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DIFFERENCES IN CONCENTRATIONS OF ALLERGENIC POLLENS AND SPORES AT DIFFERENT HEIGHTS ON AN AGRICULTURAL FARM IN WEST BENGAL, INDIA

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Abstract: The aim of the study was to assess the vertical profile of the major airborne pollen and spore concentration in the lower heights (up to six meters) and to check their allergenic potential causing respiratory allergy in agricultural workers. The study was conducted using rotorod samplers mounted at different heights at weekly intervals for two consecutive years (November 1997–October 1999). The major pollen grains and fungal spores (from mass culture) were collected in bulk and studied by skin-prick tests to detect allergenicity. Of the recorded pollen, 10 major and perennial types (e.g., Poaceae, Cheno-Amaranthaceae, Cyperaceae, *Areca*, etc.) were considered for comparative analyses. The tree pollen count showed more or less good correlation with increasing heights, whereas herb/shrub members are dominant at lower heights during all the three seasons (winter, summer and rains). The 10 major and perennial fungal spore types included Aspergilli group, *Cladosporium*, *Nigrospora*, 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. In terms of allergenicity, *Saccharum officinarum* (sugar cane) of Poaceae, showed highest reactivity (70.58%) in skin test carried out in 189 adult agricultural field workers with respiratory disorders living inside the study area. Among fungal spores, *Aspergillus japonicus* was the strongest allergen, evoking 74.07% positive reactions. *Drechslera oryzae*, the pathogen causing brown spot of rice was also found to be a potent allergen.

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Key words: aeroallergens, pollen, fungi, vertical profile, agricultural workers, occupational exposure, skin test.

INTRODUCTION

Many airborne pollen grains and fungal spores are important biopollutants responsible for human respiratory allergy. Generally, the seasonal and diurnal periodicities of aeropollen and spores are monitored by volumetric traps [2, 8] to prepare pollen/spore calendars. The dispersal of airborne fungal spores at Indian agricultural farms was volumetrically studied in crop plants [26] in relation to their pathogenic potential. A similar study on pollen grains [7] was conducted with reference to human respiratory allergy.

The main objective of the present study was to determine the concentration of dominant and perennial aeropollen and fungal spores at different heights of lower atmospheric level on an agricultural farm located in West Bengal, India. Besides the study of vertical profile, a clinical study was performed to explore the possible role

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of recorded pollen/spore types in causing respiratory allergic disorders in agricultural workers.

MATERIALS AND METHODS

Study design. In this survey, airborne pollen and spores were monitored up to 6 m height using six rotorod samplers at different levels at regular intervals for two consecutive years, i.e., from November 1997 to October 1999. Among the recorded types, 10 dominant and perennial pollen and spore taxa were studied separately to understand the vertical profile pattern (i.e., variation of concentration with heights). A correlative analysis was performed between the frequency of pollen/spore and varying heights.

Skin-prick tests were performed in 189 agricultural workers with respiratory disorders from the study region using whole pollen/spore extracts. In the case of stenopalynous (i.e., having no morphological distinction) groups of pollen/spores of the dominant representatives growing in the study area (e.g., *Oryza sativa* and *Saccharum officinarum* from the Poaceae/grass family, *Aspergillus flavous*, *A. japonicus* from the Aspergilli group, and *Alternaria alternata* from *Alternaria* genus) were selected for skin tests.

Sampling procedure. The sampling was conducted using six rotorod [22] samplers mounted on a pole at heights of one, two, three, four, five and six meters [16, 17]. The rotation speed of brass-made arms fixed on a V 12 motor with 2,800 rpm, equivalent to sampling air rate 205.74 l/min. The rotor speed was proportional to supplied voltage, which varied less than 2% during experiments.

The monitoring was carried out every week in the morning (08.00–09.00 IST) and afternoon (15.00–16.00 IST) for one-hour duration from November 1997 to October 1999. The exposed rotorod tapes were mounted and counted microscopically and converted into number of pollen/spores per m^3 according to British Aerobiology Federation guidelines [3].

In the case of fungal spores, the identification of culturable moulds to a specific level was performed by exposure at sampling site for three minutes on Petri plates with malt extract agar (MEA 2%) containing streptomycin (40 µg/ml).

The pole was situated at the centre of a square (10 × 10 m^2) plot of rice crop (*Oryza sativa* L.) at Madhyamgram field station, about 19 km north of Central Calcutta. South of the plot, there was another adjacent plot (5 × 5 m^2) of the same crop. To the west, there was a banana (*Musa paradisiaca*) orchard, together with some trees of *Bombax ceiba* and *Trema orientalis*. To the east, at a distance of 50 m, there was a mango (*Mangifera indica*) orchard. The space between the mango orchard and rice field was an area of *Amaranthus viridis*, *Chenopodium album*, *Cyperus* sp., *Justicia simplex*, some grasses and *Parthenium hysterophorus*, including a plot where sugar cane (*Saccharum officinarum*) grew in abundance. At a distance of 15–20 m to the north, there was an area of vegetation consisting of plants such as *Carica papaya*, *Cocos nucifera*, *Areca catechu*, *Catharanthus roseus*, etc. In the intermediate periods (May-July and October-December respectively) of wet and dry seasons of rice crops, the rice field was devoid of any plants.

Statistical analyses. Correlation between the frequency of pollen/spores and varying heights was studied using MINITAB computer programme for statistical analysis [19]. The significance of correlation was studied by Student's t test for paired samples.

Demonstration of the allergenic potential of identified pollen and spores

Pollen grains of Poaceae, Acanthaceae, Asteraceae, Cyperaceae, Cheno-Amaranthaceae could be identified only to family level. The same is valid for fungal spores of Aspergilli, Ascospores and Basidiospores groups. Under these circumstances, the dominant species from each group (dominant plant species from local pollen-producing flora, and dominant fungal species isolated on malt agar plates exposed in the study area) were selected for skin prick testing.

Preparation of the extracts of pollen grains. The allergenic extracts were prepared [6] from bulk of collected pollen grains with 95% purity. The extraction was made with phosphate buffered saline (PBS, 0.1 M Na-phosphate containing 0.15 M NaCl, pH 7.2) by continuous stirring at 4°C for 16 h in 1:50 (w/v) ratio. After centrifugation at 12,500 g for 40 min, the clarified extracts were dialyzed (molecular cut-off 10,000) in PBS and passed through 0.22 µm millipore filter (Millipore Corp., USA).

Preparation of the extracts of fungal spores. After the exposure of malt agar plates in the study field, the fungal isolates were transferred to other suitable media (Czapek Dox for *Aspergillus* and *Fusarium* and potato dextrose agar for others) for identification up to specific level following Samson *et al.* [24] with some modifications.

The allergenic extracts were prepared from the dominant culturable moulds, except for *Ganoderma*, where the spores were collected from sporulating fruit bodies. The mass cultures of fungi were prepared in conical flasks with sterile Sabouraud broth medium supplemented with chloramphenicol (50 mg/l). Flasks were incubated at 27 ± 2°C for three-four weeks, until proper sporulation occurred. Afterwards, the medium was decanted and the sporulating mass (without mycelia) was rinsed with distilled water and kept overnight in absolute ethyl alcohol to kill viable fungi. The pellet was then dried, powdered in grinder and defatted with diethyl ether. The allergenic extracts were prepared with 0.05 M ammonium bicarbonate buffer (pH 8.1) in 1:50 (w/v)

Table 1. Correlation between frequency of pollen/spore types in air and varying heights.

Pollen types	r value	p level	Spore types	r value	p level
1. *Areca catechu*	0.794	<0.05	1. *Alternaria* sp.	-0.972	<0.01
	0.919	<0.01		-0.991	<0.001
	0.655	>0.10		-0.989	<0.001
2. Acanthaceae	-0.901	<0.02	2. Ascospores	0.905	<0.02
	0.831	<0.05		0.835	<0.05
	0.655	>0.10		-0.101	>0.10
3. Asteraceae	-0.831	<0.05	3. Aspergilli group	0.261	>0.10
	-0.869	<0.05		0.155	>0.10
	-0.655	>0.10		-0.501	>0.10
4. Cheno-Amaranthaceae	-0.064	>0.10	4. Basidiospores	0.750	<0.10
	-0.941	<0.01		0.872	<0.02
	-0.941	<0.01		-0.835	<0.05
5. *Carica papaya*	0.655	>0.10	5. *Cladosporium* sp.	0.880	<0.02
	0.777	<0.02		0.993	<0.001
	0.831	<0.05		0.621	>0.10
6. *Cocos nucifera*	0.683	>0.10	6. *Curvularia* sp.	-0.965	<0.01
	0.887	<0.02		-0.976	<0.001
	0.828	<0.02		-0.972	<0.01
7. Cyperaceae	NA	NA	7. *Drechslera* sp.	-0.926	<0.01
	-0.876	<0.05		-0.831	<0.05
	-0.655	>0.10		-0.870	<0.05
8. *Parthenium hysterophorus*	NA	NA	8. *Fusarium* sp.	-0.177	>0.10
	0.229	>0.10		-0.799	<0.10
	-0.131	>0.10		-0.919	<0.01
9. Poaceae	-0.362	>0.10	9. *Nigrospora* sp.	0.971	<0.01
	-0.939	<0.01		0.899	<0.02
	-0.831	<0.05		0.982	<0.001
10. *Trema orientalis*	0.927	<0.01	10. *Periconia* sp.	0.927	<0.01
	0.843	<0.01		0.843	<0.05
	0.935	<0.01		0.935	<0.01

The r and p values are for winter, summer and rainy season respectively from top. The significance of correlation (p value) was determined by Student's t test. NA = not applicable.

dilution for 24 h with continuous stirring at 4°C. The suspension was centrifuged at 12,500 g for 40 min at 4°C and the supernatant was thoroughly dialyzed against PBS for overnight. After centrifugation (12,500 g at 4°C for 40 min), it was finally passed through a millipore filter.

Both the pollen grain and fungal spore extracts were stored at -70°C in sterile vials until required for skin tests.

Examined population. For skin tests, 189 agricultural workers with respiratory disorders (104 males and 85 females, ranging within 18-65 years of age) were selected from Madhyamgram and adjacent areas. The case histories of their symptoms included sneezing, cough, breathlessness, repeated cold, pain chest and rhinitis in different combinations.

Skin-prick tests. Skin-prick tests were performed on the selected individuals using histamine diphosphate (1 mg/ml) and PBS as positive and negative controls respectively. Prior testing, protein content of the extracts was measured following the method of Lowry *et al*. [15]. The tests were performed with 20 µl of solution (containing 100 µg protein per 1 ml PBS) which was placed on the ventral side of the forearm and each site was pricked with a disposable hypodermic (No. 26) needle. The wheal responses were measured after 20 min and graded into negative (no wheal, no erythema), +1 (no wheal, erythema <20 mm in diameter), +2 (no wheal, erythema >20 mm in diameter) and +3 (wheal and erythema) following Stytis *et al*. [27].

RESULTS

A total of 46 types of pollen taxa were recorded in the air of the study area in the two-year study period (list not shown). Among them, 10 common and perennial types were selected for comparative study of the vertical profile during three major seasons in a broad aspect, i.e., winter (November–February), summer (March–June) and rainy season (July–October) (Fig. 1). The results were expressed in an average count/hour/cubic meter. All selected plant types were found to flower throughout the year. There were four tree species, namely *Areca catechu*, *Carica papaya*, *Cocos nucifera* and *Trema orientalis*.

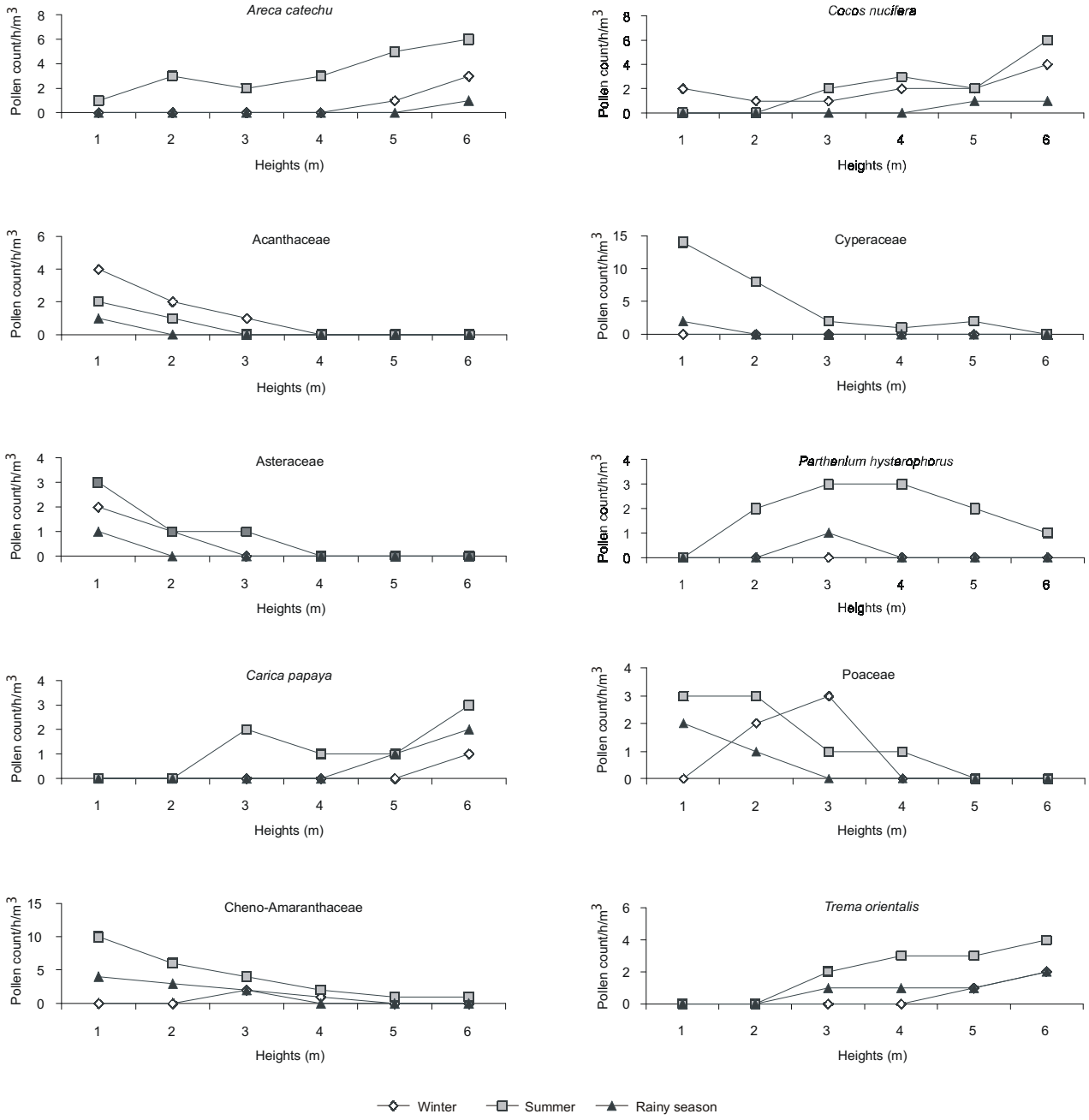

Figure 1. Vertical profile of 10 common and perennial airborne pollen types up to the height of 6 m from ground level during different seasons of the year.

These four pollen types showed significant ($p < 0.05$) positive correlation (Tab. 1) with increasing heights except *Areca* in rainy season and *Carica* and *Cocos* in winter. Among the other types, all are mainly herbaceous, except some members of Asteraceae with shrubby habit. Among them, Cyperaceae and *Parthenium* pollen was not present in air during winter and *Parthenium* showed no significant correlation (i.e., $p > 0.10$) with heights. For others, Acanthaceae showed negatively significant correlation in winter ($p < 0.02$), which was positively significant in summer and not significant ($p > 0.10$) in the rainy season. The rest of the members showed a general trend of negatively significant ($p < 0.05$) correlation with heights, except Asteraceae and Cyperaceae in rainy season, and Poaceae and Cheno-Amaranthaceae in winter. Poaceae showed the highest count at 1 m during summer and rainy season but at 3 m height during winter. For all tree members, the optimum concentration was recorded at 6 m height.

In the case of fungal spores also 10 dominant and perennial airborne types were selected for comparison out of 26 (list not shown) identified types (Fig. 2). The most dominant type was *Cladosporium*, followed by Aspergilli group, *Periconia* and *Nigrospora*. Among all 10 members,

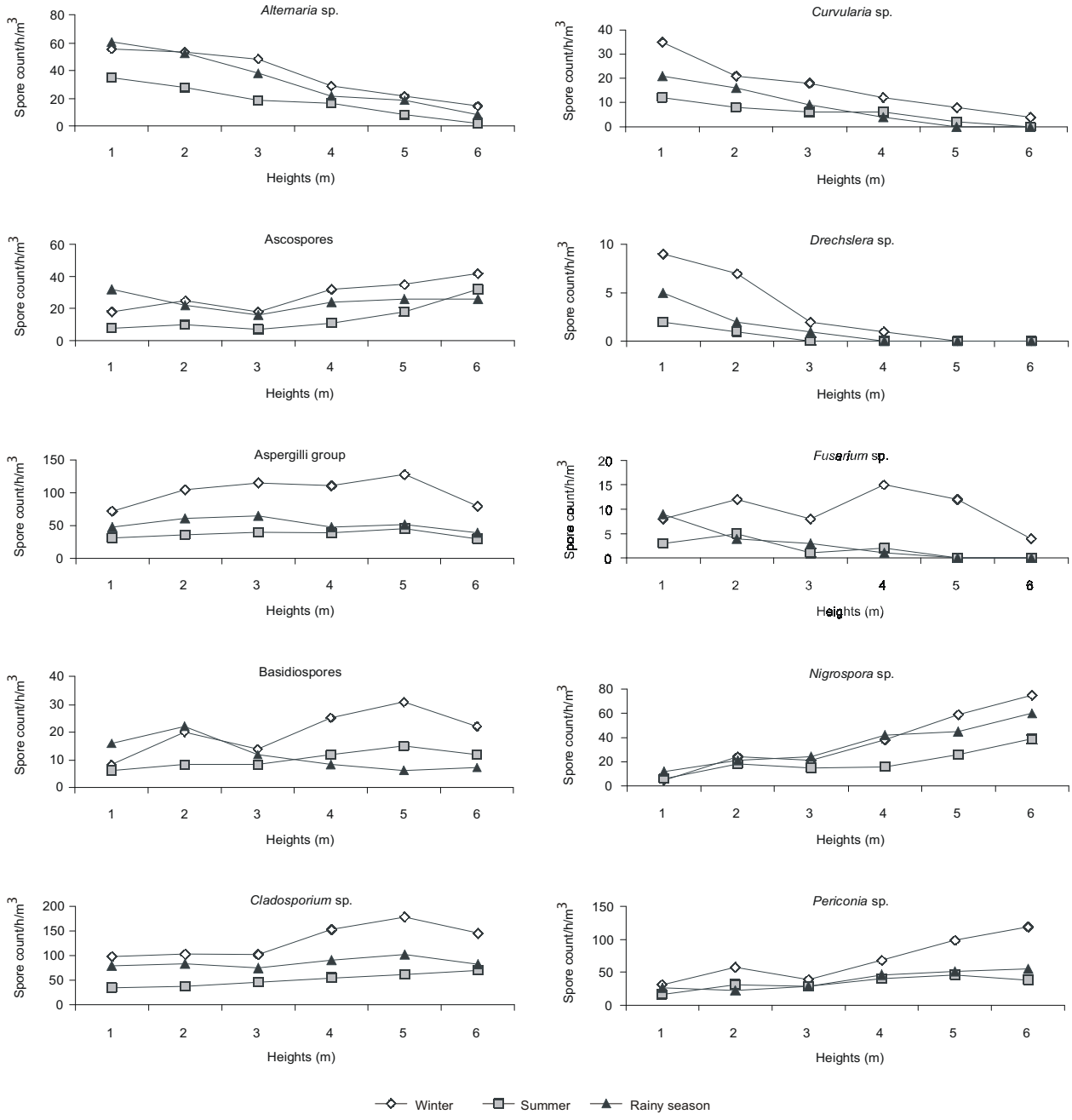

Figure 2. Vertical profile of 10 common and perennial airborne fungal spore types up to the height of 6 m from ground level during different seasons of the year.

Alternaria, Curvularia and Drechslera showed the highest concentration at the height of 1 m. For these three types, the spore count showed a significantly negative correlation ($p < 0.05$) with height (Tab. 1). *Fusarium* showed also negative correlation ($p > 0.10$, $p < 0.10$ and $p < 0.01$ in winter, summer and rainy season respectively) with increasing heights.

The correlation of frequency with height for other types, such as Ascospores (positively significant at <0.05 level in winter and summer and negatively correlated at >0.10 level in rainy season), Aspergilli group (weak positive correlation at >0.10 level in winter and summer and weak negative correlation in rainy season), *Cladosporium* (positive correlation at <0.02 level in winter and summer and >0.10 level in rainy season), *Nigrospora* and *Periconia* (with significant positive correlation at <0.05 level for all seasons), Basidiospores (positive correlation at <0.10 level in winter and summer, but significantly negative at <0.05 level in rainy season), showed a general trend of a positive nature except during the rainy season in some cases. Ascospores, *Nigrospora* and *Periconia* were more dominant at 6 m of height, whereas Aspergilli, Basidiospores and *Cladosporium* had the highest count at 5 m. In the case of *Fusarium*, the

Table 2. Results of skin-prick tests using different pollen extracts in agricultural workers with respiratory disorders.

Pollen extract	Number of examined persons	Percent of positive reactions	
		+1 Level	+2/+3 Level
1. Poaceae:			
Saccharum officinarum	102	50.00	20.58
Oryza sativa	113	21.23	4.43
2. Cyperaceae: *Cyperus rotundus*	100	44.00	18.00
3. Arecaceae: *Areca catechu*	110	40.00	10.90
4. Apocynaceae: *Catharanthus roseus*	108	37.03	9.25
5. Arecaceae: *Cocos nucifera*	107	36.44	8.41
6. Caricaceae: *Carica papaya*	100	28.00	8.00
7. Asteraceae: *Parthenium hysterophorus*	102	27.60	7.84
8. Cheno-Amaranthaceae			
Chenopodiaceae: *Chenopodium album*	105	16.19	3.80
Amaranthaceae: *Amaranthus viridis*	110	10.00	0
9. Ulmaceae: *Trema orientalis*	115	8.69	0
10. Acanthaceae: *Justicia simplex*	104	3.84	0

The pollen types studied in skin test are dominant representatives of stenopalynous families (Poaceae/Gramineae, Cyperaceae, Asteraceae and Cheno-amaranthaceae) in the adjoining study area.

optimum concentration was recorded at 4 m in winter, 2 m in summer and 1 m in the rainy season.

In the next part of this study, the allergenic potential of the selected pollen grains and fungal spores has been examined. In the case of pollen types, two members of Poaceae family were selected, namely sugar cane (*Saccharum officinarum*) and rice (*Oryza sativa*). The extract of *Saccharum* pollen evoked most positive reactions in skin-prick test (50% at +1 level and 20.58% at +2/+3 level) (Tab. 2). *Saccharum* was followed by *Cyperus rotundus* of Cyperaceae family (44% at +1 level and 18% at +2/+3 level), *Areca* and *Cocos* (Arecaceae), *Catharanthus* (Apocynaceae), *Carica* (Caricaceae), *Parthenium* (Asteraceae), *Chenopodium* (Chenopodiaceae), *Amaranthus* (Amaranthaceae), *Trema* (Ulmaceae) and *Justicia* (Acanthaceae).

In the case of fungal spores, *Aspergillus flavus, A. fumigatus* and *A. japonicus* of the Aspergilli group were identified as being the major types isolated from exposed malt agar plates and were therefore selected for the preparation of extracts for skin testing. Similarly, *Ganoderma* was selected from basidiospore producing group. In skin test, the most positive reactions were evoked by the extract of *Aspergillus japonicus*, followed by *A. fumigatus, Fusarium roseum, Aspergillus flavus, Drechslera oryzae, Periconia digitata, Curvularia lunata, Cladosporium cladosporioides, Ganoderma applanatum* and *Nigrospora oryzae* (Tab. 3). All these types were collected in bulk after identification of isolates recovered from malt agar plates exposed in the study area.

DISCUSSION

The symptoms of pollen allergy confirm a good correlation with the airborne pollen count [4]. However, the concentrations of pollen grains as well as fungal spores always vary at different heights of exposure. In this respect, the study of vertical profile, i.e., variation of concentration at different heights, is an important factor in aerobiological survey [10]. In this study, a detailed two-year survey was performed with rotorod samplers mounted at different heights. For certain limitations in the field, the height was restricted to 6 m and experiments carried out at weekly intervals. Hourly reading was performed in the morning and in the afternoon every week. It has to be admitted that rotorod samplers are not ideal for uninterrupted sampling, but under the prevailing condition these were found to be very useful for outdoor sampling with some constraints, e.g., power supply [9], so far as vertical profile is concerned. This sampler is non-directional and isokinetic at all wind speeds up to 64 km/h. If the wind speed is less than the linear velocity of the collector arms [5], the collection efficiency is supposed to be unaffected by wind turbulence.

The survey area was an agricultural farm in a suburban area with mixed vegetation including rice crop, sugar cane (*Saccharum*), mango and banana. The presence of banana and mango pollen was not recorded, probably due to their entomophilous nature.

The mast was situated in the midst of a rice crop plot to avoid the interruption caused by overhanging trees and

Table 3. Results of skin-prick tests with different fungal spore extracts in agricultural workers with respiratory disorders.

Fungal spore extract	Number of examined persons	Percent of positive reactions	
		+1 Level	+2/+3 Level
1. Aspergilli group:			
Aspergillus japonicus	108	50.92	23.15
A. fumigatus	112	46.43	18.75
A. flavus	112	48.21	16.96
2. *Fusarium* sp.: *Fusarium roseum*	102	53.92	17.65
3. *Drechslera* sp.*: Drechslera oryzae*	110	40.90	16.36
4. *Periconia* sp.*: Periconia digitata*	113	40.70	15.04
5. *Curvularia* sp.: *Curvularia lunata*	105	36.19	14.28
6. *Cladosporium* sp.: *Cladosporium cladosporioides*	100	51.00	12.00
7. *Alternaria* sp.*: Alternaria alternata*	101	47.52	11.88
8. Basidiospores: *Ganoderma applanatum*	100	36.00	11.00
9. *Nigrospora* sp.: *Nigrospora oryzae*	115	47.82	10.43

The species studied in skin test are the dominant representatives of each group recovered from malt agar plates exposed in the study area. As none representative of Ascomycetes could be mass-cultured in reliable form, this group was not studied in skin tests.

bushes around the location. The lower height was fixed at 1 m level above the rice crop canopy to minimize the biasing factors for pollen/spore count.

In general, up to the height of 6 m, the tree pollen grains were dominant at greater heights and herb members at a lower height (Fig. 1), which was probably related to the varying source heights [7]. A similar study on the vertical profile of grass pollen concentration was conducted by Rapiejko *et al.* [23] with a different method. These authors measured the concentration of pollen from 1–200 m of height from ground level and found the optimum pollen count at a lower level. This is corroborating the present finding, though restricted to lower heights.

In terms of allergenic potential (Tab. 2), *Saccharum* pollen, a Poaceae member, found to be the most significant allergenic type, followed by others. *Trema* and *Justicia* pollens were allergenically insignificant, though they were common in the air of the study area. This result is very similar to previous studies conducted in the same place [7]. It is also similar to the findings from other countries, e.g. like Poland [20], where the most frequent cause of pollen allergy in the south-eastern part of the country are pollen grains of grasses from the family Poaceae (Gramineae).

In the case of fungal spores (Fig. 2), it is very clear that the multicellular large-sized spores/conidia (*Alternaria, Curvularia, Drechslera*, etc.), were most common at lower heights. The only exception is *Fusarium* (four-celled) in winter with optimal count at 4 m level. This is probably due to the low buoyancy of large-sized spores/conidia [10]. The smaller sized spores, though all are soil-borne or growing on the crop canopy, showed a general trend of abundance at greater heights due to the easy way of dispersal of smaller spores at a higher level. Their frequency was positively correlated with heights, except for Ascospores and Basidiospores during the rainy season. This may be due to precipitation, causing their lower level dominance. Almost all the spores showed higher concentration during winter (except for *Alternaria* spores which were abundant in the rainy season), probably due to the fact that low humidity and high wind speed increase the number of released spores [14]. Generally, the rainy season is ideal for growth of fungal spores, but continuous rain removes them from the air. The spore number rises soon after the rain stops [11]. After drying out, in winter - just after the harvesting period of wet season crop of rice - the spores disperse in the air in high numbers. In summer, the spore number is low, probably due to low humidity and high temperature which is not sufficient to allow fungal growth at higher levels. This conjecture requires a detailed analysis of correlation with meteorological parameters.

In the study of allergenic potency assessed by skin-prick test (Tab. 3), the Aspergilli members occupied the first two and fourth positions while *Drechslera* was the fifth, causing 40.90% +1 level and 16.36% +2/+3 level reactions. This pathogenic fungus causing brown spot of rice [18] was thus found to be potentially allergenic. Side by side, other dominant types, such as *Nigrospora, Cladosporium, Periconia*, etc. were also found to be important allergens. Fungal spores evoked higher percentages of positive skin reactions in examined individuals compared to most of the pollen grains. Aspergilli are important as causative agents of aspergillosis and producers of aflatoxins [25], whereas

Fusarium graminearum may produce zearalenone [21]. Another spore type, *Alternaria alternata* is a predominant species and the third most important allergen in many places in the USA [1]. *Ganoderma applanatum* [13] has been found to be a common allergen in Europe.

It was reported that the allergy and asthma in farmers is more or less similar to that among the general population [12]. The results described here are expected to provide some basic information on the exposure to airborne allergens of agriculture workers from a tropical zone.

CONCLUSIONS

1. The analysis of vertical profile of airborne pollen/spores in West Bengal, India, has shown that the source height is the general determining factor for the abundance of pollen grains at a particular height, whereas the abundance of fungal spores is determined by size/shape and diameter of the spore.
2. The population of agricultural workers in India showed a remarkable sensitivity to certain pollen/spores prevalent in the air of the study area.

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