

Studies on the allergenic fungal spores of the Delhi, India, metropolitan area

Botanical aspects (aeromycology)

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The prevalence of fungal spores in the Delhi atmosphere as determined by simultaneous exposure of one slide daily and three Petri dishes a day, at intervals for one full year, is herewith presented for the first time. On the slides the spores of Alternaria sp., Cladosporium sp., small round spores, Curvularia sp., and Helminthosporium sp. were most frequent in that order and together formed 83.5 per cent of the total spore count. In the Petri dishes, colonies of Cladosporium sp., Alternaria sp., Aspergillus sp., Curvularia sp., and Fusarium sp. were most common and formed about 85 per cent of the total colony count. There were two peak periods of fungal spore concentration in Delhi: one from September to November and the other from February to April. Thus, these aeromycologic findings seem to provide an additional explanation of our earlier clinical observations that most patients get their symptoms (or aggravation of symptoms) during these two seasons. There were drawbacks to adopting one method only, either slide exposure or Petri dish exposure, and distinct advantages to using both the techniques simultaneously.

Although most writers give credit to Cadham¹ and Van Leeuwen² as the first workers to mention the possibility of fungal allergy, the names of Jimenez-Diaz³ and Hansen⁴ are also mentioned among the earliest workers. But Feinberg and associates⁵⁻⁹ (1935-37) were the first to make a systematic and comprehensive study of fungal allergy. Further researches in different parts of the world

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have established beyond doubt that fungal spores play an important role in the etiology of nasobronchial allergy.¹⁰⁻¹³

Since allergy is a common disease in India,^{14-15, 23} there is a great need for undertaking aerobiological and clinical studies in various parts of this country to find out the various aeroallergens present in the atmosphere or in the environment of the patients. Some preliminary work on fungal allergy based on slide study alone and with imported fungal antigens was reported from Jaipur.^{16, 17} However, a systematic study of aeromycology, preparation of antigens from locally isolated cultures, and their role in the etiology of respiratory allergy in the Delhi and New Delhi area has been reported by Shivpuri and associates^{14, 18-23} from time to time.

This paper presents a more comprehensive and systematic study of the prevalence of various fungal spores in the Delhi atmosphere for 12 months by simultaneous daily study of one slide and three Petri dishes at intervals. This will be followed by further communications concerning the results of skin tests supported by bronchial sensitivity and conjunctival tests, all performed with the antigenic extracts prepared from the fungal spores isolated from the Delhi atmosphere. The results of hyposensitization with fungal antigens in these patients will also be presented in a subsequent paper.

MATERIALS AND METHODS

For identification of fungal spores and for calculating their prevalence in the air, we have used simultaneous exposures of slides as well as of Petri dishes.

Table 1. *Monthly frequency on gravity slides of various serial fungal spores of*

<i>Fungi</i>	<i>Oct.</i>	<i>Nov.</i>	<i>Dec.</i>	<i>Jan.</i>	<i>Feb.</i>
1. <i>Alternaria</i> spp.	606	462	106	88	520
2. <i>Cladosporium</i> spp.	660	930	291	182	592
3. Small round spores	274	298	168	202	290
4. <i>Curvularia</i> spp.	364	224	48	53	59
5. <i>Helminthosporium</i> spp.	187	79	24	11	23
6. <i>Acrethecium</i> spp.	100	86	11	11	17
7. Fungal hyphae	70	74	33	31	33
8. <i>Nigrospora</i> spp.	37	141	42	22	20
9. Smut spores	126	95	32	18	21
10. <i>Fusarium</i> spp.	32	70	41	23	13
11. <i>Epicoccum</i> spp.	—	2	3	9	75
12. <i>Choanephora</i> spp.	37	15	1	1	19
13. Ascospores	4	3	6	1	14
14. <i>Trichothecium</i> spp.	—	—	5	5	19
15. <i>Cereospora</i> spp.	—	—	—	2	—
16. Teleutospores	—	—	—	1	—
17. <i>Pestalotia</i> spp.	—	—	—	—	—
18. <i>Septonema</i> spp.	—	—	—	—	—
19. <i>Spegazzinia</i> spp.	2	—	—	—	—
20. <i>Beltrania</i> spp.	—	—	—	—	—
21. <i>Tetraploa</i> spp.	—	—	—	—	—
22. <i>Heterosporium</i> spp.	—	—	—	—	—
23. Spoiled spores	6	4	8	5	5
24. Miscellaneous spores	2	—	—	8	1
Total	2,507	2,483	819	673	1,721

The combination of these two methods has important advantages, as will be discussed later.

The slides smeared with cotton blue-stained glycerin jelly were exposed to the atmosphere for 24 hours in a spore-catching device (by gravity) on the roof of the Patel Chest Institute building at the height of about 10 meters. The slides were carefully mounted with a drop of melted stained glycerin jelly and a cover slip (18×18 mm.) to avoid any movement of the fungal spores from their respective places. All the area under the cover slip, i.e., 3.24 cm.^2 , was studied under a high-power microscope, and various fungal spores were identified and counted.

Petri dishes (10 cm. diameter) containing rose bengal streptomycin (R.B.S.) agar medium were also exposed 3 times in a day for 5 minutes each at 7 A.M., 11 A.M., and 7 P.M. The exposed Petri dishes were incubated at $30^\circ \pm 1^\circ \text{ C.}$ for 5 to 10 days depending on the growth of the colonies. The colonies developed were counted, identified,²⁴⁻²⁶ and isolated by subculturing them on tube slants containing nutrient media. These subcultures were maintained for record purposes and were also used as a source for the mass culture of various fungi required for the preparation of antigenic extracts.

R.B.S. agar medium was prepared with the following dehydrated materials: Glucose, 10 Gm.; bacto peptone, 2 Gm.; potassium dihydrogen phosphate, 0.5 Gm.; magnesium sulfate, 0.5 Gm.; rose bengal dye, 0.05 Gm.; agar, 20 Gm.; total, 33.05 Gm.

To rehydrate the medium, 33.05 Gm. of R.B.S. agar was suspended in one

Delhi metropolitan (from Oct., 1965 to Sept., 1966)

<i>March</i>	<i>April</i>	<i>May</i>	<i>June</i>	<i>July</i>	<i>Aug.</i>	<i>Sept.</i>	<i>Total</i>
335	445	604	412	91	115	656	4,400
536	373	86	61	10	59	189	3,969
572	201	123	165	128	236	342	2,999
75	119	78	135	59	208	692	2,114
39	26	41	44	23	42	139	678
24	29	30	60	18	35	111	532
36	38	27	32	28	35	78	513
12	27	19	15	9	18	93	455
25	9	6	3	2	5	26	368
30	17	10	8	14	10	18	286
23	39	11	2	3	1	7	175
10	3	13	19	6	—	—	124
8	1	2	—	3	4	9	55
11	2	3	3	3	1	2	54
1	—	1	—	—	1	11	16
—	—	4	3	2	—	2	12
—	—	—	1	1	3	5	10
—	—	—	—	—	3	6	9
1	—	—	—	—	2	2	7
—	—	—	—	—	—	3	3
1	1	—	—	—	—	—	2
—	—	—	1	—	—	—	1
2	8	7	2	15	3	5	70
1	—	3	14	4	12	2	47
1,742	1,338	1,068	980	419	791	2,398	16,939

Table II. Colony count of different fungi at three different times of the day in

<i>Fungi</i>	<i>Time</i>	<i>Oct.</i>	<i>Nov.</i>	<i>Dec.</i>	<i>Jan.</i>	<i>Feb.</i>
1. <i>Cladosporium</i> spp.	7 A.M.	225	315	160	115	177
	11 A.M.	217	373	198	125	169
	7 P.M.	316	442	156	86	297
	Average	253	377	171	109	214
2. <i>Alternaria</i> spp.	7 A.M.	58	96	41	57	68
	11 A.M.	115	142	68	33	203
	7 P.M.	69	101	54	40	126
	Average	81	113	54	43	132
3. <i>Aspergillus</i> spp.	7 A.M.	60	120	70	110	30
	11 A.M.	60	117	23	111	33
	7 P.M.	56	141	40	70	57
	Average	59	126	44	97	40
4. <i>Curvularia</i> spp.	7 A.M.	51	33	16	15	11
	11 A.M.	101	61	35	24	32
	7 P.M.	87	32	19	15	19
	Average	80	42	23	18	21
5. <i>Fusarium</i> spp.	7 A.M.	28	23	14	13	14
	11 A.M.	38	40	19	14	24
	7 P.M.	41	27	11	11	20
	Average	36	30	15	13	19
6. <i>Penicillium</i> spp.	7 A.M.	19	8	7	70	15
	11 A.M.	20	5	7	25	9
	7 P.M.	13	7	8	29	5
	Average	17	7	7	41	10
7. <i>Acrothecium</i> spp.	7 A.M.	6	8	2	3	0
	11 A.M.	14	23	3	3	3
	7 P.M.	13	12	1	2	1
	Average	11	11	2	3	1
8. Yeast	7 A.M.	21	12	10	1	5
	11 A.M.	12	4	2	7	19
	7 P.M.	19	10	6	5	3
	Average	17	9	6	4	9
9. <i>Helminthosporium</i> spp.	7 A.M.	11	5	5	2	0
	11 A.M.	45	14	9	9	2
	7 P.M.	21	9	2	6	5
	Average	26	9	5	6	2
10. <i>Phoma</i> spp.	7 A.M.	7	4	1	3	7
	11 A.M.	7	14	2	5	12
	7 P.M.	3	12	4	5	1
	Average	6	10	2	4	7
11. <i>Fusidium</i> spp.	7 A.M.	6	19	9	7	8
	11 A.M.	4	14	9	4	0
	7 P.M.	5	12	3	5	8
	Average	5	15	7	5	5

The annual total of all 3 times for the rest of the fungi are: *Rhizopus* spp. (77), *Paecilomyces* spp. (40), *Chaetomium* spp. (39), *Neurospora* spp. (38), *Syncephalastrum* spp. (20), *Cephalo* spp. (4), *Stachybotrys* spp. (4), *Pyrenochaeta* spp. (4), *Blakeslea* spp. (2), *Thielavia* spp. (2),

each month (From Oct., 1965, to Sept., 1966)

<i>March</i>	<i>April</i>	<i>May</i>	<i>June</i>	<i>July</i>	<i>Aug.</i>	<i>Sept.</i>	<i>Total</i>	<i>Grand total of all 3 times</i>
126	22	7	9	3	7	43	1,209	4,201
207	80	11	13	2	7	19	1,421	
168	27	8	19	1	13	28	1,571	
167	43	9	14	2	9	33	1,400	
115	89	148	150	36	29	54	941	3,533
206	212	194	134	52	70	45	1,474	
153	124	152	154	43	50	52	1,118	
158	142	165	146	44	50	50	1,178	
63	60	93	140	99	80	39	964	2,577
56	84	56	69	53	51	31	744	
49	60	90	145	55	63	43	869	
56	68	80	118	69	65	38	859	
23	23	29	47	6	32	67	353	1,372
42	58	27	35	16	66	95	592	
45	21	22	34	4	51	78	427	
37	34	26	39	9	50	80	457	
26	19	22	46	12	25	24	266	914
43	47	40	34	16	22	22	359	
40	18	19	36	21	30	15	289	
36	28	27	39	16	26	20	305	
4	5	6	11	3	9	5	162	366
3	2	2	6	1	7	7	94	
8	6	2	12	1	13	6	110	
5	4	3	10	2	10	6	122	
6	2	7	17	3	6	10	70	318
8	16	9	16	6	14	15	130	
11	7	15	8	11	10	27	118	
8	8	10	14	7	10	17	106	
6	1	0	1	2	17	5	81	252
18	6	0	1	0	19	7	95	
3	1	3	4	0	7	15	76	
9	3	1	2	1	14	9	84	
7	3	8	3	0	3	6	53	244
13	6	9	7	1	4	4	123	
4	1	4	2	1	6	7	68	
8	3	7	4	1	4	6	81	
3	1	1	1	1	0	2	31	137
12	11	0	8	0	1	0	72	
2	0	3	2	0	1	1	34	
6	4	1	4	0	1	1	46	
5	1	0	0	0	2	0	57	135
6	1	2	0	0	0	0	40	
3	2	0	0	0	0	0	38	
5	1	0	0	0	1	0	45	

spp. (64), *Trichothecium* spp. (60), *Trichoderma* spp. (48), *Corticium* spp. (45), *Nigrospora* spp. (8), *Starkeomyces* spp. (8), *Pullularia* spp. (5), *Actinomyces* (5), *Memnoliella* *Histoplasma* sp. (1), *Mortiriella* sp. (1), *Heterosporium* sp. (1), *Scopulariopsis* sp. (1).

Continued on following page.

Table II. *Cont'd*

<i>Fungi</i>	<i>Time</i>	<i>Oct.</i>	<i>Nov.</i>	<i>Dec.</i>	<i>Jan.</i>	<i>Feb.</i>
12. <i>Epicoccum</i> spp.	7 A.M.	0	1	6	2	12
	11 A.M.	0	3	3	1	13
	7 P.M.	0	2	5	3	12
	Average	0	2	5	2	12
13. <i>Candida</i> spp.	7 A.M.	4	2	0	0	0
	11 A.M.	53	5	1	0	0
	7 P.M.	2	12	0	0	0
	Average	20	6	0	0	0
14. <i>Mucor</i> spp.	7 A.M.	4	1	4	0	0
	11 A.M.	2	2	2	1	0
	7 P.M.	2	2	1	0	0
	Average	3	2	2	0	0

liter of distilled water and boiled to dissolve the medium completely, distributed into Petri dishes and/or tubes, and sterilized in the autoclave for 15 minutes at 15 pounds of pressure. Then 20 units penicillin and 40 μ g of streptomycin per liter were added, after cooling the medium to 45 to 50° C., under aseptic conditions. These Petri dishes and tube slants were stored in the refrigerator and were exposed or inoculated as required.

RESULTS

From October, 1965, to September, 1966, a total of 236 slides were exposed. These slides captured a total of 16,939 fungal spores, thus giving an average of 22.2 spores per square centimeter per slide.

The most frequent spores were those of *Alternaria* sp. (25.9 per cent), *Cladosporium* sp. (23.4 per cent), small round spores (17.7 per cent), *Curvularia* sp. (12.5 per cent), and *Helminthosporium* sp. (4.0 per cent). These comprised about 83.5 per cent of the total aerial fungal spores. Only 0.8 per cent of the spores could not be identified, out of which 0.5 per cent were broken or spoiled spores, while 0.3 per cent were not very characteristic (Table I).

During the same period, 814 Petri dishes containing nutrient media were also exposed. A total of 14,791 colonies appeared in these Petri dishes, giving an average of 18.2 colonies per Petri dish.

The most common colonies were those of *Cladosporium* sp. (28.4 per cent), *Alternaria* sp. (23.9 per cent), *Aspergillus* sp. (17.4 per cent), *Curvularia* sp. (9.3 per cent), and *Fusarium* sp. (6.2 per cent). These accounted for about 85 per cent of the total colony count (Table II).

The monthly variation in spore counts, with respect to rainfall and temperature during the year, is given in Table III.

DISCUSSION

For identification and calculation of frequency of aerial fungal spores, a few workers have used a single technique. Some have used the slide-exposure

March	April	May	June	July	Aug.	Sept.	Total	Grand total of all 3 times
4	2	0	0	0	0	0	27	102
15	3	0	0	0	0	0	38	
13	0	2	0	0	0	0	37	
11	2	1	0	0	0	0	34	
0	0	0	0	0	0	0	6	82
0	0	0	0	0	3	0	62	
0	0	0	0	0	0	0	14	
0	0	0	0	0	1	0	27	
0	2	1	2	6	3	5	28	81
2	0	5	3	1	13	2	33	
0	0	2	3	3	4	3	20	
1	1	2	3	3	7	3	27	

Table III. *Monthly variations in total spore counts with respect to rainfall and temperature*

Months (1965-1966)	Rainfall* (mm.)	Temperature* (C.)			Spore count
		Maximum	Minimum	Mean	
Oct.	—	33.3	19.3	26.30	2,507
Nov.	—	29.8	12.8	21.30	2,483
Dec.	0.1	23.2	6.1	14.65	819
Jan.	3.0	23.3	6.3	14.80	673
Feb.	25.4	26.2	12.0	19.10	1,721
March	0.1	30.5	14.4	22.45	1,742
April	1.3	36.1	21.6	28.85	1,338
May	58.5	39.2	25.4	32.30	1,068
June	162.9	38.1	26.9	32.50	980
July	88.8	36.9	27.5	32.45	419
Aug.	211.9	32.8	25.7	29.25	791
Sept.	68.1	33.7	23.4	28.55	2,398

*Courtesy—Meteorological Department, Ministry of Tourism and Civil Aviation, Lodi Road, New Delhi-3.

technique alone,^{17, 27} while others have only exposed Petri dishes containing nutrient media.^{18, 28-31} Either technique, if used singly, has significant drawbacks. With the first method (slides only)^{17, 27} these are:

1. There are spores of several fungi with a similar appearance, which thus cannot be identified to their generic level (e.g., *Apergillus* sp., *Penicillium* sp., *Mucor* sp., *Paecilomyces* sp., etc.)

2. Some spores are too small or too transparent or do not have characteristics sufficiently individual to use them as means of identification (e.g., yeast sp., *Phoma* sp., *Neurospora* sp., *Candida* sp., etc.).

3. There are some viable fungal hyphae which also cannot be identified on slides.

4. The material on the slides may be washed away by rain or blown away with strong wind.

The culture-plate technique shows a large variety of colonies, but there are also some serious drawbacks:

1. The Petri dishes were exposed for only 5 to 15 minutes a day by most of the earlier workers.^{18, 28-31} Therefore, the Petri dish count may not yield a representative sample of fungal spores present during the entire 24 hours and will give the frequency of the spores only for the particular time at which they were exposed.²⁹

2. Some spores of obligate parasitic fungi (e.g., rust and smut spores) for which nutrient media will not sustain growth cannot be identified on Petri dishes and can be identified only on slides.

3. There are some spores which are often found in groups, and in Petri dishes one spore or a group of 5 to 100 spores will produce only one colony (e.g., *Cladosporium* sp., *Alternaria* sp.), while they can be counted individually on slides.

Consequently, we have used a combination of both techniques and have exposed slides as well as Petri dishes, simultaneously. Moreover, the Petri dishes were exposed 3 times in a day (7 A.M., 11 A.M., and 7 P.M.) for 5 minutes each. The object of exposing three Petri dishes a day was to get, as far as possible, the appropriate frequency of fungal spores unidentifiable on slides but which can be identified after they have grown in the exposed Petri dishes by microscopic morphological studies.

It appears likely that the reports on the incidence of various fungal spores in the atmosphere by Indian and foreign workers who have used either only gravity slides or only Petri dishes may not represent the true prevalence of the fungal spores in the air, as is obvious from the analysis of our data. It will be seen from Table I that colonies of *Aspergillus* sp. form 17.4 per cent of the total of 14,791 colonies on the Petri dishes, whereas there is no mention of *Aspergillus* sp. in 16,939 spores counted on gravity slides. The reason is that spores of *Aspergillus* sp., though frequent in the air, cannot be identified on the exposed slides because of their insignificant size and their resemblance to the spores of *Penicillium* sp., *Paecilomyces* sp., *Rhizopus* sp., etc. Therefore, we have included them under the "small round spores" category in Table I.

When we compare the frequency of those genera which are identifiable on slides with the colony count of Petri dishes, we see that even after exposing three Petri dishes a day there is some difference in the percentages. Therefore, a combination of both the methods is essential.^{10, 32} Slide exposure is necessary to get the frequency of those fungal spores identifiable on slides, whereas Petri dishes should be exposed, as many times a day as possible, to get a more correct 24 hour picture of the various fungal spores unidentifiable on the slides. However, it must be emphasized that even the combination of both of these methods does not give a precise quantitative measurement of the spore content of the air, which is only possible by employing a volumetric device, which was not available in India despite our best efforts.

Fungal spores are frequent throughout the year in the Delhi atmosphere (Fig. 1). Seasonal variations were noted in the prevalence of fungal spores in the atmosphere which probably are due to factors such as temperature, rain, and other climatic conditions. The peak periods of fungal spore concentration are

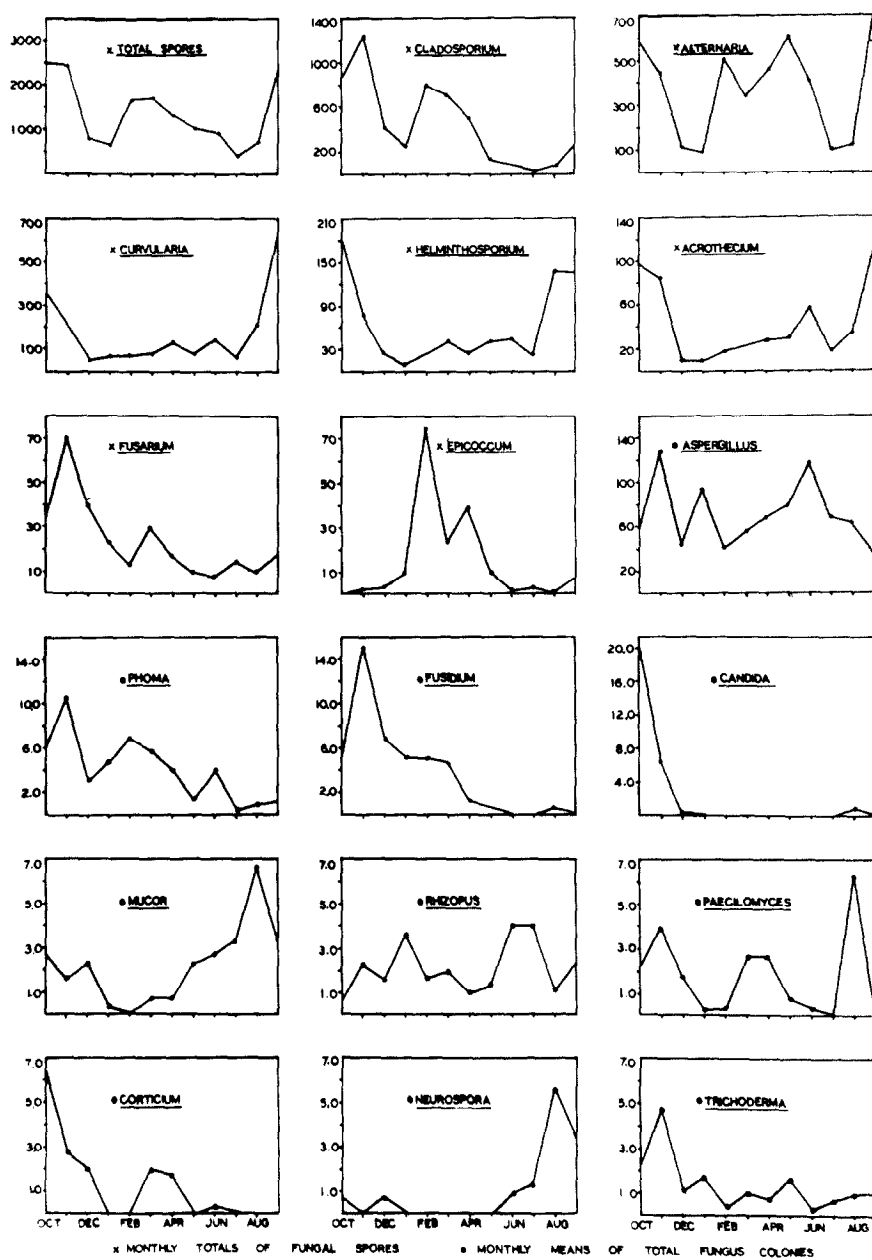


Fig. 1

Monthly concentration of various fungal spores in Delli (metropolitan) atmosphere from Oct., 1965, to Sept., 1966.

the months of September to November and February to April (autumn and spring, respectively). In the rainy season, the fungal spore content of the atmosphere is low because most of the fungal spores are washed down by the rain (this may not be true for covered spaces like homes of patients, factories, etc., for which the results will be reported later). Just after the rainy season, when the sun shines the whole day and the wind blows, the fungal spore concentration gradually increases and reaches its maximum in the months of September to November. After November, there is a substantial decrease in fungal spore concentration in December and January probably due to the lower temperatures. Again the spore count increases as the average daily temperature increases, i.e., from mid-February to mid-April. Thereafter the concentration again starts decreasing gradually probably due to a further increase in temperature over the optimum for fungal growth (Fig. 1).

Individual genera of fungi (Fig. 1) could be categorized into four groups: (1) those having their highest prevalence during September to November, e.g., *Acrothecium* sp., *Candida* sp., *Curvularia* sp., *Fusidium* sp., *Fusarium* sp., *Phoma* sp., *Trichoderma* sp.; (2) those having their peak periods during February, March, and April, e.g., *Epicoccum* sp. and *Paecilomyces* sp. (but the prevalence of *Paecilomyces* spores was also substantially high in the months of August and November); (3) in addition, some fungal spores found to have peak periods during the months of July, August, and September, e.g., *Mucor* sp. and *Neurospora* sp.; (4) also certain fungal spores found in substantial amounts almost all year round, e.g., *Alternaria* sp., *Aspergillus* sp., and *Rhizopus* sp. (but the frequency of *Alternaria* sp. spores was substantially lower during December, January, July, and August).

Following his clinical observations for several years, Shivpuri^{19, 20} reported that in Delhi the overwhelming majority of patients with respiratory allergy get their initial symptoms (or aggravation) in September, October, and November. Their next period of severe symptoms in mid-February to mid-April. Furthermore, in patients who get their symptoms irregularly all year round, the previously mentioned two seasons are their worst months.

It is interesting to note that these seasons correlate closely with the periods of highest atmospheric mold prevalence (Fig. 1). Thus our independent aerobiological observations seem to provide an explanation for the earlier clinical observations as to why patients get their symptoms (or aggravations) during these months. Our studies in aeropalynology have shown that pollens also increase a great deal during these months. Therefore, it stands to reason that both the pollen grains as well as the fungal spores which attain their peak during these two seasons contribute jointly or separately to the aggravation of symptoms.

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REFERENCES

1. Cadham, F. T.: Asthma due to grain rusts, *J. A. M. A.* 83: 27, 1924.
2. Van Leeuwen, W. S.: Bronchial asthma in relation to climate, *Proc. Roy. Soc. Med.* 17: 19, 1924.

3. Jimenez-Diaz, D., and Sanchez Cuenca, F.: Arch. med. **28**: 284, 1924, *Quoted in* Feinberg, S. M., and Durham, O. C.: Allergy in practice, Chicago, 1944, Year Book Medical Publishers, Inc., p. 280.
4. Hansen, K.: On mold asthma, Verhandl. deutsch. Gesellsch. inn. Med. **40**: 204, 1928.
5. Feinberg, S. M.: Mold allergy: Its importance in asthma and hay fever, Wisconsin M. J. **34**: 254, 1935.
6. Feinberg, S. M., and Little, H. T.: Studies on the relation of microorganisms to allergy. II. Role of yeasts in allergy (preliminary report), J. ALLERGY **6**: 564, 1935.
7. Feinberg, S. M., and Little, H. T.: Studies on the relation of microorganisms to allergy. III. A year's survey of daily mold spore content of the air, J. ALLERGY **7**: 149, 1936.
8. Feinberg, S. M.: Seasonal hay fever and asthma due to molds, J. A. M. A. **107**: 1861, 1936.
9. Feinberg, S. M.: Asthma and allergic rhinitis from molds; an analysis of 90 cases, Lancet **57**: 87, 1937.
10. Feinberg, S. M., and Durham, O. C.: Allergy in practice, Chicago, 1944, Year Book Medical Publishers, Inc., p. 216.
11. Sheldon, J. M., Lovel, R. G., and Mathews, K. P.: A manual of clinical allergy, Philadelphia, 1953, Lea & Febiger, Publishers.
12. Naranjo, P.: Etiological agents of respiratory allergy in tropical countries of Central and South America, J. ALLERGY **29**: 362, 1958.
13. Hyde, H. A., Richards, M., and Williams, M. D.: Allergy to mould spores in Britain, Brit. M. J. **1**: 886, 1956.
14. Shivpuri, D. N., and Singh, K.: Studies in local allergens in 324 patients with bronchial asthma and rhinitis in New Delhi/Delhi-India, in Proc. Twenty-first Tuberculosis and Chest Disease Workers' Conference, Calcutta, Feb., 1966, p. 189.
15. Vishwanathan, R.: Definition, incidence, aetiology and natural history of asthma, Indian J. Chest Dis. **6**: 109, 1964.
16. Bhargava, R. K., Kasliwal, R. M., Sethi, J. P., and Sogani, I. C.: Prevalence of respiratory allergy to molds in Jaipur, Indian J. Chest. Dis. **3**: 81, 1961.
17. Gupta, K. D., Sogani, I. C., and Kasliwal, R. M.: Survey of allergenic aerial mold spores at Jaipur, Indian J. Chest Dis. **2**: 237, 1960.
18. Sandhu, D. K., Shivpuri, D. N., and Sandhu, R. K.: Studies on the airborne fungal spores in Delhi—their role in respiratory allergy, Ann. Allergy **22**: 374, 1964.
19. Shivpuri, D. N., and Dua, K. L.: Results of hypo-sensitization on 250 patients with bronchial asthma in respiratory allergy, Ann. Allergy **22**: 632, 1964.
20. Shivpuri, D. N., and Singh, M. P.: On etiologic diagnosis of offending allergens in 200 patients with bronchial asthma and/or allergic rhinitis, J. Palynology, India **1**: 90, 1965.
21. Agarwala, M. K., Mukerji, K. G., and Shivpuri, D. N.: Studies in the allergenic fungal spores in Delhi atmosphere, in Aspects of allergy and applied immunology, Delhi, 1968, New Heights Publishers, p. 91.
22. Shivpuri, D. N., Menon, M. P. S., Prakash, D., Singh, K., and Agarwala, M. K.: Clinical research in common allergens in Delhi atmosphere, in Aspects of allergy and applied immunology, Delhi, 1968, New Heights Publishers, p. 98.
23. Shivpuri, D. N.: Studies in allergy and allergens in India: An introduction, in Aspects of allergy and applied immunology, Delhi, 1968, New Heights Publishers, p. 71.
24. Barnett, H. L.: Illustrated genera of imperfect fungi, Minneapolis, 1958, Burgess Publishing Company.
25. Funder, S.: Practical mycology, Oslo, Norway, 1935, Broggers Boktrykkeris.
26. Laboratory procedures in clinical mycology, TM 8-227-8, Headquarters, Dept. of Army, 1964.
27. Walkington, D. L.: A survey of hay fever plants and important atmospheric allergens in the Phoenix, Arizona, metropolitan area, J. ALLERGY **31**: 25, 1960.
28. Rajan, B. S. V., Nigam, S. A., and Shukla, R. K.: A study of the atmospheric fungal flora at Kanpur, Proc. Indian Acad. Sc. **35**: 33, 1952, b.
29. Rooks, R., and Shapiro, R. S.: The value and limitations of mold surveys by the culture plate method, J. ALLERGY **29**: 24, 1958.
30. Goodman, D. H., Northey, W. T., Leathers, C. R., and Savage, T. H.: A study of airborne fungi in the Phoenix, Arizona, metropolitan area, J. ALLERGY **38**: 56, 1966.
31. Turner, P. D.: The fungal air spora of Hong Kong as determined by the agar plate method, Trans. Brit. Mycol. Soc. **49**: 255, 1966.
32. Merksamer, D., and Sherman, H.: An evaluation of atmospheric mold spores as allergens in New York metropolitan area: A seven-year study, J. ALLERGY **29**: 60, 1958.