

Short communication

Comparative aerobiology, allergenicity and biochemistry of three palm pollen grains in Calcutta, India

Pampa Chakraborty, Swati Gupta-Bhattacharya, Sunirmal Chanda*

Division of Palynology and Environmental Biology, Department of Botany, Bose Institute, 93/1 Acharya Prafulla Chandra Road, Calcutta 700 009, India

Received 10 April 1995; revised 6 December 1995; accepted 5 February 1996

Abstract

A Burkard volumetric trap was used at Salt Lake City, Calcutta, to record the occurrence and frequency of three common palm pollen, namely, *Areca catechu*, *Borassus flabellifer* and *Phoenix sylvestris* for two consecutive years (July 1988–June 1990). The meteorological factors responsible for the frequency of relevant airborne pollen grains were analysed. The allergenic potential of these pollen types was investigated by skin-prick tests on adult respiratory allergic patients. These were also chemically analysed in terms of total carbohydrate, lipid and soluble protein. Total soluble protein of the above types was used in 11% SDS-polyacrylamide gel electrophoresis to study the range of molecular components.

Keywords: Aerobiology; Palm pollen grains; Allergy

1. Introduction

The contribution of airborne pollen grains has been studied from July 1988 to June 1990 at the eastern fringe of the metropolitan city of Calcutta, a newly developed township known as Salt Lake City, using a Burkard automatic volumetric sampler to record seasonal variations. From this record, a detailed study of the pollen grain from *Cocos nucifera*, a very common fruit palm, has already been discussed by Gupta et al. (1994).

Three more palm pollen types, originating from *Areca catechu* L. (Areca palm), *Borassus flabellifer* L. (Palmyra palm) and *Phoenix sylvestris* Roxb. (Date sugar palm) were found to be potent in causing respiratory allergy for susceptible individuals. Following aerobiological and clinical studies regarding allergenic potential, comparative chemical analyses of these types were done to study the total amount of carbohydrate, lipid and soluble protein. The total soluble protein was studied using 11% SDS polyacrylamide gel elec-

trophoresis. These three palms produce edible fruits, thus playing a significant role in the Indian economy. All three types are cultivated in many places and grown wild in Calcutta and also throughout the country.

2. Materials and methods

A 2-year recording of the incidence of relevant palm pollen grains in Salt Lake City was carried out using a Burkard volumetric sampler. Sampling height was 3 m above ground level.

Meteorological data were collected at Alipore Meteorological Centre, Calcutta, consisting of average temperature, wind velocity, rainfall and relative humidity.

The antigenic extracts were prepared from bulk uncontaminated pollen grains following the method of Shivpuri (1962), Sheldon et al. (1967) and Gupta and Chanda (1991). Strict sterility checking was made. The extract was diluted in 1:50 ratio in phosphate buffered saline (pH 7.2). The skin-prick tests were performed on adult respiratory allergic patients at the Allergy Unit of the Institute of Child Health, Calcutta (Table 1).

* Corresponding author.

Table 1

Results of skin-prick tests demonstrating the allergenic potential of the three palm pollen types

Pollen type	Total no. of patients	No. of the patients showing			% of +ve response
		+ 1 level response	+ 2 level response	+ 3 level response	
<i>Areca catechu</i>	593	115	3	0	19.89
<i>Borassus flabellifer</i>	500	106	9	0	21.20
<i>Phoenix sylvestris</i>	530	200	27	3	43.39

Table 2

Results of chemical analysis

Pollen type	Total carbohydrate (%)	Total lipid (%)	Total soluble protein (%)
<i>Areca catechu</i>	5.20	12.00	20.00
<i>Borassus flabellifer</i>	10.00	3.80	26.80
<i>Phoenix sylvestris</i>	17.88	12.70	11.00

The total carbohydrate content was estimated according to the method of Morris (1948). The samples were prepared following the method of Crook and Johnston (1962). Total lipid was estimated according to Itoh and Kaneko (1974) (Table 2).

Total protein was estimated following the method of Lowry et al. (1951) using bovine serum albumin standard curve (Table 2). Protein extraction was made at 4°C in Tris–HCl buffer, pH 7.6.

Finally, 11% SDS-polyacrylamide gel electrophoresis was performed for total soluble protein according to the method of Laemmli (1970). The extraction of the soluble protein was made in sample buffer (0.125 M Tris–HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β -mercaptoethanol, 0.1% bromophenol blue dye) with heating for 2 min at 100°C before loading. The amount of protein was 50 μ g per channel and the total current was 14 mA. The gel run was made in Tris–glycine buffer

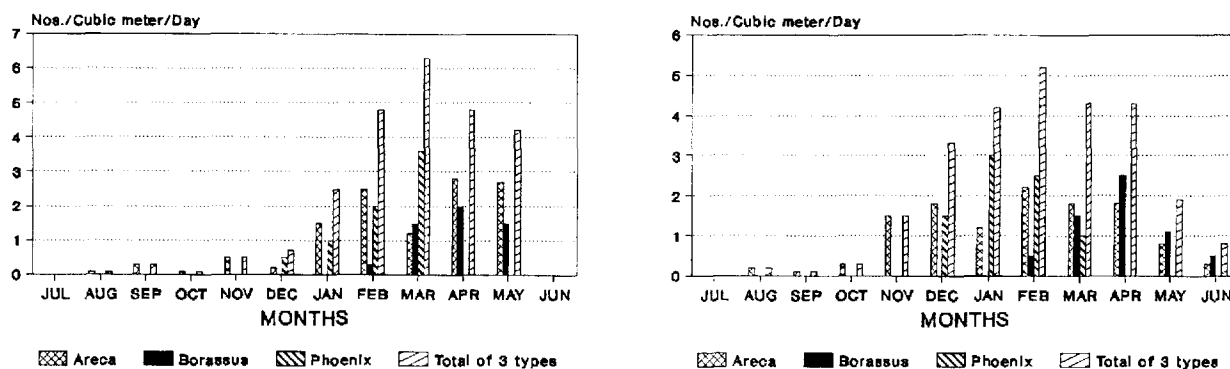


Fig. 1. (a) Average monthly concentration of three palm pollen (1988–1989). (b). Average monthly concentration of three palm pollen (1989–1990).

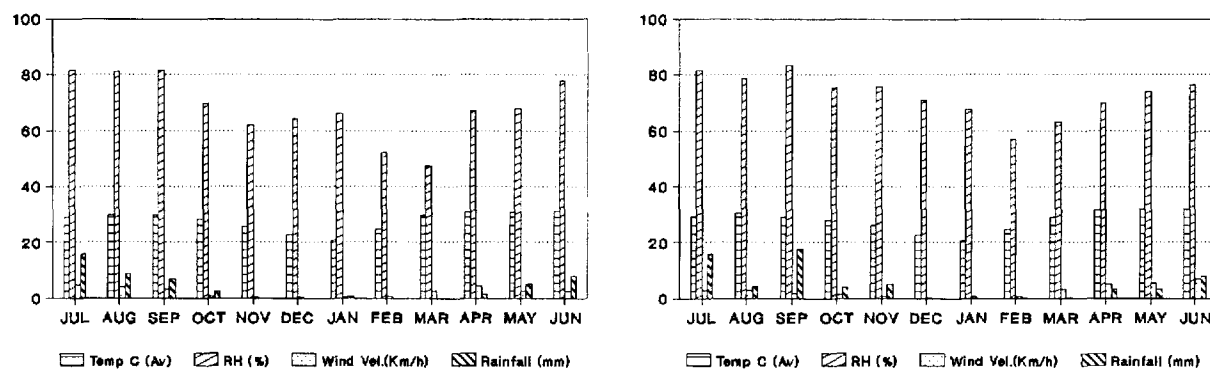


Fig. 2. (a) Meteorological parameters (1988–1989). (b) Meteorological parameters (1989–1990).

(pH 8.3) with 0.1% SDS. The gel was calibrated with a marker protein obtained from Sigma Chemical Co. (St. Louis, MO).

3. Results and discussion

3.1. Aerobiological studies

A 2-year survey (July 1988–June 1990) was carried out to record the occurrence and frequency (Fig. 1a,b) of the relevant palm pollen types with reference to meteorological parameters (Fig. 2a,b). The occurrence of pollen grains were expressed as No./m³/day as a monthly average for each case.

The presence of *Areca catechu* pollen was recorded frequently, but not in July 1988 and June–July 1989. The maximum frequency was found in April 1989 and February 1990.

The presence of the pollen of *Borassus flabellifer* was restricted to February–May 1989 and also June 1990. The maximum frequency was recorded in April 1989 and April 1990.

Pollen grains from *Phoenix sylvestris* were recorded from December to March for both 1988–89 and 1989–90 surveys. The highest frequency was noted in March 1989 and January 1990.

The period from February to March shows the presence of all three palm pollen types, which corroborates with the findings of Gupta et al. (1994), and demonstrates an optimum level in terms of the total count of relevant pollen types. This period is characterised by moderate temperature (24–29°C), low relative humidity (47–63%), low–moderate windspeed (0.6–3.4 km/h) and very low precipitation (trace–0.3 mm). For *Borassus* and *Phoenix* pollen, this is the flowering period. *Areca*, however, flower year-round, but showed no record in July 1988 and June–July 1989 probably due to heavy rainfall, high relative humidity and moderate–high windspeed. In general, the total count was also very low during June–September probably for the same reason. The count came to nil in July 1988 as well as in 1989.

3.2. Skin-prick test

While performing skin-prick tests, the maximum allergenic sensitivity was noted for the pollen of *Phoenix*, with a relatively large number of +2 and +3 level responses. The response was restricted to +1 and +2 levels for *Areca* and *Borassus*. The results were analysed according to Stylys et al. (1982).

3.3. Chemical analyses

The preliminary chemical studies on these pollen types revealed that the percentage of carbohydrate and lipid was highest in *Phoenix sylvestris*. The total amount of soluble protein, however, was optimum in the pollen of *Borassus flabellifer*.

The result of 11% SDS-PAGE analysis for soluble protein (Fig. 3) demonstrates a number of bands in all three cases. The highest limit was beyond 123 kDa except for *Phoenix*. In all cases the lowest limit was beyond 34 kDa. In *Areca* and *Phoenix*, the number of bands was 15–20, whereas the number was eight in *Borassus*.

The cumulative result of the study shows that the relevant pollen types occur significantly in the total airborne population in Salt Lake City, Calcutta, with the potential to elicit an allergenic response in susceptible patients.

Acknowledgements

We are thankful to the Director, Institute of Child Health, Calcutta and also Dr (Mrs) I. Roy, Dr (Mrs) S. Chatterjee and all staff members of the same Institute, for their kind help in performing clinical investigations.

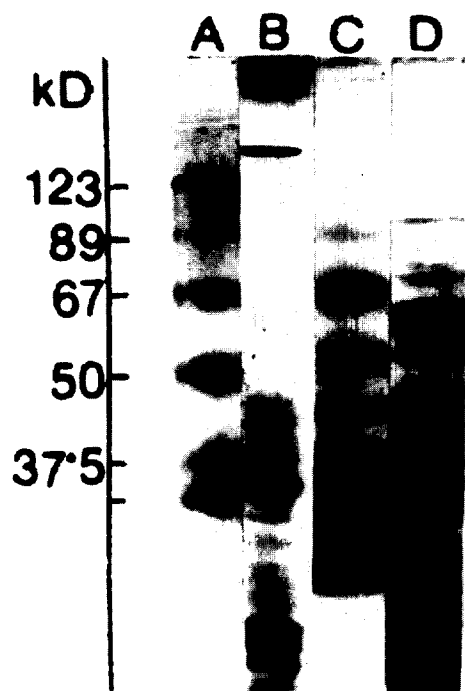


Fig. 3. 11% SDS-PAGE of total soluble protein of three palm pollen. A, molecular weight marker; B, *Areca catechu*; C, *Borassus flabellifer*; D, *Phoenix sylvestris*.

References

- Crook, E.M. and Johnston, I.R. (1962) The qualitative analysis of the cell walls of selected species of fungi. *Biochem. J.* 83, 325.
- Gupta, S. and Chanda, S. (1991) Aerobiology and some chemical parameters of *Parthenium hysterophorus* pollen. *Grana* 30, 497–503.
- Gupta, S., Bhattacharya, K. and Chanda, S. (1994) Pollen grains of *Cocos nucifera* L., a dominant aeroallergen from India. *Ann. Agric. Environ. Med.* 1, 28–32.
- Itoh, T. and Kaneko, H. (1974) Yeast lipids in species variation. I. Simple method for estimating the cellular lipids. *Yakugaku* 23, 350–354.
- Laemmli, U.K. (1970) Cleavage of structural protein during assembly of the head of the bacteriophage T₄. *Nature* 227, 680–684.
- Lowry, C.H., Rosenbrough, M.J., Farr, A.L. and Randall, R.I. (1951) Protein measurement with folin phenol reagent. *J. Biol. Chem.* 193, 256–275.
- Morris, D.L. (1948) The qualitative determination of carbohydrate with Drewood's anthrone reagent. *Science* 107, 301–302.
- Sheldon, J.M., Lovell, R.G. and Mathews, K.P. (Eds.) (1967) *A Manual of Clinical Allergy*. W.B. Saunders, Philadelphia, PA.
- Shivpuri, D.N. (1962) Comparative evaluation of the sensitivity of common methods of diagnostic antigen tests in the patients of respiratory allergy. *Indian J. Chest Dis.* 4, 102.
- Stylis, D.P., Stobo, J.D., Fudenberg, H. and Wells, J.V. (1982) *Basic and Clinical Immunology*, 4th edn. Lauge Medical Publication, Maruzen Asia (Pvt.) Ltd., 409 pp.