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Fomes pectinatis: an Aeroallergen in India

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SUMMARY **This work is focused on the aerobiology and allergenicity of *Fomes pectinatis* in India. The atmospheric concentration of *Fomes* basidiospores was recorded and the antigens were prepared from spore (FSE) and whole body (FWBE) materials. The intradermal (ID) and prick (PT) skin tests were conducted on 172 patients having respiratory allergy. The period from July to October has been recorded as having a higher concentration of *Fomes* spores. The maximum counts (67 spores/m^3) were observed from the North Delhi site in the month of July, 1989, compared with 550 spores/m^3 in the South Delhi site. Marked skin positivity (2+ and above) varied from 9.8% to FSE to 22% to FWBE. Nine out of twelve ID positive patients (2+ to 3+) to FSE also gave PT positive response. For FWBE, similar ID and PT response was obtained in 80% of cases. The soluble protein content of FSE was 0.37 mg/ml, whereas, for FWBE it was 0.70 mg/ml. It was observed through ELISA that almost all patients had significantly raised FP specific IgE levels in their sera. The current study, therefore, indicates that *Fomes pectinatis* may be a prevalent aeroallergen in India.**

Basidiomycetes are now established as aeroallergens in many parts of the world.[1] It was in the early seventies that a relationship between a high atmospheric concentration of basidiospores and high asthma admissions in hospitals was recorded in New Orleans.[2] Although common forms of Basidiomycetes such as bracket and polypore fungi, mushrooms and puffballs constitute about 8% of the total basidiomycetes,[3] the literature reveals only *Ganoderma* as prevalent bracket aeroallergens.[4-6] It became apparent that there was a need to study other members of this basidiomycete group. Though *Fomes* has been a suspected aeroallergen,[7] there is no report available on its airborne concentration and allergenicity. The lack of such information and the luxuriant growth of this polypore (*Fomes pectinatis*) along with *Ganoderma lucidum* during the rainy season in Delhi prompted us to find out their atmospheric concentration in Delhi and its role as an aeroallergen in India for the first time.

MATERIALS AND METHODS

Aerobiological survey

The concentration of *Fomes pectinatis* in the atmosphere of the Delhi metropolis was recorded for two consecutive years from October, 1989 to September, 1991, in five different localities as described in our earlier communication.[6] In brief, Burkard personal volumetric sampler was operated thrice a month at each site every tenth day for 10 minutes between 10.00-12.00 hour of the day. Exposed slides were scanned using a Zeiss Axioscope light microscope. Spore identification of *Fomes* was based on the shape, size and ornamental pattern of the spore, which is elliptic

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and transparent and brown in colour, and also from personal communication with Dr. Levetin, U.S.A. Spore counts were expressed as the number of spores per cubic meter of air sampled (spores/m^3).

Preparation of antigen

Collection of* Fomes *fruit bodies

Fomes pectinatis (FP) is a commonly occurring species in and around Delhi during the period July-October. The fruit bodies containing spores on their outer surface were collected from the field and their identification was confirmed from Literature[8-10] and personal communication (Dr. K.G. Mukerji, Department of Botany, Delhi University, India). The spores were manually scraped from the upper surface of fruit bodies after they shed spores and stored in the laboratory for 3-4 days at 37°C. Microscopic examination revealed 99% purity of the spore material which was used for antigen extraction. Fruit bodies with residual spores were dried at 37°C for 72 hours and crushed into smaller pieces and pulverized for antigen preparation.

Extraction of antigen

In brief, the spore and whole body materials were defatted in diethyl ether and extracted in 1:20 (w/v) concentration in 0.05 M ammonium bicarbonate buffer (pH 8.1) containing PMSF (phenylmethanesulphonylfluoride) and toluene as anti-protease and anti-bacterial agents, respectively. The extracts were centrifuged (13,500 x *g*) after continuous stirring for 16 hours in cold and supernatant was dialyzed using 7500 molecular weight cut-off membrane against distilled water. The dialyzed solution was centrifuged and filtered through 0.22 μm membrane filter in aseptic conditions. Aliquots were lyophilized and stored at -20°C. The spore and whole body antigens were designated as FSE and FWBE, respectively.

Protein estimation

The soluble protein of FSE and FWBE were precipitated using 15% PTA in hydrochloric acid (10% strength) and assayed by the method outlined by Lowry *et al.*[11]

Skin tests

The details of antigen preparation for skin tests have been explained in our earlier communication.[12] Briefly, FSE and FWBE were tested intradermally (ID) on 172 patients having respiratory allergy symptoms. A detailed medical and personal history was recorded before conducting these tests. Prick skin tests (PT) were also performed on 10 ID negative and 38 ID positive patients to confirm the allergenicity of both extracts. For prick test, lyophilized antigens were reconstituted in glycerinated phosphate buffer saline (1:1). Tests were performed and graded as per the method outlined by Singh *et al.*[13] (Table 1).

Fourteen asymptomatic individuals having no family history of allergy were also tested to act as control, and to rule out any non-specific skin reaction.

Serum

Blood samples were drawn from both skin test negative and positive cases. Expressed sera were cleared of cell debris and stored at -20°C until further use.

ELISA

Indirect ELISA conditions were standardized as in the case of *Ganoderma lucidum*.[12] Both extracts were coated onto 96 well NUNC ELISA plates. The bound antigen was probed with patients'

Table 1 Criteria for grading skin prick test reaction

Grade	Size of wheal (mm)	Remarks
Negative	Same as buffer	1. Patients either reacted to saline or did not react to saline
1+	2-3 mm	2. Allowance was made to size of wheal in recording the reaction
2+	4-5 mm	
3+	5-8 mm	3. Erythema was present, but grading was done based on wheal size
4+	> 8 mm with pseudopodia	

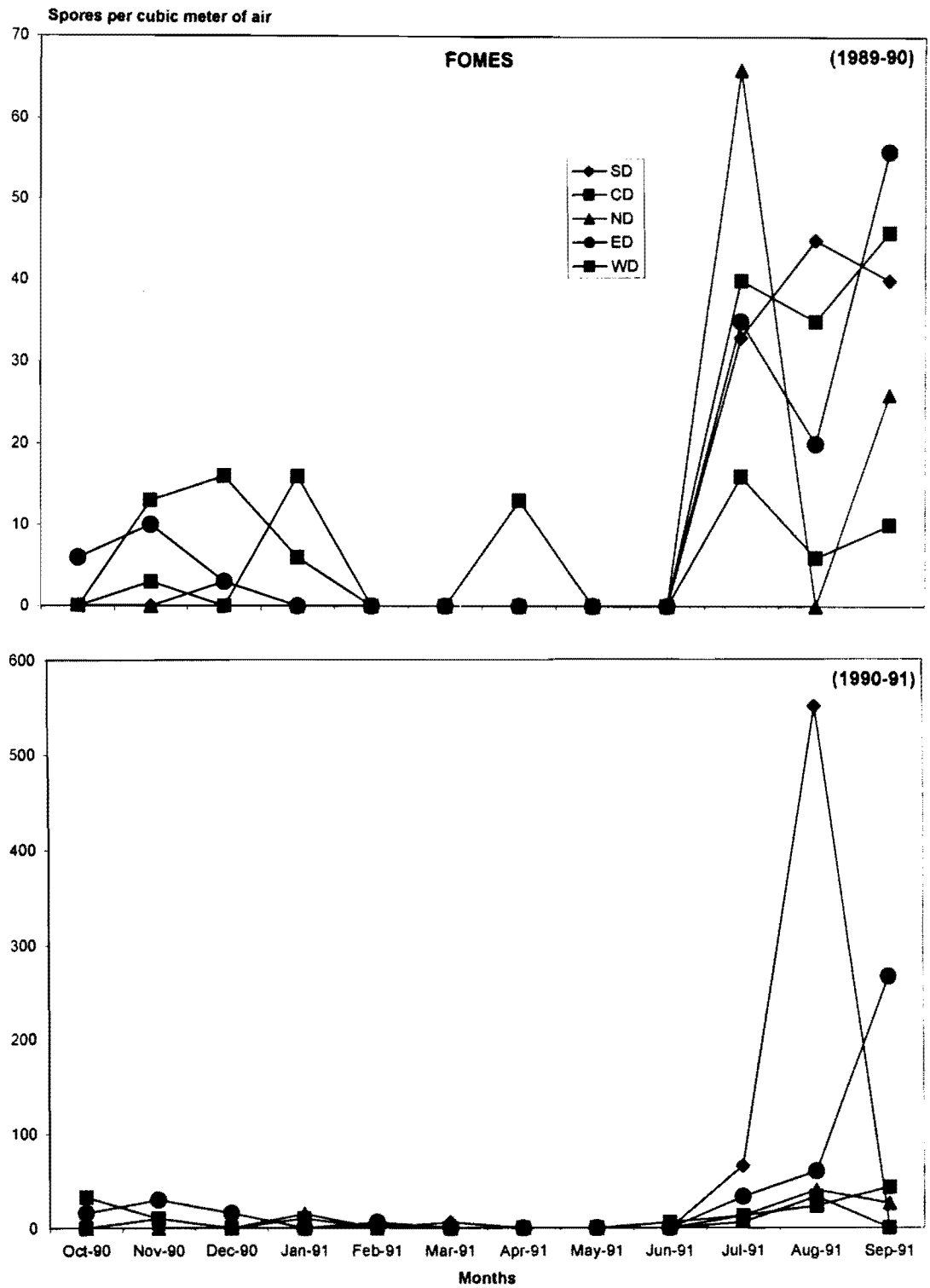

Fig. 1 Monthly spore concentration of *Fomes* at human height as recorded by Burkard personal slide sampler operated at five sites in Delhi metropolis; SD = South Delhi, CD = Central Delhi, ND = North Delhi, ED = East Delhi and WD = West Delhi.

serum followed by alkaline phosphatase labeled antihuman IgE. Antigen-serum IgE-anti IgE complex was then detected by adding PNPP substrate. Colour was allowed to develop and optical density (OD) was taken at 405 nm using NUNC immunoreader.

ELISA analysis

Two different approaches were followed to analyze the ELISA results,[12] however, in the current study the *in vitro* positivity results are based on the standard deviation method.[14]

RESULTS

Atmospheric prevalence

The concentration of *Fomes pectinatis* in different months at various sites during 1989-90 and 1990-91 are depicted in Fig. 1. The period from July to October recorded higher concentration of *Fomes* spores. The maximum counts (67 spores/m^3) were observed from the North Delhi site in the month of July, 1989. This peak shifted to the month of August with 550 spores/m^3 from the South Delhi site. May and June were free from *Fomes* basidiospores in both years at all the sites.

Patient symptoms and skin reactivity

Results of intradermal skin tests conducted with FP extracts are shown in Table 2. Markedly positive skin reactions (2+ and above)

Table 2 Results of intradermal skin test conducted with *Fomes pectinatis* extracts on 172 patients with respiratory allergy

Antigens		Skin positivity				
		1+	2+	3+	1+ to 3+	2+ to 3+
FSE	No.	33	17	0	50	17
	%	(19.18)	(9.88)	(0.00)	(29.06)	(9.88)
FWBE	No.	33	35	3	71	38
	%	(19.18)	(20.34)	(1.74)	(41.27)	(22.09)

Table 3 Prevalence of marked skin positivity (2+ and above) to *Fomes pectinatis* among patients having different clinical symptoms

Symptoms	Number of patients	2+ and above reaction	
		FSE	FWBE
Bronchial asthma	34	2 (5.9%)	7 (20.6%)
Allergic rhinitis	23	4 (17.4%)	6 (26.0%)
Asthma/rhinitis	115	11 (9.6%)	25 (21.7%)

varied from 9.8% to FSE to 22% to FWBE. The skin reactivity was variable according to the symptoms of patients; the results are given in Table 3.

The comparative results of ID and PT are given in Table 4. Nine out of twelve ID positive patients (2+ to 3+) to FSE also gave PT positive response. For FWBE, similar ID and PT responses were obtained in 80% of cases.

None of the control individuals reacted to either of the ex-

Table 4 Comparative results of prick test conducted on 48 patients with different degrees of intradermal reactivity

Skin response	FSE		FWBE	
	ID	PT	ID	PT
Negative	24	23	11	13
1+	12	16	16	12
2+	12	7	20	21
3+	0	2	1	2
1+ to 3+	24	25	37	35
2+ to 3+	12	9	21	23

Table 5 Patients showing identical and non-identical skin reactions to FSE and FWBE extracts

FSE	FWBE			
	Negative	1+	2+	3+
Negative	90	18	13	1
1+	9	12	11	0
2+	2	3	11	2

Total identical: negative 90/122 (73.77%); positive 23/50 (46.0%)
Total non-identical: negative 32/122 (26.22%); positive 27/50 (54.00%)

Table 6 Presence of specific IgE against *Fomes pectinatis* antigens as assessed by ELISA analysis

Antigen	Sera tested	Observations		Mean O.D.		Significance (P)
		P	C	P	C	
FSE	16	16	6	0.660	0.033	$p < 0.01$
FWBE	31	28	10	0.565	0.025	$p < 0.01$

P = ELISA positive cases; C = control; OD = optical density

Table 7 Results of FP non-specific IgE in ID positive cases classified by the type and nature of respiratory symptoms

Symptom	FSE		FWBE	
	Sera tested	No. positive	Sera tested	No. positive
Bronchial asthma	2	2	5	5
Allergic rhinitis	4	4	4	3
Asthma and rhinitis	9	8	22	19
Total cases	15	14	31	28
Seasonal	4	4	10	9
Irregular	1	0	4	3
Perennial	9	9	15	14
NDH*	1	1	2	2
Total cases	15	14	31	28

*no definite history

tracts. The comparison of skin reactivity to FSE and FWBE is analyzed in Table 5.

The soluble protein content of FSE was 0.37 mg/ml whereas for FWBE it was 0.70 mg/ml.

ELISA

The results of *Fomes* antigens specific for IgE ELISA in markedly ID positive cases of 172 patients are given in Tables 6 and 7. It was observed that almost all patients had significantly raised IgE levels in their sera.

DISCUSSION

In our earlier communication we have reported the presence of many basidiospores in the environment of Delhi constituting about 8% of the total airborne mycoflora. In the current study it was found that *Fomes* basidiospores occur in ambient air of Delhi as in other studies.[7,15] Their atmospheric concentration is very low when compared with that of other basidiospores,[16-18] however, we expected the concentration to be similar to *Ganoderma* basidiospores. Irrespective of concentration it was found that *Fomes pectinatis* follows the same meteorological pattern as that of other basidiospores and occur in the months of high humidity and temperature in India.[6,19]

The extracts prepared from spores and whole body elicited markedly positive skin reactions among patients with respiratory allergy. The low positivity to spore extract might be due to the reduced exposure of patients to *Fomes* basidiospores, since it usually grows on tree trunks and spores are liberated at higher than human breathing levels. Alternatively, they might be less allergenic.[20] On the other hand, faster degradation of the mature fruit bodies releases mycelium and residual spores once it is detached from the tree. This probably leads to higher exposure to mycelial allergens than the spores. Even though spore extract showed low skin reactivity sensitization due to spores cannot be ruled out.

As the pattern of skin reactivity to *Fomes* spores and whole body extracts is different from other basidiomycetes, it needs elaborated studies to find out as to what parts of this fungus are the actual sensitizing aeroallergens.[12,21-23] Our earlier suggestion that the suitability of starting material varies with the genus in the basidiomycetes group is further substantiated by this study. At the same time the overlapping of skin sensitivity reflects the presence of some common components in FSE and FWBE.

Many patients who had symptoms during the basidiospores season showed positive reaction to spore extracts. Patients showing positivity to fruit body extracts had exaggerated symptoms in months

following the spore season and thereafter. We observed that these fruit bodies either fall from the trees by the end of autumn or are detached by gardeners and dumped together. Thereafter, disintegration probably results into post season sensitization.

The skin response also varied with the age of the patients, the same being high in younger patients who had a higher prevalence of rhinitis compared with other age groups. These observations further substantiate our earlier reports that rhinitis patients showed higher reactivity to basidiomycete antigens.

High serum levels of FP specific IgE were found in patients having marked skin positivity. ELISA positivity to spore extract among patients with irregular and seasonal symptoms probably reflect their exposure to spore allergen in the *Fomes* season. On the other hand higher positivity to FWBE among perennial patients suggest less frequent but greater exposure to allergens in the environment. It is expected that once an individual is sensitized to cap allergens which contain both spores as well as spores with mycelium will have frequent symptoms. Tarlo *et al.*[23] has reported less circulatory IgE in perennial patients. Higher specific IgE levels were detected in patients having asthma and rhinitis in case of spore allergens and in rhinitis against cap allergens. Interestingly, perennial patients had poorer responses than seasonal and irregular patients to allergens of both extracts. This suggested a higher exposure to cap allergens. The current study therefore indicates that the level of exposure to basidiospore allergen is as important as the allergen itself.

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