

Aerobiology, biodiversity and chemistry of plant trichomes in the tropics at Bodh Gaya, India – a biopollutant and the suspected human allergen

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Abstract

Aerobiology, diversity and chemistry of some air borne plant trichomes were studied at Magadh University campus, Bodh Gaya, India between September 1995 and August 1996. Thirty-three morphotypes of trichomes were tapped using a Gravity Air sampler. The different types of trichomes showed pronounced seasonality in their distribution pattern. The simple non-septate trichomes of grasses were predominant throughout the year. The other commonly observed trichomes were comparable to that of *Chrysanthemum*, *Tridax*, *Solanum*, *Amaranthus* and *Martynia*. Trichomes. The Shannon diversity index of trichomes fluctuated widely (1.063–2.867) during different months of the year. This suggests that seasonality had definite bearing on the composition of aerobiota. Relative humidity was found to play greater role in the case of a number of morphotypes (67%), diversity index (50%) and species richness (61%) but in the case of index of dominance (62%), rainfall played a greater role at $P < 0.01$ level. The trichomes of a few selected plant species, the pollen grains of which are reported allergens, were chemically analysed by collecting them in bulk. They were found to be fairly rich in their total protein (9.58 to 93.60 mg/g) and carbohydrate (10.0 to 20.20 mg/g) contents.

1. Introduction

Trichomes are minute epidermal outgrowth of plants, which are constantly present in the air (Sinha and Mishra, 1994) along with fungal spores and pollen-grains. It is suspected that the trichomes of plant pollens which are reported allergens may cause allergy in Human beings. This may be due to the presence of some specific chemicals contained in the parent plants. Positive results have been observed by Sahay (1998) in the case of grass trichomes. However, extensive aerobiological, biodiversity, biochemical and clinical investigations of trichomes is required before their allergenic nature can be established with certainty. Metcalfe (1963) and Metcalfe and Chalk (1965) observed wide morphological variation in the trichomes of ground flora. Ramayya (1972) studied the classification and phylogeny of trichomes of

angiosperms. Lavania (1990) recorded the morphological variations of trichomes of *Solanum* in India. Extensive aerobiological investigations of air trichomes were conducted by Sinha (1986), Sinha and Mishra (1994) and Mishra and Sinha (1988) with a view to exploring the incidence of some allergenic trichomes in the ambient air of Bodh Gaya. Chopra (1958) chemically analysed the stinging hairs of *Urtica dioca* and reported the presence of acetyl choline, histamine and 5-hydroxy-tryptamine (5-HT) which cause irritation and symptoms of urticaria. Kanchan and Jayachandra (1980) identified the presence of chemical inhibitors like parthenine, caffeic acid, vanillic acid and fumaric acid from the trichome leachates of *Parthenium hysterophorus*. During the study of ultrastructure, development and secretion of *Inula viscosa* hairs, Warker and Fahn (1981) reported that head cells of the trichomes secrete lipids, poly-

saccharides and proteins. Sahay (1998) chemically analysed grass trichomes and observed fairly large concentration of protein and carbohydrate with allergenic potential. However, detailed studies pertaining to aerobiology, diversity and chemistry and of airborne plant trichomes are meagre.

The present paper deals with the aerobiology, diversity and chemistry of some suspected allergenic plant trichomes of Bodh Gaya. This is aimed at exploring the etiological role of trichomes in causing allergy.

The main objectives of the study are:

1. To observe the range of morphological variations and seasonality in airborne plant trichomes, an important component of the aerospora.
2. To document the diversity index, index of dominance and species richness of the trichomes.
3. To analyse the chemical component of a selection of suspected allergenic trichomes of ground flora trapped from the air.

2. Methodology

(A) Survey of hairy ground flora and preparation of reference slides of trichomes

116 hairy ground flora plants growing in the nearby areas of Magadh University, Bodh Gaya were surveyed. As the prevalence of trichomes was reported in drier months (Sinha and Mishra, 1994) reference slides were prepared during these months. For some larger plants like *Moringa*, *Peltaphorum*, *Cassia* etc. the same were prepared throughout the year. The epidermal peels of hairy aerial parts of the plants (i.e. leaves, stems, flowers, fruits and seeds) were removed with the help of surface sterilized forceps and scissors. Such dissected peels were stained in alcoholic safranin and mounted in lactophenol. Every precaution was taken to avoid entrance of air bubbles inside the coverglass and the slides were sealed with quick fix. Camera lucida sketches were drawn and measurement of 3 largest and the smallest trichomes were taken into account to record the range of variations in length.

(B) Bulk collection of trichomes

Since trichomes are minute and tender appendages of plants, their bulk collection is very difficult and time consuming. They are variously located on different

aerial parts of the plants. Therefore, three separate methods were employed for different groups of plants to make their bulk collection possible.

(a) *Tridax procumbens*, *Echinops echinatus*, *Parthenium hysterophorus*

Hairy seeds were taken from the plants and the pappus was removed with the help of sterilized forceps. 5 grams of pappus was collected, dried at $30 \pm 1^\circ\text{C}$ in an incubator for 24 hours and stored in properly sealed sterilized polybags.

(b) *Calatropis procera*, *Cryptostegia grandiflora*, *Martynia diandra*, *Muconia sativa*, *Solanum elongata*, *Cicer arietinum*

Several plants were collected and kept for 48 hours at room temperature. Hairs were scraped with the help of sterilized scalpels. 5 grams of trichomes were collected, dried and stored in properly sealed sterilized polybags.

(c) Grasses i.e. *Cynodon dactylon*, *Oryza sativa*, *Saccharum munja*, *S. officinarum*, *S. spontaneum*, *Triticum vulgare*

Several inflorescences were collected and kept for 48 hours at room temperature. These were broken into small pieces and ground in mixer-cum-grinder at the rate of 1000 to 5000 rpm. The light hairy masses formed the uppermost lump followed by the pieces of rachis and seeds. Hairy masses were separated with the help of sieves. 5 grams of trichomes were collected and stored in sterilized polybags.

(C) Aerobiological trapping of trichomes of ground flora from the air and estimation of morphological and seasonal variations

Aerobiological trapping was done twice a week between September 1995 and August 1996 using a Gravity Air sampler. Trichomes settle under gravitation on the sticky slides exposed in the atmosphere and were counted. The number of trichomes trapped in a month is the mean average value of four observations of the month. Camera lucida sketches of trichomes were drawn and identified with the aid of literature and reference slides. Quantitative estimation was expressed in terms of number per cm^2 and percent occurrence. The calculations were made as mentioned below:

(a) Number per cm^2

Length of coverglass = 60 mm

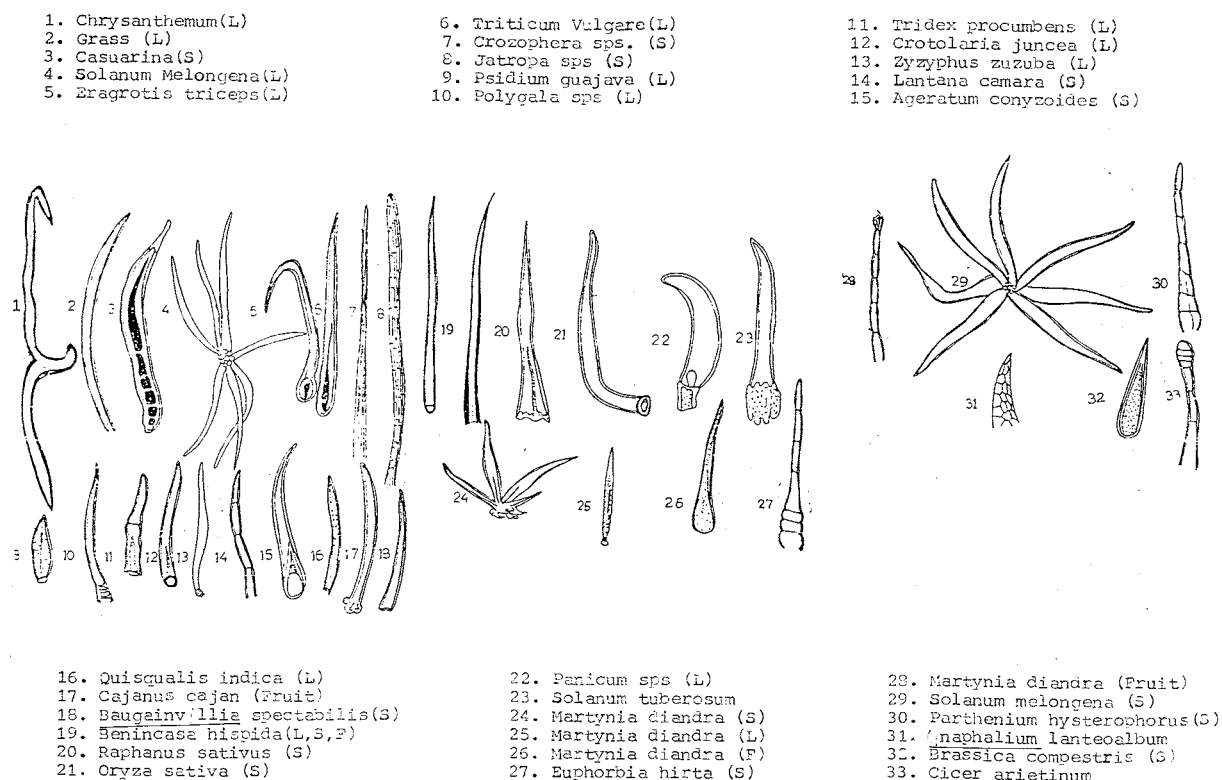


Figure 1. Morphotypes of plant trichomes.

Breadth of coverglass = 22 mm

Area covered by the cover glass = 60×22
= 1320 mm²
= 13.2 cm²

Therefore,

Number cm² =
Average number of trichomes trapped in a month
13.2

(b) Percent occurrence

Average number of trichomes in month
Average number of total aerobiota* in that month
 $\times 100$

*Aerobiota observed and included were fungal spores, trichomes, insect scales, insect legs, Algal filaments and protozoan cysts.

(D) Diversity indices

(a) Species diversity was calculated using the formula given by Shannon (Magurran, 1988).

$$H = \sum p_i \ln p_i$$

Where H = Shannon species diversity index

$$p_i = n_i/N$$

where n_i = No. of trichomes of a species

N = Total number of trichomes of all species

(b) Index of dominance was computed by $C_d = \sum (n_i/N)^2$

(c) Species richness was calculated by a variety index
(d). (Odum, Cantlon and Kornicker, 1960)

$$d = S - 1/\log N$$

Where S = Species Number.

(E) Chemical analysis of the trichomes

Carbohydrate content was estimated using the method of Morris (1948). Total soluble protein was analysed by Lowry et al. (1951) method using bovine serum

albumin standard curve. 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).

(F) Statistical analysis

Stepwise multiple regression equation was calculated using a computer programme package to assess the relative role of rainfall, relative humidity and temperature in determining no. of morphotypes, no. per cm² percent occurrence, species diversity, index of dominance and species richness of plant trichomes.

3. Results and discussion

Aerobiology

A perusal of plate (A) revealed that out of 33 morphotypes recorded, simple, non-septate trichomes of grasses were present throughout the year. This may be due to their abundance in the air as the vegetation around the experimental site was dominated by grasses. Other common trichomes observed were comparable to peltate trichomes of *Chrysanthemum*, uniseptate trichomes of *Tridax procumbens*, stellate trichomes of *Solanum melongena*, panicoid trichomes of *Panicum* species, serrate trichomes of *Amaranthus viridis* and glandular trichomes of *Martynia diandra*.

Seasonal variation of trichomes was very pronounced and meteorological factors appeared to be the main determinant. Higher values of trichomes morphotypes were obtained during summer months of March to June (16–26). It seems probable that plant parts along with trichomes were dried as a consequence of high temperature and relatively dry air. Such dried trichomes became lighter in weight and were easily dispersed into the ambient air due to trampling under foot by man and animal of the plant surfaces at various stages of their growth and development. They float in the air either individually or in agglomerated form depending upon their shapes, sizes, as well as meteorological conditions. However, decrease in temperature and relative humidity during the winter months of November to February (10–14) could not cause much decline in the trichome morphotypes. It might be due to low relative humidity alone with high wind velocity which resulted in the detachment of trichomes from the aerial parts of the plants and dispersed them into the ambient air but not to the

Table 1. Species diversity (H), Index of dominance (Cd), & species richness (d) of air-richomes

Months	H	Cd	d
September	1.486	0.291	27.84
October	2.141	0.239	37.84
November	1.777	0.252	52.63
December	1.533	0.190	55.80
January	2.131	0.184	56.22
February	1.820	0.122	56.05
March	2.364	0.111	71.49
April	2.867	0.148	75.00
May	2.561	0.079	102.61
June	2.614	0.108	79.67
July	1.918	0.347	20.18
August	1.063	0.411	13.56

extent as it was done during the summer season. By contrast, increase in relative humidity (80.33%) along with high rainfall (252.23 mm) during wet months of July to October might have been responsible for the decline in morphotypes (4–7). A positive correlation was observed between rainfall and trichomes quality ($p < 0.01$). Gregory (1973), Sinha (1986) and Sinha and Mishra (1994) opined that rain scrubbing seems to be an ideal method of deposition for air particulates.

Diversity

Table 1 indicates that the trichomes Shannon diversity index (H) values fluctuated widely (between 1.063 to 2.867) during different months of year. The maximum value was observed during summer months (2.36–2.87) while the minimum value was recorded during the rainy season (1.06–2.11). The peak value was witnessed in the month of April (2.87). On applying stepwise multiple regression equation (Table 2), it is clear that relative humidity explained 59% of the variation in Shannon diversity index at $p < 0.01$ level during different months. Rainfall and temperature did not play any significant role in the aforementioned variations. The values obtained during species richness also supported the data. The environment showed higher values of species richness (d) during the summer months (71.49–102.61) while the minimum value was observed during wet months (13.50–37.13). The peak value of the same was recorded in the month of May (102.61). These observations indicated that diversity and species richness are interdependent and directly proportional to each other. However, the

Table 2. Stepwise multiple regression equation relating rainfall (RF), relative humidity (RH) & temperature (T) with trichome morphotype, percent occurrence, no. per cm², diversity, species richness & dominance (The squared multiple correction co-efficient (R²) are also Shown)

Variables	Equation	R ²
No. of morphotypes	3.96 – 0.322 RH	0.67
Percent occurrence	no variable meet the criteria	
No. per cm ²	do	
Shannon diversity index (H)	3.38 – 0.121 RH	0.50
Species richness (S)	129.60 – 1.17 RH	0.61
Index of dominance	0.15 + 0.0001 RF	0.62

R² values are significant at P < 0.01 level.

Table 3. Chemistry of plant trichomes (mg/g)

S. No. Name of plant	Total protein	Total cabohydrate		
		Total sugar	Reducing sugar	Starch
1. <i>Calotropis procera</i>	24.25 ± 0.002	12 ± 0.001	8.0 ± 0.002	49 ± 0.001
2. <i>Cicer arietinum</i>	64.20 ± 0.005	12 ± 0.01	10.0 ± 0.01	46 ± 0.002
3. <i>Cryptostegia grandiflora</i>	43.75 ± 0.008	10 ± 0.009	8.0 ± 0.007	42 ± 0.001
4. <i>Cynodon dactylon</i>	52.40 ± 0.008	14 ± 0.003	8.0 ± 0.004	39 ± 0.002
5. <i>Echinops echinatus</i>	22.43 ± 0.005	12 ± 0.004	8.0 ± 0.009	54 ± 0.002
6. <i>Martynia diandra</i>	82.40 ± 0.002	10 ± 0.005	8.9 ± 0.005	41 ± 0.009
7. <i>Muconia sativa</i>	67.60 ± 0.003	14 ± 0.003	6.4 ± 0.006	44 ± 0.001
8. <i>Oryza sativa</i>	74.20 ± 0.008	12 ± 0.004	4.7 ± 0.004	17 ± 0.002
9. <i>Parthenium hysterophorus</i>	68.40 ± 0.001	16 ± 0.002	11.2 ± 0.01	41 ± 0.01
10. <i>Saccharum munja</i>	28.25 ± 0.001	20 ± 0.009	10.2 ± 0.009	42 ± 0.007
11. <i>S. officinarum</i>	29.75 ± 0.002	20 ± 0.009	10.2 ± 0.009	53 ± 0.001
12. <i>S. spontaneum</i>	93.60 ± 0.005	15 ± 0.003	4.5 ± 0.006	39 ± 0.001
13. <i>Solanum melongena</i>	57.90 ± 0.006	12 ± 0.003	10.0 ± 0.004	57 ± 0.001
14. <i>Tridax procumbens</i>	9.58 ± 0.001	16 ± 0.002	10.0 ± 0.002	59 ± 0.001
15. <i>Triticum vulgare</i>	84.20 ± 0.002	16 ± 0.003	52.0 ± 0.001	43 ± 0.001

positive relationship recorded above between species richness and Shannon diversity index is not true in all seasons especially during winters where the relation was opposite (Table 1). Here the relative humidity influenced 61% of variations at p < 0.01 level that occurs in species richness. Rainfall and temperature did not show any significant relation. Index of dominance (cd) of trichomes of different plant species varied widely (0.079 to 0.412) during different months of the year. Higher values were observed during rainy season where it varied between 0.291 (September) to 0.122 (February) to 0.252 (November). The minimum value was recorded between 0.079 (May) to 0.148 (April). From Table 1, it is evident that the index of dominance was mainly determined by rainfall where

it influenced 61% of the variations in the distribution of air-trichomes, at P < 0.01 level. Relative humidity and temperature did not play any significant role in determining their dominance in the atmosphere.

Chemistry

On studying the chemistry of selected plant trichomes (Table 3), it was observed that trichomes had a fairly rich proportion of total protein and carbohydrate contents. Protein content in grasses, namely *Cynodon dactylon*, *Oryza sativa*, *Saccharum munja*, *S. officinarum*, *S. spontaneum* and *Triticum vulgare*, varied from 29.75 mg/g to 93.60 mg/g. Leguminous plants such as *Cicer arietinum* (64.20 mg/g) and

Table 4. Seasonal variations of air-trichomes along with meteorological data from September' 95 to August' 96

Month	Meteorological data			Seasonal variations of air – trichomes			
	Temperature		RH	RF	No. of morphotypes	Percent occurrence	Number
	Max.	Min.	%				
September	32.67	25.25	082	182.6	6	38.30	1.742
October	31.74	21.10	075	95.25	7	45.10	2.121
November	27.55	12.66	064	00.00	10	38.58	2.280
December	24.29	9.09	069	14.95	12	24.00	2.037
January	20.26	7.00	077	14.95	13	22.00	1.899
February	26.14	11.55	060	29.30	14	20.00	1.758
March	34.24	16.91	037	00.00	16	24.00	1.830
April	39.45	23.59	033	00.35	23	34.00	1.515
May	42.36	26.35	046	00.05	26	34.00	1.946
June	37.23	27.65	066	189.30	19	30.00	1.973
July	33.52	25.45	084	320.01	7	16.00	0.995
August	32.41	26.13	081	417.35	4	10.00	1.041

Muconia sativa (67.60 mg/g) also showed high protein content. But in *Calotropis procera* (24.25 mg/g) and *Cryptostegia grandiflora* (43.75 mg/g), the members of Asclepiadaceae family, values were on the low side. A great deal of variation was also observed in the trichomes of plants belonging to asteraceae family. The trichomes of *Parthenium hysterophorus* showed fairly high protein content (68.40 mg/g) while *Tridax procumbens* had a low value of 9.58 mg/g. Further, the range of variation of starch in the trichomes of studied species was from 17 mg/g (*Oryza sativa*) to 57.0 mg/g (*Solanum melongena*). The amount of total sugar varied from 10 mg/g (*Cryptostegia grandiflora*) to 20.2 mg/g (*Saccharum munja*). Again the amount of reducing sugar was the least in *Saccharum spontaneum* (4.5 mg/g) and maximum in *Parthenium hysterophorus* (11.20 mg/g). Gupta et al. (1994) and Chakraborty et al. (1996) while studying the allergenic nature of pollen grains of various plants observed that pollens with an average of 13.3 mg/g of protein and 11.0 mg/g of carbohydrate behaved as allergens. Therefore, it may be expected that trichomes of tested plants with higher concentration of protein and carbohydrate may also be allergenic in nature. However, still there are reports where pollens with even more concentration of protein and carbohydrate are non allergenic in nature. Hence, before ascertaining the allergic nature of trichomes extensive clinical investigations are required to be conducted.

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