay fever, and naso-bronchial allergies, as well as eye and skin conditions like conjunctivitis, dermatitis, and eczema. Symptoms of pollen allergies, known as pollinosis, include breathlessness, and itching of the nose and eyes. Local factors such as wind speed, humidity, climate, and season affect the retention of pollen and spores in the webs.

The pollen grains and spores of various plants have been identified as potential allergens such as *Ageratum conyzoides, Albizia lebbeck, Holoptelea integrifolia*, Poaceae (Grasses), *Delonix regia, Dillenia pentagyna, Tectona grandis, Amaranthaceae, Asteracese* sps, *Ailanthus excelsa, Ricinus communis, Eucalyptus globulus, Terminalia arjuna, Tinospora cardifolia, Azadirachta indica, Neltuma juliflora, Peltophorum pterocarpum, Bombax ceiba, Senna occidentalis, Senna siamea, Xanthium strumarium, Cyperus rotundus*[16,17] and the fungal spores *Alternaria, Nigrospora* and *Curvularia lunata*[18,19].

The plants have a high potential of causing pollen allergy to susceptible individuals in comparison with the entomophilous plants. The airborne pollen is essential for understanding and treating allergic diseases related to pollen hypersensitivity.

The analysis of spiderwebs from various monuments in Hyderabad has provided a comprehensive under-

standing of the prevalence and distribution of allergenic pollen and spores in the region.

Pollen Prevalence at Monuments

Herbaceous plant pollen were found to be particularly dominant in the Arts College, followed by the Qutub Shahi Tombs, Golconda Fort, Charminar, and the University College of Science. This dominance suggests that these locations contain significant amounts of herbaceous vegetation, which contributes to the airborne pollen load. The high prevalence of herbaceous pollen, especially from plants like Ageratum conyzoides and members of the Poaceae family (grasses), is noteworthy, as these are known allergens capable of causing respiratory issues such as bronchial asthma and hay fever.

On the other hand, tree pollen was more prevalent in the University College of Science, Charminar, Golconda Fort, Qutub Shahi Tombs, and Arts College. The identified tree species, including *Albizia lebbeck, Holoptelea integrifolia*, and *Delonix regia,* are recognized for their allergenic potential. The presence of tree pollen in these monuments indicates a significant contribution from the surrounding flora, which can lead to seasonal allergies among individuals who are sensitive to tree pollen.

Allergenic Pollen Types: Production, Peak Periods, Trapping in Spiderwebs, and Impact on Tourists

The identified allergenic pollen types from various plants in Hyderabad's monuments pose significant health risks to susceptible individuals. These pollen grains are produced in varying quantities depending on the plant species, environmental conditions, and seasonal variations (Fig. 4.7). Their behavior in the environment, including their trapping in spiderwebs and subsequent

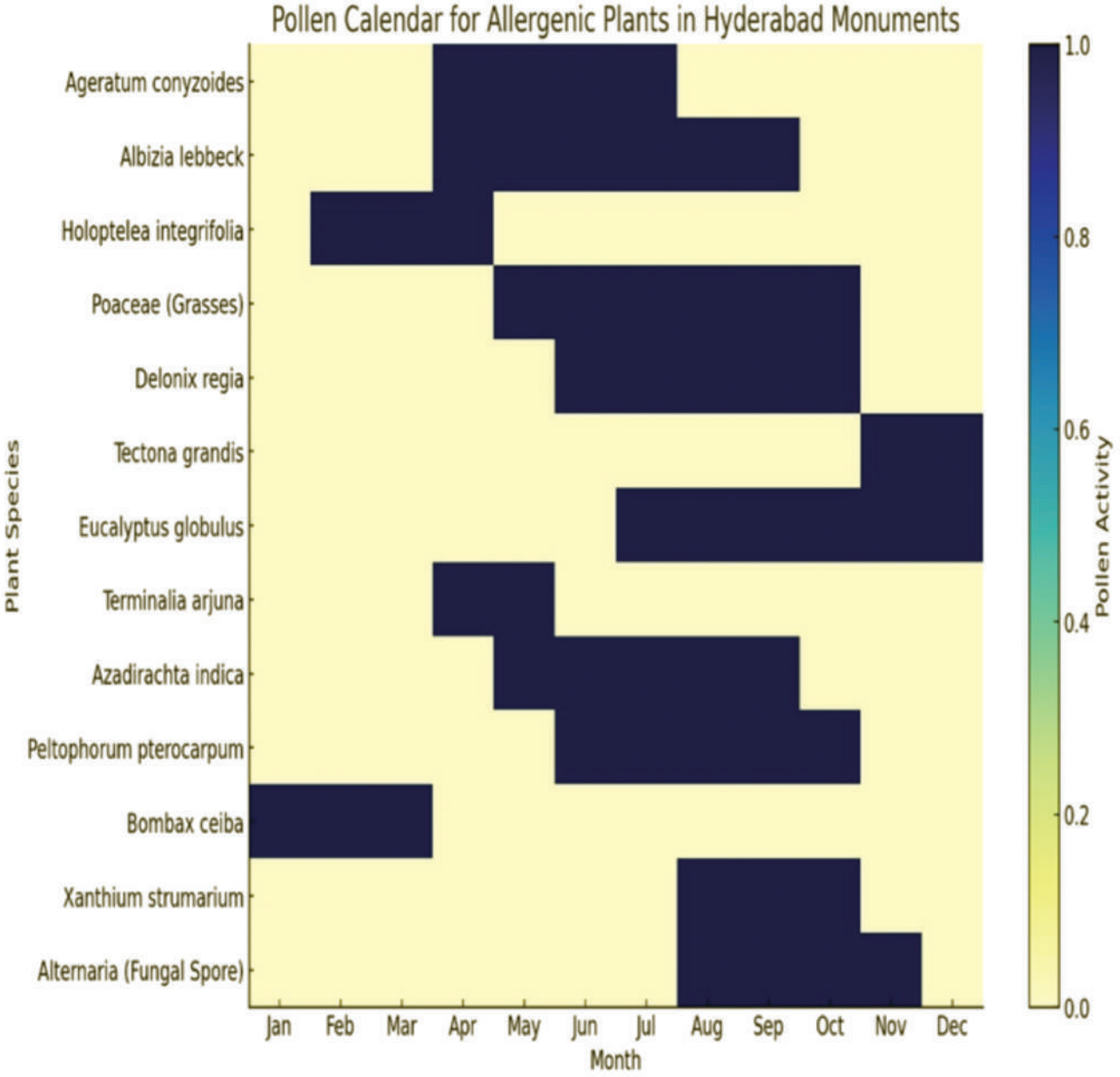


Fig. 4.7: Showing Pollen calender of allergic plants at various monuments of Hyderabad

release into the air, plays a crucial role in determining their impact on both local residents and tourists.

CONCLUSION

The study of pollen and spore analysis from spiderwebs in Hyderabad's monuments highlights the widespread presence of allergenic pollen and fungal spores, which pose significant health risks to susceptible individuals. Due to the historical and cultural significance of these monuments, they attract both tourists and locals, making awareness of pollen allergies essential for public health. While pollen is an inevitable part of the environment, understanding its impact and taking appropriate precautions can help mitigate the associated health risks.

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Research Article

CHRONOBIOLOGY OF *AILANTHUS EXCELSA* ROXB. (HEAVEN TREE): INVESTIGATING CIRCADIAN PATTERNS IN POLLEN DISPERSAL AND PHENOLOGICAL DYNAMICS

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Ailanthus excelsa Roxb. (Simaroubaceae), commonly known as the 'Heaven Tree,' is an exotic avenue tree recognized for its rapid growth and environmental adaptability. However, it is also a significant airborne pollen allergen, contributing to respiratory disorders in susceptible individuals. This study investigates the chronobiology of airborne allergens by examining the flowering phenology and circadian rhythm of pollen dispersal in A. excelsa. The phenological study reveals peak blooming occurs from January to March, with the highest bloom intensity in February. Floral buds take 10-15 days to transition to full bloom, and over 80% of buds open between 08:00-10:00 hrs. Pollen dispersal peaks between 08:30-09:30 hrs., with a single flower releasing an average of 16,270,000 tri-colporate pollen grains. Pollen grains were trapped using a 'Rotorord' sampler at 24-hour intervals, and concurrent meteorological data (temperature, humidity, wind speed, and light intensity) were recorded to correlate environmental factors with pollen dispersal. The findings enhance our understanding of the temporal dynamics of allergen release, supporting the development of targeted allergy management strategies. This research has implications for public health and urban planning, particularly in regions where *A. excelsa* is common.

Key Words: *Ailanthus Excelsa*, Chronobiology, Circadian Patterns, Pollen Dispersal, Phenological Dynamics.

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INTRODUCTION

The present study deals with the chronobiology of airborne pollen allergens by examining the circadian rhythm of pollen dispersal in *Ailanthus excelsa* Roxb. (Simaroubaceae), which is an exotic avenue tree commonly known as the 'Heaven Tree[1,2]. From a medical, mainly clinical perspective, it is imperative to understand the specifics regarding pollen and spore loads in the atmosphere. The flowering periods of higher plants recur cyclically in each season, yet the timing of blossoming may vary annually across different geographical regions. Drawing upon disparities noted in multiple years of airborne pollen observations[3–10], pollen calendars are devised as indispensable tools for allergy diagnosis and management. Allergic conditions such as bronchial asthma, allergic rhinitis, and atopic dermatitis are experiencing a significant surge worldwide, encompassing developing nations. Over 30% of the population is afflicted by one or more allergic ailments. Among the principal causative agents identified are pollen grains. It can be inferred that there were two peaks of pollen count annually, one during March to April and other during August to October.[11]

Through literature scrutiny, it is evident that allergenic proteins are present in *A. excelsa* Roxb. pollen. Among these pollen proteins, designated as AE1-AE9, AE6 (59.7 kDa), and AE8 (45.0 kDa) have been substantiated as the primary allergenic determinants. In comparison, AE1 (97.4 kDa), AE2 (92.6 kDa), AE4 (73.3 kDa), AE5 (64.8 kDa), AE7 (52.4 kDa), and AE9 (42.7 kDa) contribute minimally to allergenicity[12]. This investigation has adeptly observed the allergenic potential, pollen dispersal, and circadian periodicity of these allergenic potent pollen grains. The findings of this study hold promise for further refinement in pollen calendar development.[4,11,13,14]

The knowledge about floral phenology and flower visitor interaction is significant for studying insect – mediated pollen dispersal in air. *A. excelsa* is recognized for its fast growth and adaptability in various environments, making it a potential contributor to airborne pollen allergens. This prominent aeroallergen may be responsible for individuals afflicted with naso-bronchial disorders[2,15,16]. The allergenic potential of *A. excelsa* pollen in provoking various respiratory disorders in susceptible individuals has been previously documented.

Several plant species show their pollination dynamics as ambophily in nature i.e either anemophily (wind-mediated pollination) or entemophily (insect – mediated pollination).

The amount of pollen in the atmosphere depends on several atmospheric factors like humidity, temperature, air current, etc. Pollen distribution/ dispersal role is vital for public health, and one allergenic potential pollen dispersal study can be helpful for those suffering from that type of pollen allergy[2,7,11,17–20].

METERIALS AND METHODS

Experimental Site and Environment

Present investigation was conducted on the phenology, atmospheric pollen dispersal, and insect mediated pollen dispersal and phenology of *A. excelsa* Roxb. in the University campus of Visva-Bharati, Santiniketan (87°42´ E and 23°42´ N), West Bengal, India.

The atmospheric pollen incidence of *A. excelsa* was recorded by trapping pollen grains using a 'Rotorod' sampler[21]. The hourly atmospheric pollen incidence was determined. The sampler was positioned 5 m from the ground level, 1 m, and 6 m from the source plant. Pollen production was measured using the standard methodology.[32]

The percentage of atmospheric pollen incidence was calculated using the following formula.[30,31]

Percentage of particular pollen grain = No. of particular pollen grains ÷ Total no. of pollen grains trapped × 100

The atmospheric pollen frequency was recorded during the peak flowering period, and the observations were correlated with the meteorological factors (Humidity, temperature). Climatological Information and weather forecast data were collected (Fig. 5.3) from the site of study and India Meteorological Department (Sriniketan 42708).

RESULTS AND DISCUSSION

Ailanthus excelsa Roxb., commonly known as the 'Tree of Heaven[1]', is a rapidly growing deciduous tree reaching heights of 20-25 m, characterized by a straight cylindrical trunk and light grey stem bark. It is a prevalent avenue tree in various regions of India. The leaves are pinnately compound, reaching lengths of up to 90 cm, with 8-14 pairs of leaflets. The small yellowish flowers are arranged in panicles, and the fruits consist of single-seeded samaras. Observations on the flowering phenology (Table 5.1) revealed that bud emergence coincides with the onset of new leaf emergence. The inflorescence, a cymose panicle often found in the axils, exhibits maximum bud development in February. Floral bud opening

Table 5.1: Flower phenology of *Ailanthus exelsa* Roxb

Floral characters	Observation
Flowering period	January-March
Peak flowering period	March
Odour and Nectar	Present
No of flowers/inflorescence	210-300
Anthesis period	06:00-07:30 hour
Flower type	Actinomorphic
Flower colour	Whitish
Pollen production/flower	16,27,000 (Approx.)
Pollen type	Tri-colporate

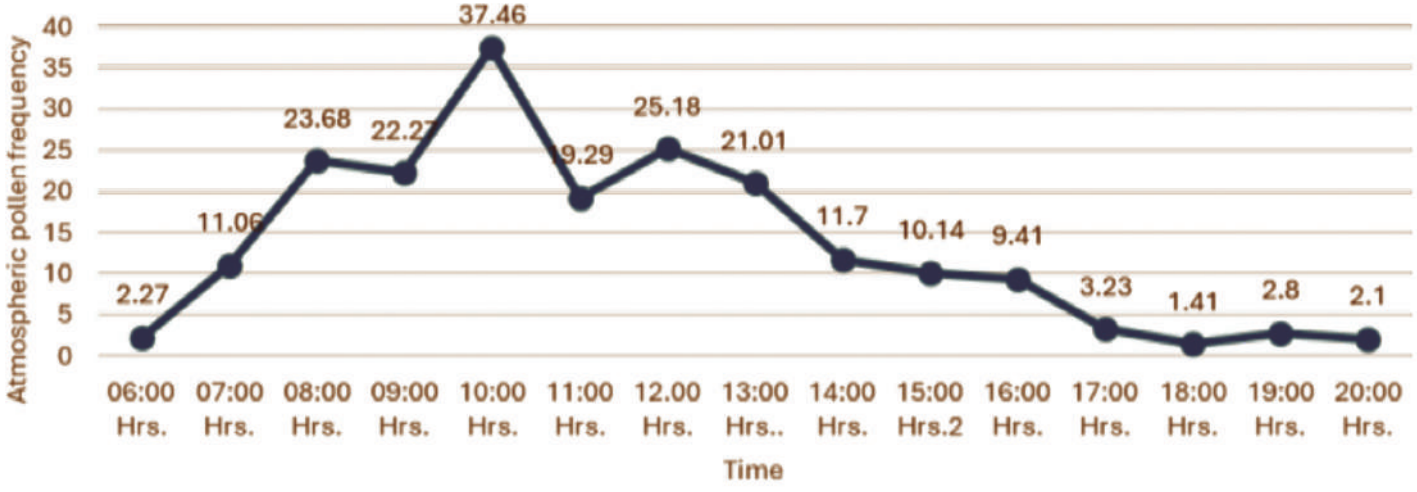

Fig. 5.1: Atmospheric pollen frequency 1m distance from the source plant

commences in mid-February, with an asynchronous flowering pattern observed, indicating varying stages of flower development on the same tree. The peak flowering period spans was 10-15 days, concluding by the end of March, with slight variations observed among different plants within the same season.

This study involved the observations of approximately 70 randomly selected *A. excelsa* plants across different locations at university campus. Flower openings are initiated between 0600 and 0730 hours, with peak flowering ranging from 80-90 percent, occurring between 08.00 and 10.00 hours.

Pollen dispersal of the species reaching a maximum of 97.27%, exhibits a pronounced peak between 08.00 and 10.00 hours within a 24-hour circadian cycle at a 1m distance from the source plant (Fig. 5.1). Predominantly mediated by wind[6,13,22,23], with 37.46% dispersal occurring at 6m from the source plant (Fig. 5.2), this process is crucial for subsequent pollination events. Each flower produces approximately 16,27,000 pollen grains, comparable to dust particles, facilitating both wind and insect-mediated transfer mechanisms. The pivotal role of wind in pollen dispersal is underscored by its sensitivity to meteorological conditions[8–10,10,13,24–27]. Higher humidity levels lead to a decrease in pollen frequency in the air, whereas lower humidity levels are associated with increased dispersal distances. These findings highlight the intricate interplay between environmental factors and pollen transport dynamics, shedding light on the mechanisms underlying the dispersal of pollen from plants.

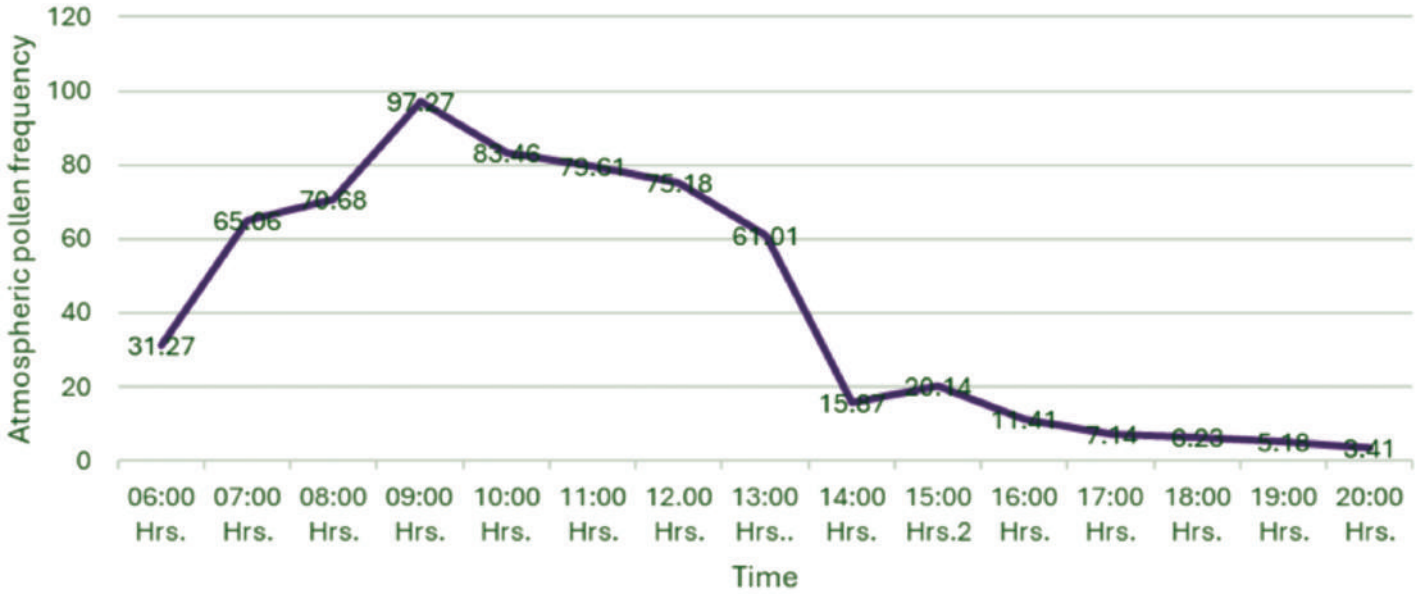

Fig. 5.2: Atmospheric pollen frequency 6 m distance from the source plant

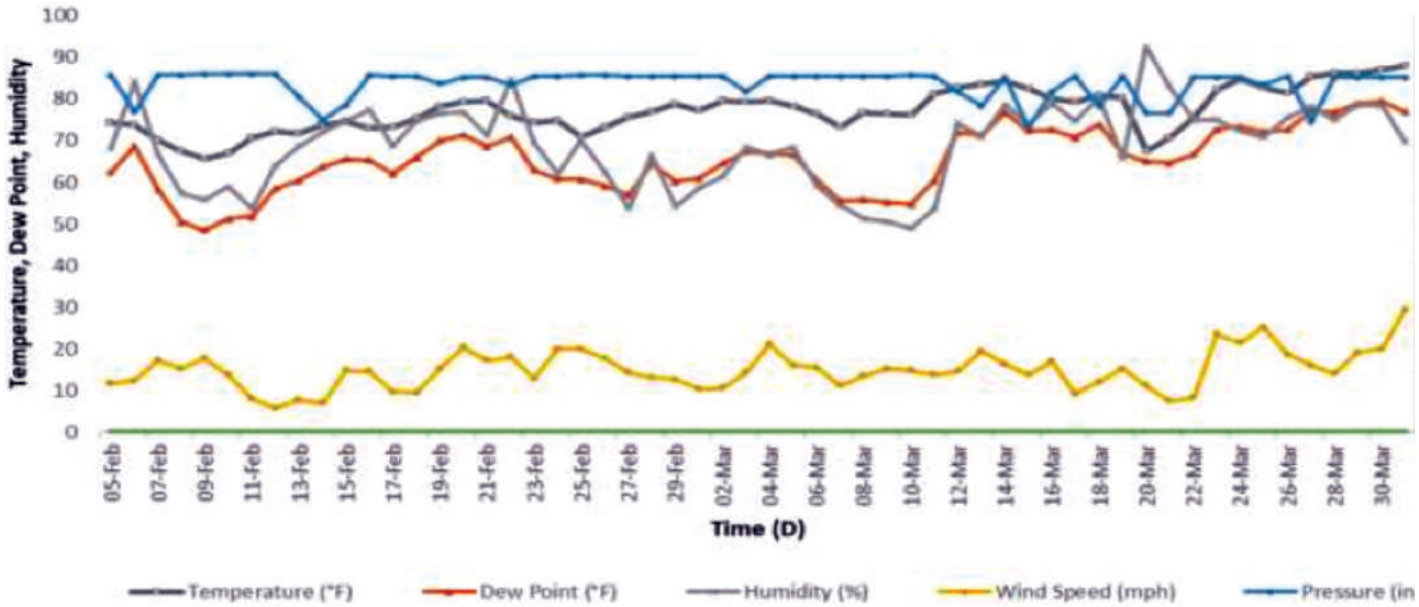

Fig. 5.3: Meteorological data during the flowering period of *A. excelsa*

The investigation into the circadian pollen dispersal of the plant holds profound significance, particularly in the context of rising allergy prevalence among individuals and its impact on clinicians[6,10,13,18,25,26,28]. Anther dehiscence typically occurs upon desiccating anther tissues, a process influenced by environmental factors such as

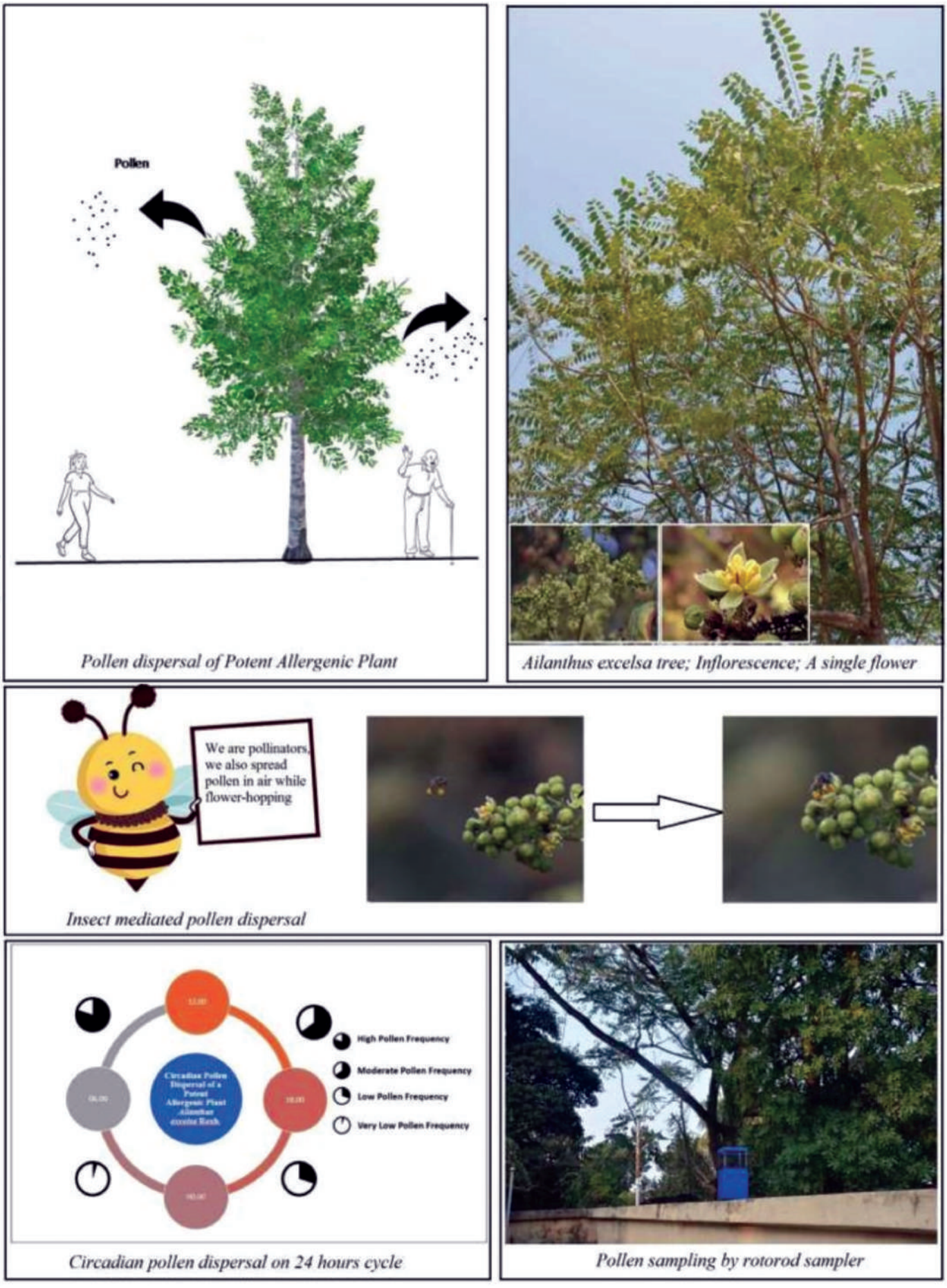

Fig. 5.4: Circadian pollen dispersal, pollen trapping and phenology of Ailanthus excelsa

temperature and humidity. Pollen grains in the air primarily rely on temperature and relative humidity. Throughout February and March, data on temperature and relative humidity for each hour, along with the atmospheric pollen frequency, over seven days characterized by varying weather conditions were collected. During the early stages of flowering, specifically at 10:00 hr, a 37.46% atmospheric pollen incidence was recorded (Fig. 5.2), coinciding with slightly lower temperature and relative humidity levels (average 28.2°C; RH 67%).

As the flowers opened, various Hymenoptera species, including *Apis dorsata, Amegilla* sp., *Tetragonula* sp., *Ceratina* sp., and *Megachile* sp., were the predominant visitors. During their visits, these bees' movement and wing vibrations facilitated pollen dispersal into the air. This mechanical action of pollen release increased the likelihood of cross-pollination in the surrounding area. From an aerobiological perspective, the airborne pollen also posed a potential allergenic risk, contributing to the local pollen load (Fig. 5.4).

As a potent allergenic plant, understanding the temporal patterns of pollen release is crucial for assessing the risk of allergen exposure. This research provides valuable insights into the dynamics of pollen dispersal, contributing to our comprehension of the factors influencing airborne allergen incidence (Fig. 5.4). Moreover, the findings of this study are instrumental in the creation of pollen calendars, which serve as essential tools for allergy management and public health planning. By identifying peak periods of pollen dispersal, clinicians and individuals susceptible to allergies can better prepare and manage their symptoms, ultimately leading to improved quality of life.

CONCLUSION

This study will contribute to a deeper understanding of the allergenic plant pollen dispersal behaviour of *Ailanthus excelsa* Roxb. These findings hold practical significance for individuals grappling with allergies. The temporal patterns of pollen release observed in this research are pivotal in crafting effective preventive measures and interventions. Furthermore, the study underscores the significance of monitoring pollen dispersal as a key indicator for assessing the air quality index. Such insights provide a scientific foundation for future endeavours to enhance pollen forecasting, thereby improving public health outcomes and aiding allergy management efforts. The significance of both wind and insect-mediated pollen dispersal, alongside the impact of dew on pollen release, underscores the comprehensive nature of this study in elucidating the mechanisms underlying the dispersal of *Ailanthus excelsa* Roxb. pollen.

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Research Article

EFFECT OF PARTICULATE POLLUTANT DEPOSITION ON BIOAEROSOL MONITORING OF KOLKATA CITY: A COMPARATIVE STUDY WITH SUBURBAN AREA

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Bioaerosols are important factors to cause human respiratory allergy and allergic asthma. To get quantitative interpretation of aeroallergen exposure in a particular geographic area, regular volumetric monitoring of aerobioparticles is necessary. The significance of airborne biopollutant monitoring is very much dependent on its reliability. During aerobiological monitoring with volumetric suction sampler, deposition of particulate pollutants often causes interruption in data recording and as a consequence, data interpretation becomes difficult and leads to erroneous or misleading conclusions. In the present study we have conducted aerobiological studies (2018-2019) in the city of Kolkata usingBurkard 7-day volumetric sampler and found interferences due to particle (e.g., soot, dusts, automobile exhausts, etc.) deposition during prolonged dry periods in winter. To make an analysis of such data loss, comparative study in the same period was made with the data recorded from a less industrialized suburban site (control data), where atmospheric particle deposition was less. In dry days in winter, airborne pollen/spore data loss ranged from 55-100% during peak period of depositions in the city, when compared to suburban area ($p < 0.05$).

Key Words: Bioaerosol monitoring, particle deposition, airborne pollen and spore, data loss.

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INTRODUCTION

Aerobiology is the study on airborne bioparticles, in terms of their origin, dispersal and effect on plants as well as human being. Airborne bioparticles are important contributors of aerosol, among which pollen grains and fungal spores are very significant[1]. These pollen and spores often act as trigger of allergic reaction in human body. Hence aerobiological data are very important to predict the atmospheric allergen exposure to help the susceptible individuals suffering from respiratory allergy and asthma[2]. On the other hand, botanists, mycologists, plant pathologists and ecologists utilize aerobiological data to study the factors influencing reproductive biology of present day flora with their dispersal and distributional pattern[3,4].

For an aerobiological monitoring, a volumetric sampler is necessary for accurate quantitative determination of atmospheric bioaerosol composition, highlighting airborne pollen grains and fungal spores. Burkard spore traps are widely used for determining the bioaerosol composition of atmosphere. These volumetric samplers are based on Hirst spore trap[5,6]. Air is drawn at 10 lit/minute and airborne particles are deposited on a sticky tape mounted on a rotating drum. Microscopic analysis of the tape provides airborne concentration of particulate matters. Sampling efficiencies are generally reported to be dependent on particle size, wind velocity, type of adhesive[7] for this sampler. When there is no change in the wind speed, the sampling efficiency had been reported[5,7] to be more than 90%.

In an urban atmosphere, the air contains a range of particulate matters (soot materials, automobile exhausts, dust, etc.), which get deposited on the sticky tape during sampling and affect the counting and identification of airborne pollen and spores[8]. As a result, we get a high number of unidentified categories of bioparticles, which sometimes cannot even be counted due to masking effect of deposition.

In the present study, during an aerobiological survey of Kolkata city, there is a first time the report on the effect of particulate matter deposition hampering the accuracy of aerobiological data collection to record the incidence of pollen grains and fungal spores.

METERIALS AND METHODS

Study site

Aerobiological study was conducted in two sites for comparative study using Burkard 7-day volumetric samplers. The first one is in the Central Kolkata, which is situated in a residential area approximately 4 km east of industrial section of the city (Fig. 6.1). For comparative study, control data were collected from another sampling site from northern suburban area of Kolkata city. This site is situated in 19 km north of central Kolkata, using another Burkard sampler.

Aerobiological sampling

Aerobiological study was carried out from January 2018 to December 2019 continuously for two years in the two sites. The Burkard spore traps were set for seven day sampling. The coated tapes were changed weekly and cut into seven daily segments and examined microscopically. The microscopic counts were converted into atmospheric concentration and expressed as number per cubic meter according to the guideline of The British Aerobiology Federation[9]. After two-year comparative study of total airborne pollen and spore counts in both the sites, we found a marked effect of particulate matter deposition affecting the monitoring result in Calcutta site when compared with that of suburban area.

In this study we selected the Burkard tape samples from Kolkata city in 2019, just when the particle deposition greatly interrupted the counting of trapped particles by masking them (especially in dry days). The data were compared with the normal samples of same time period from a comparatively less polluted suburban area (in term of particle deposition) to get an idea about the loss of data for such factor.

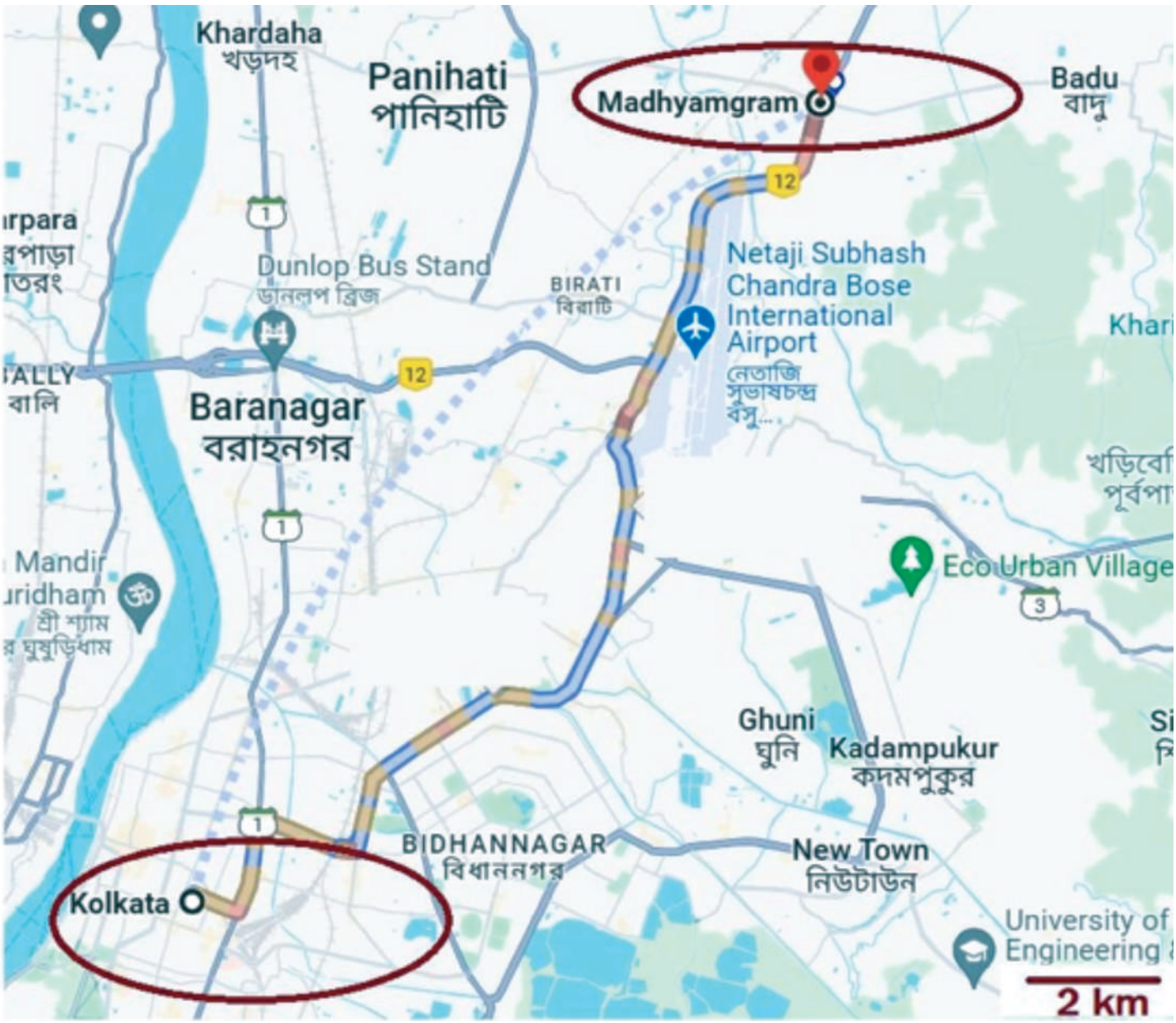

Fig. 6.1: Location map of the aerobiological sampling sites in the city of Kolkata and suburban areas

Data on meteorology and suspended particulate matters

Meteorological data of Kolkata Metropolis were collected to record daily temperatures (maximum and minimum), relative humidity and rainfall. Data on suspended particulate matters were recorded from the air quality information page of West Bengal Pollution Control Board.

RESULTS

The Aerobiological sampling has been conducted in the two sites (Kolkata city and suburban area) from January 2018 to December 2019. For airborne pollen grains (Fig. 6.2), the mean10-day average in the air of Kolkata city showed the count to be less in number when compared with suburban area. During dry days (especially from the end of October to April) the pollen count of Kolkata is 8-75% lower in comparison.

In case of fungal spores too (Fig. 6.3), the same data showed decrease in count during dry days, though it is lesser in comparison to pollen records (0.5-38%). In this case maximum decrease was found during the peak period of the total spore count (October).

It has been observed that the maximum degree of particulate matter deposition occurs during dry days without any rainfall for a prolonged period in every year. It was also been found that a remarkable part of sampling surface of Burkard tape is covered with dust, soot or other particles to interfere pollen and spore counting. The period with high degree of data loss due to such reason was recorded, which is really worth to mention and an

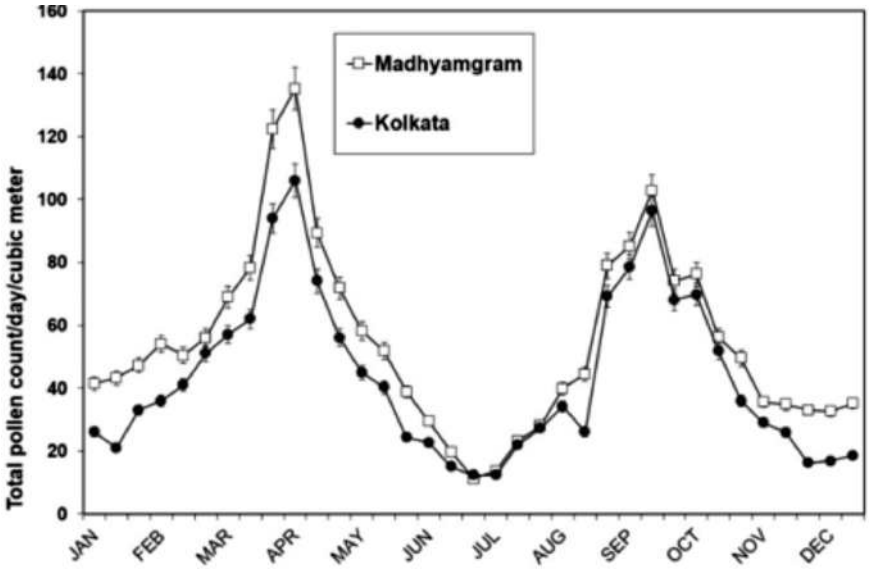

Fig. 6.2: Variation of total pollen concentration in the air of Kolkata metropolis and suburban area (Madhyamgram), showing the mean 10-day average

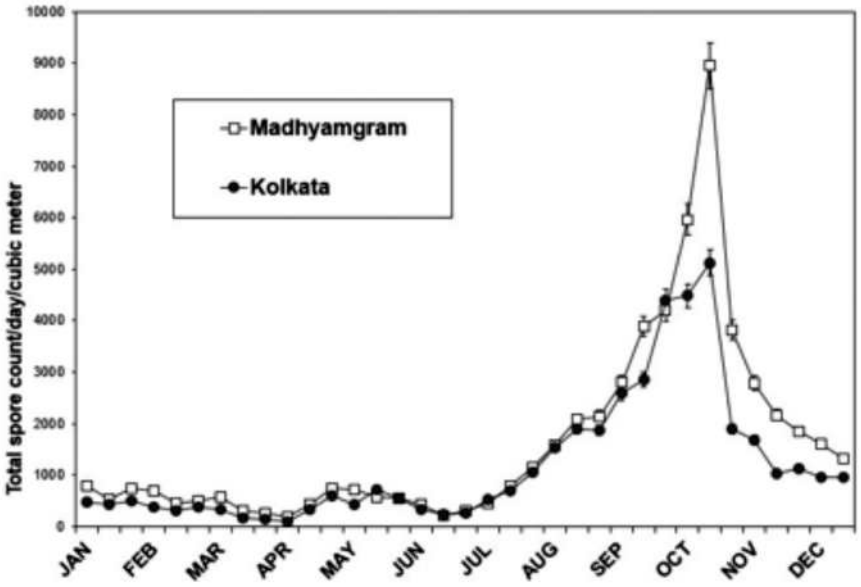

Fig. 6.3: Variation of total fungal spore concentration in the air of Kolkata metropolis and suburban area (Madhyamgram), showing the mean 10-day average.

Exposed tape of Burkard 7-day spore trap in dry season with distinct particulate deposition

Exposed tape of Burkard 7-day spore trap, with less particulate deposition

Fig. 6.4: Typical example of Burkard tape segment with evident particle deposition level (at top) in city, compared to suburban area (at bottom), during prolonged dry days (January 11-17- 2019) in winter, when there is no rain for a long period.

account on this was tried to be made in the present study.

Figure 6.4 depicts typical example of seven day Burkard tape segment with remarkable level of particle deposition in the city area, compared to suburban site, during prolonged dry days (January 11-17, 2019) in winter, when there is no rain for a long period. The last rainfall was recorded 59 days back.

Average temperature in that week ranged from 18.5ºC to 20.45ºC, average humidity from 56.5% to 72%, suspended particulate matters from 378-488μg/cubic meter of air (Table 6.1).

In respect of the occurrence of the airborne suspended particulate matter, 24-hourly variation for continuous one month period from 16th Dec. 2018 to 15th Jan., 2019, the maximum level was recorded mostly 11 PM to 1 AM with up to 1100 μg/m^3 concentration (Fig. 6.5).

Corroborative to the occurrence of variable level of particle matter in air, in the seven-day segment several dark coloured zones (A) were found, having VERY HIGH degree of particulate pollutant deposition intervening with HIGH (B), MODERATE (C), LESS (D) and VERY LESS (E) amount of particle depositions at different times of the days of the week (Fig. 6.6). Presumably these are soot and dust particles along with pollen grains and spores when viewed under microscope (Fig. 6.6) with different levels of particle deposition.

Table 6.1: Weather data and suspended particulate matters in the atmosphere of Kolkata city from 11-17th January, 2019

Date	Temperature (°C)		Relative Humidity (%)		Suspended Particulate Matter (SPM, µg/m^3)
	Maximum	Minimum	Maximum	Minimum	
11-01-19	11.9	26.1	37.0	92.0	488
12-01-19	11.9	26.2	29.0	92.0	404
13-01-19	12.6	26.4	40	91.0	406
14-01-19	14.2	26.7	50..0	94.0	424
15-01-19	15.9	23.7	34	85	434
16-01-19	13.2	24.5	37	76	378
17-01-19	13.2	23.8	37	78	442

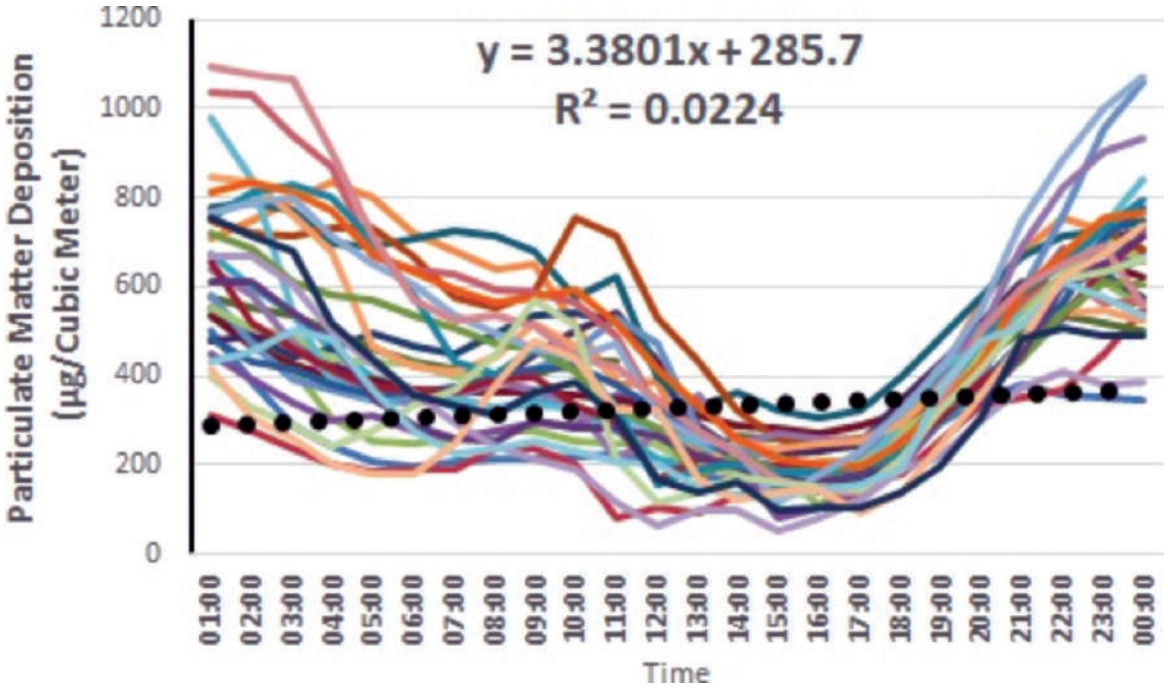

Fig. 6.5: Hourly variation of particulate deposition for one month recorded in dry days throughout December 16, 2018 – January 15, 2019 in Kolkata city, with dotted trend line.

In the very highly polluted zone (Particle deposition level A), it is impossible to see the pollen or spore and the whole data have to be almost lost during this period of the day.

In case of particle deposition level B, 75-80% of data was lost, when compared with the control data of the same time period collected from suburban area of Kolkata city. In this suburban area (19 km north from

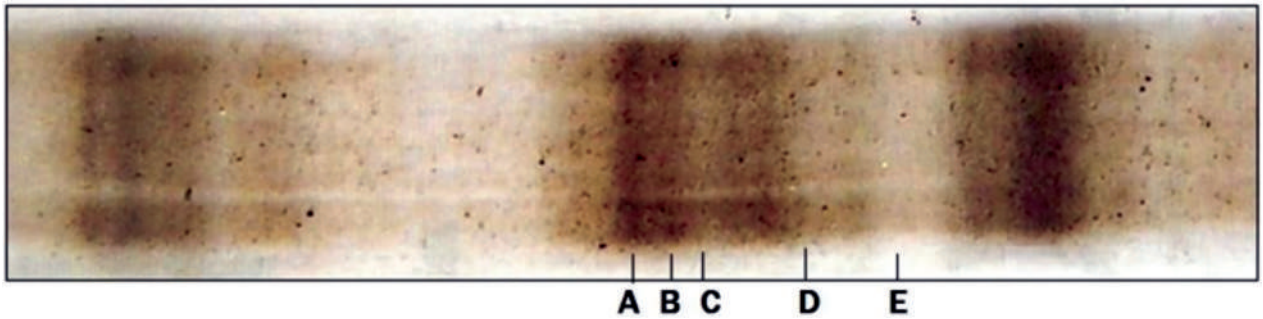

Fig. 6.6: Hourly variation of particulate deposition for one month recorded in dry days throughout December 16, 2018 – January 15, 2019 in Kolkata city, with dotted trend line.

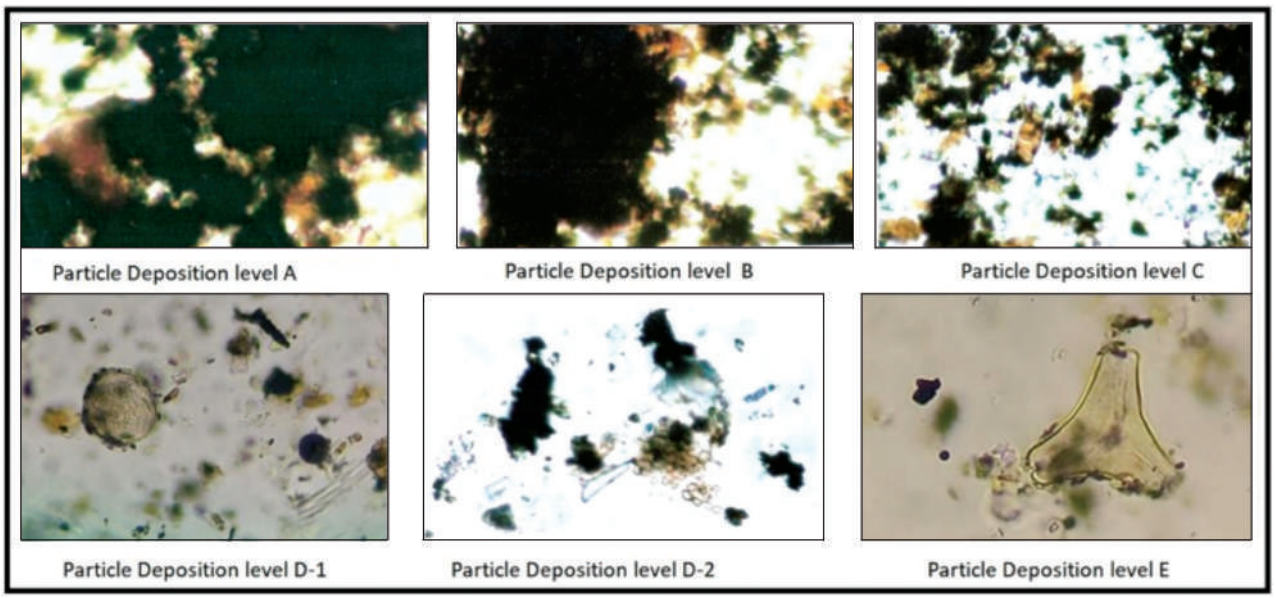

Fig. 6.7: Photomicrographs (X800) of field areas observed in the Burkard tape segments for pollen/spore monitoring with different grades (A-E) of particulate deposition in Kolkata City, during dry period.

Table 6.2: Results of pollen/spore counting in number/cubic meter/hour in different ranges of particulate matter deposition

Level of Particle of Deposition	Pollen/Spore Count (No./m³/h)	Comment on Data	Comparative Prediction of Percentage Data Loss
A. Very high	Nil	Cannot be counted	100
B. High	2.6 - 20	Very few grains were visible	75-80
C. Moderate	8.8 - 56	Few grains were visible	55
D. Less	17.2 - 80	Almost all pollen/spores are visible	1-2
E. Very less	9.2 - 220	Pollen/spores were clearly visible for counting	Nil

the study area) there is deposition on tape segment during monitoring, but it did not affect the counting though there is some loss of data due to power cut.

In similar comparison, 55% data loss in moderately deposited zone at particle deposition level C, 1-2% loss in less deposited zone at particle deposition level D for both pollen grains (D-1) and fungal spores (D-2). Data loss due to particulate deposition in the microscopic field goes gradually down to zero level (Fig. 6.7, Table 6.2) where there is very less interference at Particle deposition level E.

DISCUSSION

Biological particles or aerosols carrying allergenic proteins produce allergic symptoms and asthma in sensitive individuals[10]. Among the factors implicated to the recent epidemic of bronchial asthma, environmental allergenic particles are most important[11]. Sensitization to aeroallergens is a very significant determinant for asthma hospitalization[12]. Aerobiological monitoring provides the basic data for the determination of qualitative as well as quantitative allergen exposure in a specific environment. Natural environment and bioaerosols are often complex and no one sampling procedure can realistically be sufficient to detect all the bioparticulate matters present in a given volume of air. Bioaerosols are usually mixed with gaseous pollutants and other non-viable and inorganic particles with radii ranging over eight orders of magnitude and concentrations over 30[13,14]. These may limit the value of the sampling systems[15]. In outdoor, air masses are too large to be examined in their entirety. So, regular sampling is necessary and results must be extrapolated to draw conclusions on the whole. Errors in the sampling data collection thus lead to erroneous conclusion[16]. It is very important because a single bioparticle can initiate an infection, pathogenicity or allergic reaction on living organisms.

Different spore traps yield different types of information and none gives a complete picture of the bioaerosols. Microscopic assessment is least selective allowing all particulates to be counted and classified but requires highly skilled identification knowledge. Our sampler helped us in that way, but even then identification of all species is seldom possible and often genera (e.g. Aspergilli group) cannot be identified.

Regarding sampling location, it is desired that the site should be representative of the study area without any contamination of non-bioparticulates. This criterion can not be satisfied in urban zones, where pollutants from factories and vehicles can be heavy, Especially in a city like Kolkata (formerly Calcutta), where the urban agglomeration is one of the largest in India with a population of more than 14 millions (2001 census and further)[17]. We found a high deposition of soot particles, which are composed of agglomerated particles and organic materials. Because of their loose structure, soot particles are deposited to impact the surface in-elastically and stick to it. Their large cross-sectional area also can make an effect on aerosol monitoring[18]. In addition oil droplets also appear to impact the sampling surface in- elastically and stick to the surface. Above all these factors, in cities buildings, trees and other obstructions effect sampling but no parameters have been identified that could allow adequate prediction[19].

CONCLUSION

Regular aerobiological monitoring is necessary and results must be extrapolated to draw conclusions on the whole to get a better view of the real-time atmospheric

exposure for the bioparticles like pollen grains and spores in urban environment.

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Short Communication

EFFECT OF TEMPERATURE, RELATIVE HUMIDITY AND RAINFALL ON THE FUNGAL SPORE COUNT IN JOWAR FIELD OF MANGALVEDHA TEHSIL OF SOLAPUR DISTRICT (MAHARASHTRA, INDIA)

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Overall, temperature, relative humidity and rainfall interact to shape fungal spore counts in the environment. Interactions among these variables were found to be influential. Each factor contributes to fungal growth and spore dynamics in distinct ways and their combined effects are crucial for understanding and managing fungal-related issues. Seasonal variation shows impact on aeromycological composition of air. There is remarkable increase in number of spores after irregular precipitation. Number of fungal spores in air increases when relative humidity is greater than 70%. There is gradual decrease in fungal spores as relative humidity is below 70% from November to January. As the Relative humidity decreases below 50% and Temperature is between 25-30 degrees, number of fungal spores tends to increase. Thus, the study highlights the significant influence of temperature, relative humidity and rainfall on fungal spore count and each of the factors plays a distinct yet interconnected role.

Key Words: Fungal spores, Temperature, Relative humidity, Rainfall, Solapur District, Maharashtra.

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INTRODUCTION

Fungal populations play a significant role in various ecosystems, influencing both ecological dynamics and human activities. The abundance and diversity of fungal species are known to be highly sensitive to environmental conditions, particularly temperature, relative humidity and precipitation[1]. Understanding these relationships is essential for managing fungal-related challenges in agriculture, public health, and industry. Fungal overgrowth can cause crop diseases, respiratory issues, and product contamination, making effective management vital[2].

Temperature directly affects fungal growth rates and reproductive cycles[3]. Each fungal species has an optimal temperature range for growth, beyond which growth rates decline. Similarly, relative humidity is a key factor in fungal proliferation, as it influences spore germination, colonization and survival[4]. High humidity levels can create favorable conditions for fungal growth, while lower humidity may inhibit it. Rainfall impacts fungal dynamics by contributing to soil moisture and providing a medium for spore dispersal, further affecting fungal distribution and abundance[5]. Variations in these climatic variables can lead to significant changes in fungal community composition and abundance[6]. For example, increased temperatures and humidity have been linked to outbreaks of pathogenic fungi, while variations in rainfall can alter fungal distribution patterns across different environments[7,8].

This study aims to investigate the effects of temperature, relative humidity and rainfall on fungal counts, focusing on how these factors interact to influence fungal populations. By analyzing data across different climatic conditions, this research seeks to enhance our understanding of fungal ecology and provide insights that could inform strategies for managing fungal-related challenges in various sectors.

METERIALS AND METHODS

The present study was carried out at Mangalvedha area (Maharashtra, India: Lat 17.53288° and Long 75.46333°)

for the period of Rabi season from 4th October 2022 to 12th February 2023. These aeromycological investigations were carried out by using continuous volumetric Tilak air sampler located in the various fields of jowar, in Mangalvedha taluka[9]. During this study air sampler was placed at 3 feet height above the ground level. Tilak air sampler is the most popular air sampler that is electrically operated device, it works continuously 24 × 7 whole week and samples are collected every week. The rotating drum of the sampler requires seven days to complete one rotation. The samples were collected from the cello tape fixed on the drum to catch the spores by applying melted glycerin jelly. The cello tape was replaced after collection of samples. Collected cello tape was mounted on the slides and examinations were done under the compound microscope. Identification of spores was done with the help of morphological characters and by using standard books of Aeromycology, monographs etc. During this study Ainsworth's (1971) classification is used.

RESULTS

The present aeromycological investigation was performed at Mangalvedha area during Rabi season from 4th October 2022 to 12th February 2023. Mainly the impact of temperature, precipitation (rainfall) and relative humidity were the parameters considered for the study. Observations were made on weekly basis.

The effect of average temperature and precipitation on the number of fungal spores represented in Fig. 7.1.

Influence of relative humidity on fungal spore count was depicted in Fig. 7.2. Gradual decrease in fungal spore count was observed as relative humidity is below 70% from November to January. As the relative humidity decreases below 50%, number of fungal spores found to be increased.

DISCUSSION

The findings of this study highlight the impact of temperature, relative humidity and rainfall on the fungal spore counts, underscoring the importance of these environmental variables in fungal ecology. Temperature has a clear impact on fungal spore counts. Fungal populations generally increase with rising temperatures up to an optimal point, beyond which counts decline. This response is indicative of the temperature sensitivity of fungal growth and reproduction[3]. Higher temperatures can accelerate metabolic processes, leading to increased fungal growth and sporulation[6]. However, extreme temperatures may inhibit fungal activity by denaturing essential cellular proteins or disrupting metabolic pathways, leading to decreased fungal viability[4,7].

Relative humidity proved to be a significant determinant of fungal counts in our study. Our data align with earlier studies that emphasize the critical role of humidity in fungal proliferation[4]. Elevated humidity levels facilitate spore germination and fungal colonization by maintaining adequate moisture levels on surfaces and in the air, thereby enhancing fungal growth[1]. Conversely, low humidity conditions were associated with reduced

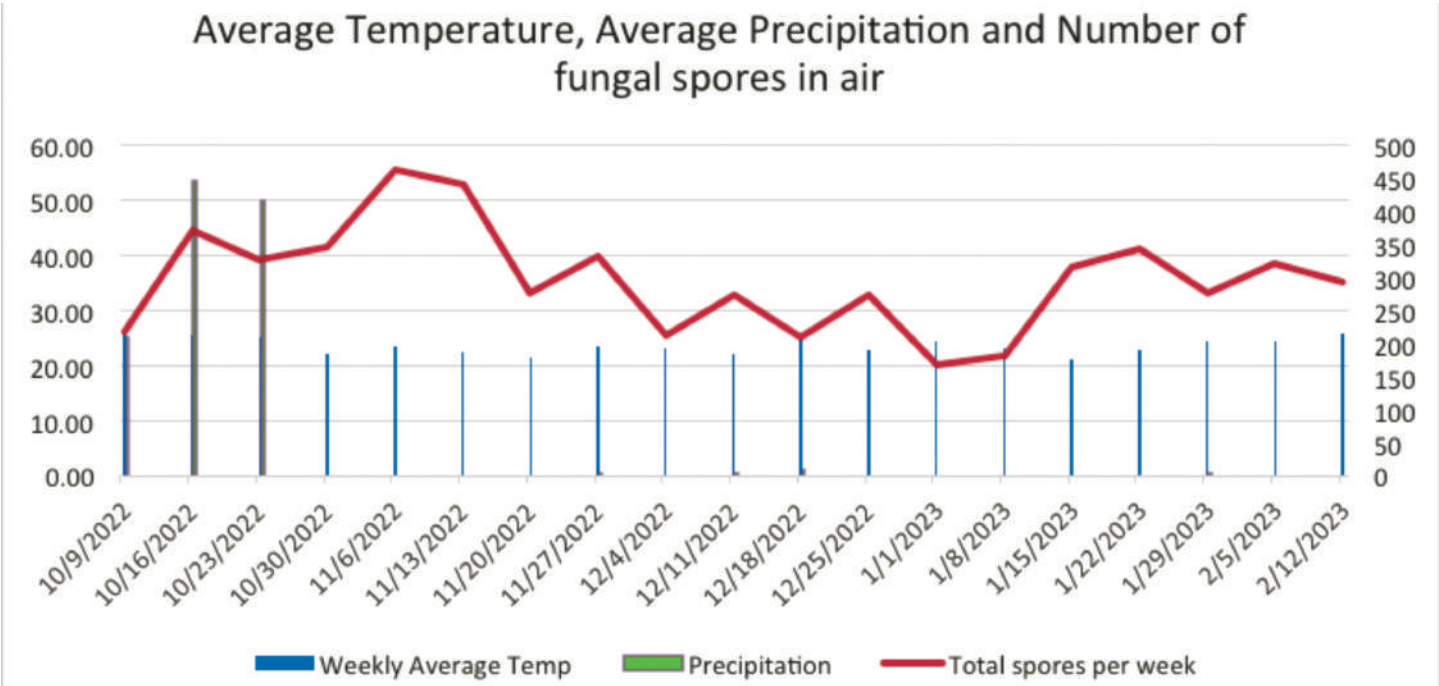

Fig. 7.1: Effect of average temperature and precipitation on fungal spore count.

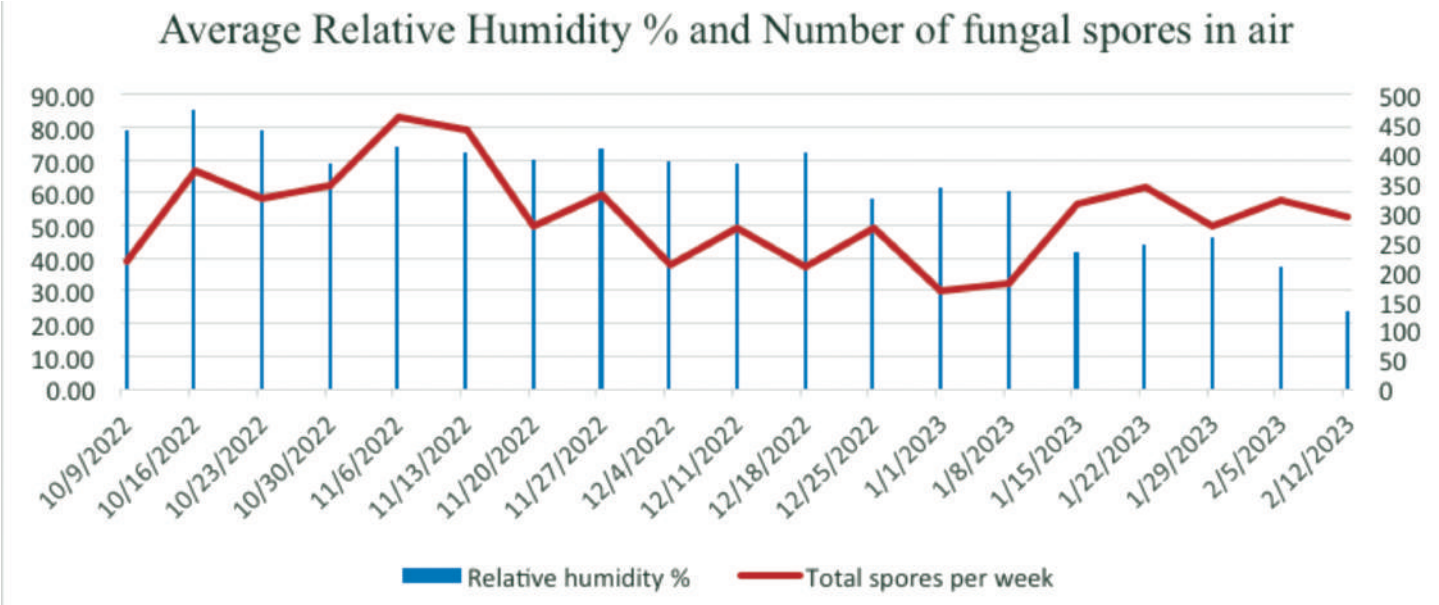

Fig. 7.2: Effect of average relative humidity on fungal spore count.

fungal counts, likely due to desiccation stress and lower spore viability. These findings are consistent with the notion that fungi require specific moisture thresholds to thrive and propagate effectively.

Rainfall was found to have a dual effect on fungal counts. On one hand, increased rainfall contributes to higher soil moisture and promotes fungal growth by creating an environment conducive to spore dispersal and colonization[5]. On the other hand, excessive rainfall can lead to waterlogging and anaerobic conditions, which may negatively impact certain fungal species by reducing oxygen availability and altering nutrient dynamics[8]. Thus, while moderate rainfall tends to enhance fungal populations, extreme precipitation events can disrupt fungal communities by creating inhospitable conditions.

The interactions between temperature, relative humidity and rainfall further complicate the effects on fungal counts. For instance, the combined effect of high temperature and high humidity often results in exponential fungal growth, as both conditions are conducive to fungal activity[2]. However, this interaction can be influenced by rainfall patterns, which can either exacerbate or mitigate the effects depending on the timing and intensity of precipitation. These complex interactions suggest that predicting fungal counts requires a nuanced understanding of how these variables interplay under varying climatic scenarios.

The insights gained from this study have practical implications for managing fungal-related issues in agriculture, public health and industry. For instance, in agricultural settings, monitoring and managing temperature and humidity levels can help in predicting and controlling fungal diseases[7]. Additionally, understanding the impact of rainfall patterns can aid in designing effective irrigation and drainage systems to mitigate fungal risks[5]. Future research should focus on exploring the mechanistic pathways through which these environmental variables influence fungal physiology and community dynamics. Long-term studies incorporating seasonal and geographical variations could provide a more comprehensive understanding of how changing climate conditions will impact fungal populations globally.

CONCLUSION

In summary, our study confirms that temperature, relative humidity and rainfall significantly influence fungal counts, each playing a distinct yet interconnected role. These findings contribute to a deeper understanding of fungal ecology and highlight the need for integrated approaches in managing fungal-related challenges in various sectors.

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