

Aerobiology, allergenicity and biochemistry of three pollen types in Berhampore town of West Bengal, India

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Abstract

An aerobiological survey was performed in Berhampore town of West Bengal, India, to know the frequency of three common airborne pollen, namely *Acacia auriculiformis*, *Eucalyptus citriodora* and *Madhuca indica* using an ASTIR one day volumetric sampler. *Acacia* pollen showed its peak concentration in September, followed by *Madhuca* in April, while *Eucalyptus* showed its two peaks between September–October and January–April. Meteorological factors like temperature, RH, rainfall played an important role in release and dispersal of pollen. Skin prick tests with the antigenic extracts of the three pollen types, showed their allergenic potentialities. The highest markedly positive reactions were exhibited by *Eucalyptus* (34.04%), followed by *Madhuca* (22.93%) and *Acacia* (21.87%). 30–60% (NH₄)₂ SO₄ cut fraction (Fraction II) of each pollen type showed maximum positivity in skin prick test. Biochemical analysis showed that *Acacia* pollen was richer in protein and carbohydrate, than the other two types. The total protein component of the above types were studied by SDS-PAGE showing different protein bands with a range of molecular weight 29–110 KD. In isolated fraction II (allergenically most potent) of *Eucalyptus* and *Madhuca* different protein band of 43–200 KD were obtained, while a single protein band of 57 KD was obtained for *Acacia*. The IgE specific allergenic reactivity was confirmed by Dot-blotting technique.

1. Introduction

There are noticeable variations in pollen allergic symptoms in relation to place and climatic conditions. In India allergenically potent pollen producing plants are fairly common. Aerobiological survey in relation to pollen allergy has been confined to only some parts of India, thus providing an inadequate image considering the largeness of our country and diversity of plant assemblages and ecotype. In India more than 10% of the population is estimated to suffer from major allergic or upper tract respiratory diseases. Considering the above facts the present investigation has been undertaken to know the seasonal distribution of three common allergenic pollen grains namely, *Acacia auriculiformis* (**Akashmoni**), *Eucalyptus citriodora* (**Eucalyptus**) and *Madhuca indica* (**Mahua**) in the air of Berhampore town, a centrally located

representative part of West Bengal, about 200 Km North-East of Calcutta. Following aerobiological and clinical studies regarding allergenic potential, comparative biochemical analyses of these types were also done to know the amount of carbohydrate, lipid and soluble protein. The protein profile was studied by SDS Poly Acrylamide Gel Electrophoresis.

2. Materials and methods

The incidence of airborne pollen grains in Berhampore town was carried out for two years (May 1995–April 1997) using an ASTIR one day volumetric sampler (Astir India Co. Ltd., New Delhi), placed at a height of 4m above ground level. The sampler (suction rate 10 l/min.) was run for 10 minutes twice in a day between 7 to 8 hr. and 18 to 19 hr. at four days

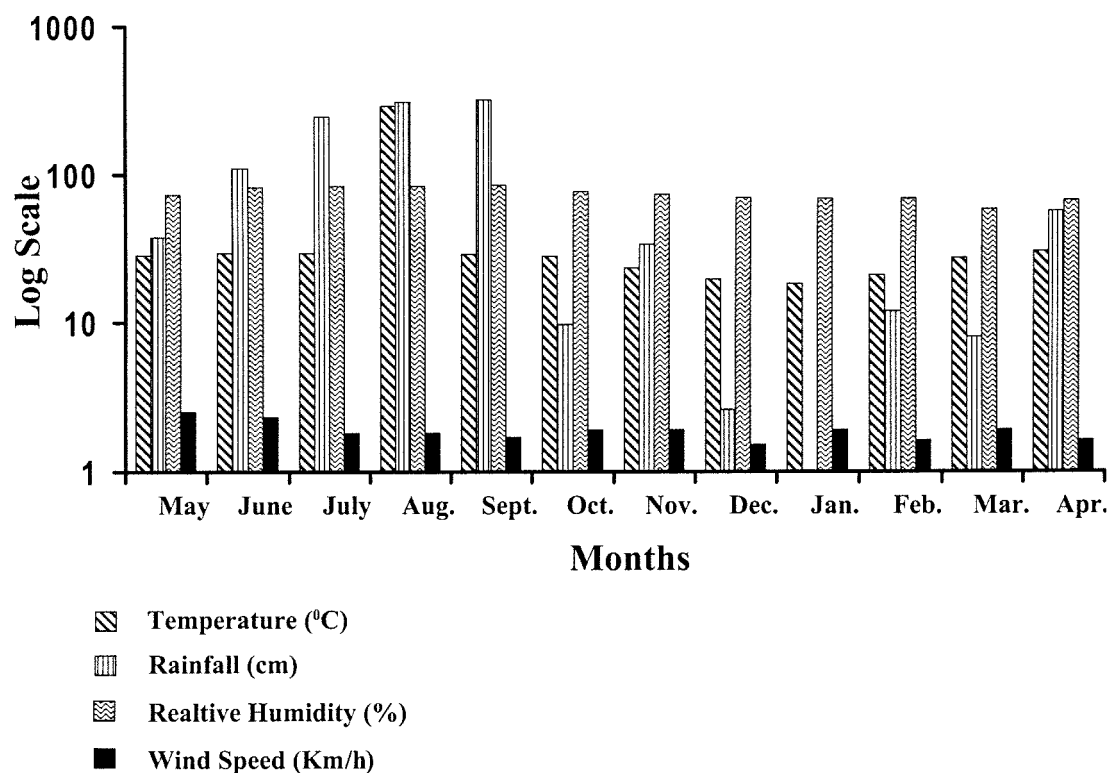


Figure 1. Meteorological parameters of Berhampore Town (1995–1997).

interval. The main type of vegetation around the trap covers plants, which mainly are tropically rain-fed, coming from the avenue trees, gardens and city parks. The meteorological data of Berhampore town was collected from the Pulses and Oil Seeds Research Station, Berhampore, which is about 1 km. far from the sampling site.

To test allergenicity, allergenic extracts were prepared from bulk uncontaminated pollen grains following the method of Shivpuri (1962), Sheldon et al. (1967) and Gupta and Chanda (1991). The extract was diluted in 1:50 ratio in phosphate buffer (pH 7.5) and sterility was checked before skin prick test on adult respiratory allergic patients at the Allergy Unit of the Institute of Child Health, Calcutta. A control test was performed with sterile buffer saline. The results were analysed according to Stytis et al. (1982).

Total soluble carbohydrate content was estimated according to the method of Duboise et al. (1956). Total lipid was estimated using the method of Itoh and Kaneko (1974). Total soluble protein was measured following the method of Lowry et al. (1951).

After biochemical analysis the whole extracts of each pollen type was fractionated in the range of 0–30%, 30–60% and 60–90% saturation of ammonium sulphate. These fractions were referred to as FrI, FrII and FrIII respectively. The allergenic activities of the fractions were determined by skin prick test.

Finally 11 and 15% SDS-PAGE were performed for total soluble protein, according to the method of Laemmli (1970). The extraction of soluble protein was made in sample buffer (0.5 M Tris HCl, pH 6.8; 2% SDS; 10% glycerol, 5% β -mercaptoethanol; 0.1% bromophenol blue dye) heating for $2\frac{1}{2}$ minutes at 100°C before loading. The amount of protein was 40 μ g per channel and the total current was 14mA. The gel run was done in Tris glycine buffer (pH 8.3) with 0.1% SDS. The gel was calibrated with a marker protein obtained from Genei Pvt. Ltd. Bangalore. 11% SDS-PAGE was also carried out in case of the most active $(\text{NH}_4)_2\text{SO}_4$ cut fraction (FrII) of each pollen type.

IgE specific Dot blotting of the three pollen allergens were performed with sensitive patients' sera

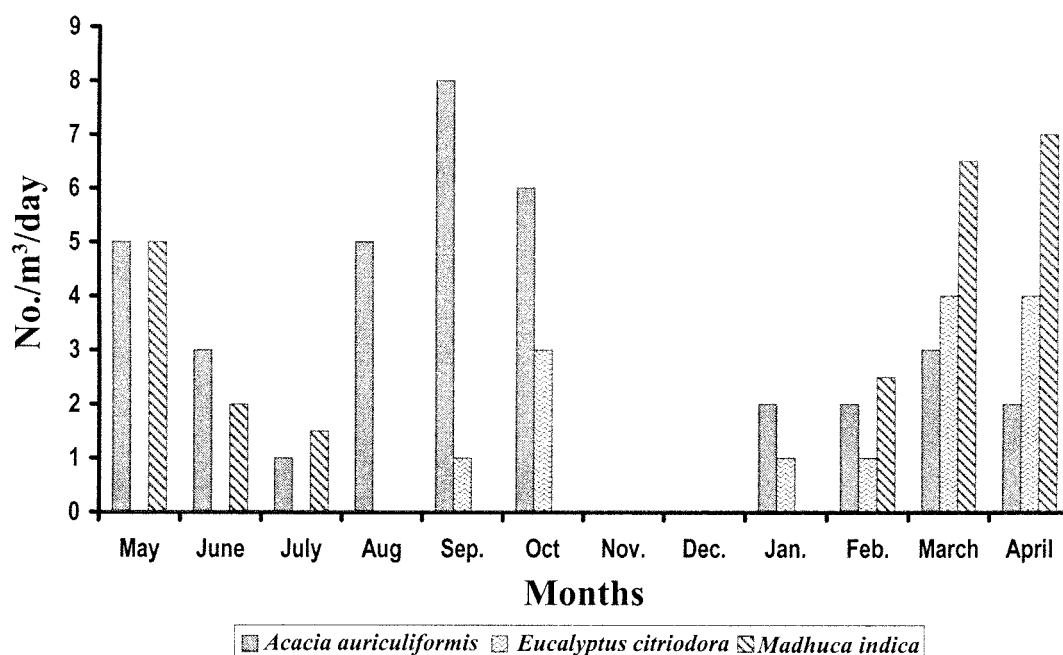


Figure 2. Average monthly concentration of three pollen types (1995–1997).

Table 1. Result of skin prick tests using whole pollen extract

Pollen type	Total No. of patients	No. of patients showing			% of +ve response
		+1 level response	+2 level response	+3 level response	
<i>Acacia auriculiformis</i>	96	18	2	1	21.87
<i>Eucalyptus citriodora</i>	235	67	11	2	34.04
<i>Madhuca indica</i>	109	22	3	—	22.93

following the method of Singh and Knox (1985) to observe the IgE specific cross reactions.

3. Results and discussion

3.1. Aerobiological studies

Aerobiological survey was carried out to record the occurrence and frequency of the pollen types with reference to meteorological parameters. The concentration of pollen grains was expressed as P/m³/day as a monthly average for each case (Figure 2). *Acacia auriculiformis* pollen was present almost the year all round with a maximum frequency between September and October, while the count came to nil between November and December (Figure 2).

The incidence of *Eucalyptus citriodora* pollen was recorded in September–October and January–April, while *Madhuca indica* pollen was found from the last week of February to July (Boral et al., 1999). Meteorological parameters (Figure 1) like temperature, rainfall, relative humidity and wind speed are responsible for fluctuations in pollen concentration (Andersen, 1980; Bricchi et al., 1992; Hjelmroos, 1992). From India, Agashe and Alfadil (1989), Banik and Chanda (1990) and Boral et al. (1999) have correlated atmosphere biopollutants with meteorological parameters. As in any other part of the world, including tropical parts of India, the composition of airspora changes quantitatively and qualitatively with the season (Hirst, 1953; Gregory, 1961). The maximum concentration of *Madhuca* pollen was noted between March and May (Boral et al. 1999) due to little rain (5–8 cm),

Table 2. Results Of skin prick test using fractions of the three pollen antigen

Pollen type	(NH ₄) ₂ SO ₄ cut fractions	Total No. of patients	% Positive response
<i>Acacia auriculiformis</i>	AF ₁ (0–30%)	12	8.33
	AF ₂ (30–60%)	12	83.33
	AF ₃ (60–90%)	12	66.66
<i>Eucalyptus citriodora</i>	EF ₁ (0–30%)	17	17.65
	EF ₂ (30–60%)	17	88.23
	EF ₃ (60–90%)	17	35.29
<i>Madhuca indica</i>	MF ₁ (0–30%)	21	19.04
	MF ₂ (30–60%)	21	66.66
	MF ₃ (60–90%)	21	47.61

Table 3. Chemical analyses of the three pollen grains

Pollen type	Total soluble carbohydrate (%)	Total lipid (%)	Total soluble protein (%)
<i>Acacia auriculiformis</i>	3.2	19	25.2
<i>Eucalyptus citriodora</i>	1.75	25	7.8
<i>Madhuca indica</i>	2.7	24	7.7

moderately low relative humidity (60%) and relatively high temperature (35 °C). For *Madhuca*, this was the peak flowering period. *Eucalyptus* flower twice and their air concentrations more or less coincide with their flowering period (Figure 2). *Acacia* flower the year all round with two peaks, and their absence during November and December is perhaps due to an abrupt decrease of rainfall and temperature with comparatively low RH (Figure 1).

3.2. Skin prick test

From the skin prick test with the whole pollen extract, the maximum allergenic sensitivity was noted in *Eucalyptus citriodora* (34.04%) with a relatively large number of +2 and +3 level response (Table 1). In *Acacia auriculiformis* (21.87% positivity) the response was also observed up to +2 and +3 level. While the response (22.93% positivity) was restricted to +1 and +2 level for *Madhuca indica* (Boral et al., 1999). When the skin prick test was performed with the three (NH₄)₂SO₄ cut fractions (i.e. FrI, FrII and FrIII) of each pollen type on sensitive patients, FrII elicited maximum percentage (83.33% in *Acacia*, 88.23% in *Eucalyptus* and 66.66% in *Madhuca*) of

positive allergic reactions compared to FrI and FrIII (Table 2). The result of *Madhuca* is found to be consistent with the earlier work (Boral et al., 1999)

3.3. Chemical analysis

The preliminary chemical studies of these pollen types (Table 3) revealed that the percentage of carbohydrate was moderately high in *Acacia auriculiformis* and *Madhuca indica* (Boral et al., 1999). The amount of total soluble protein was found to be high in *Acacia auriculiformis* (25.2%), while the percentage of lipid in all the species was moderate (19–25%).

The results of 11% SDS-PAGE and 15% SDS-PAGE (in case of *Acacia auriculiformis*) of total soluble protein demonstrate a number of bands in all the cases (Figure 3). The number of bands was 4 in *Acacia auriculiformis*, 16 in *Eucalyptus citriodora* and 6 in *Madhuca indica* (Boral et al., 1999) ranging from 29 KD to 125 KD molecular weight (Figure 3). 11% SDS-PAGE of the most active fraction of *Eucalyptus* (EF₂) revealed a total of 6 major protein bands showing a range of molecular weight between 54–136 KD (Figure 4). The most active fraction (MF₂) of *Madhuca* showed 7 major protein components

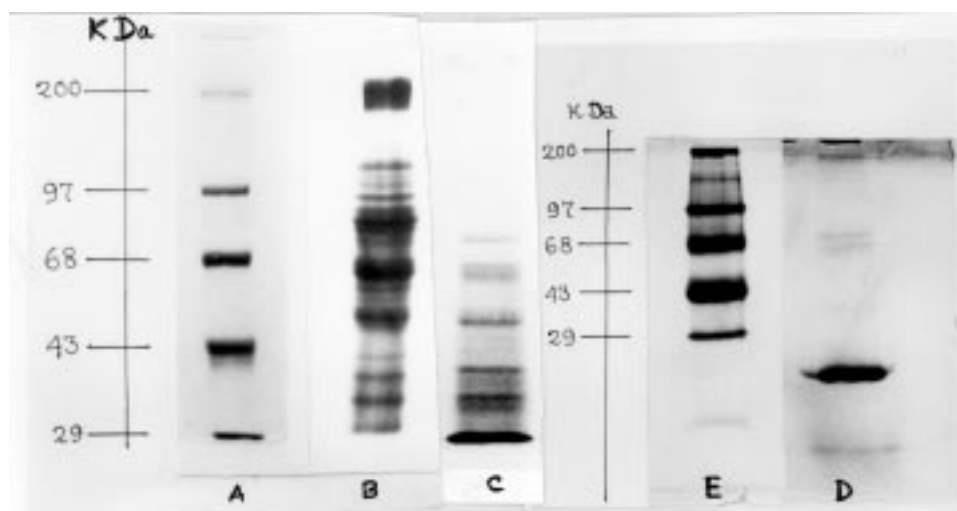


Figure 3. SDS-PAGE of total soluble protein of three pollen grains.
A. & E. Molecular weight marker.
B. Extract of *Eucalyptus citriodora* pollen protein (11% SDS-PAGE).
C. Extract of *Madhuca indica* pollen protein (11% SDS-PAGE).
D. Extract of *Acacia auriculiformis* pollen protein (15% SDS-PAGE).

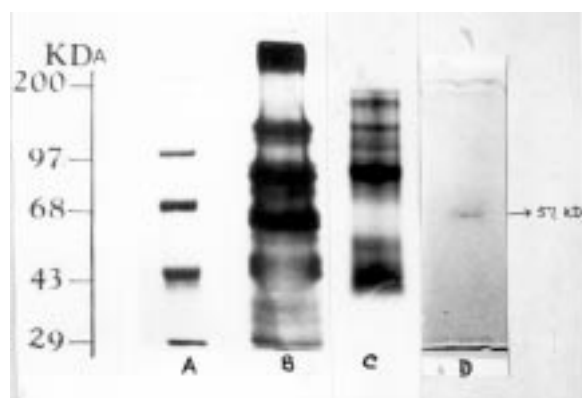


Figure 4. 11% SDS-PAGE of most active allergenic fraction (FrII) of three pollen.
A. Molecular weight marker.
B. Most active fraction (EF₂) of *Eucalyptus citriodora*.
C. Most active fraction (MF₂) of *Madhuca indica*.
D. Most active fraction (AF₂) of *Acacia auriculiformis*.

between the molecular weight range of 43–200 KD, while the most active fraction (AF₂) of *Acacia* showed a single protein band of 57 KD (Figure 4).

3.4. Dot blotting

The results of Dot blotting analysis, which is a simple and reliable immunochemical technique, confirmed the allergenic activity of the pollen antigen with the respective sensitive patients' sera. Here allergic

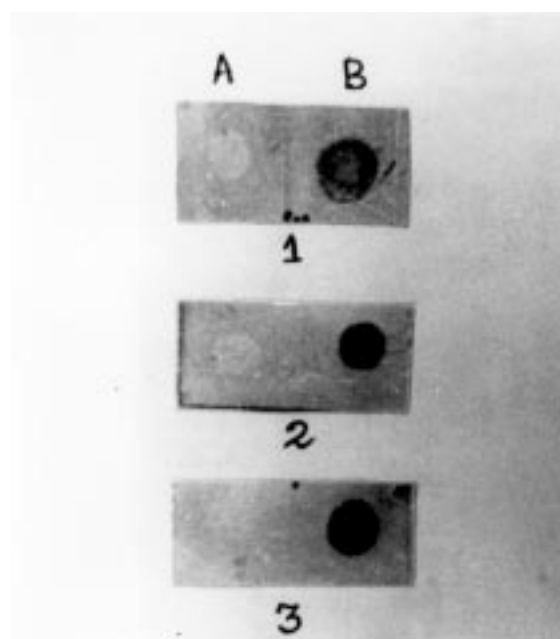


Figure 5. Allergenic relationship between pollen antigenic extract with allergic patient sera by IgE specific Dot Immunoblotting. The allergen spots are marked as: A = Negative spot, B = Positive Spot.

The pollen antigenic extracts are of

1. *Acacia auriculiformis*
2. *Eucalyptus citriodora*
3. *Madhuca indica*.

patients' sera gave specific spot with specific pollen antigen, while negative control sera did not show any spot (Figure 5).

Results of our study confirms that *Acacia auriculiformis*, *Eucalyptus citriodora* and *Madhuca indica* (Boral et al., 1999) are common airborne pollen grains in India causing allergic symptoms in susceptible patients. Among the three pollen grains *Eucalyptus* is found to be the most potent aeroallergen.

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