Aeromycological survey at Lucknow

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Khandelwal, Asha 1992. Aermycological survey at Lucknow. *Geophytology* **21** : 199-206.

The present paper embodies the account of fungal population present in the air at Birbal Sahni Institute of Palaeobotany, Lucknow for period of one year (1970 - 1971) by employing petriplate exposure and slide exposure methods. The petriplate exposures revealed the presence of twenty species of *Aspergillus* which alone constituted about sixty percent of the total annual colony count while, the slide exposures showed the dominance of *Alternaria* (32.8%) followed by *Puccinia* uredospores (18.6%) and *Helminthosporium* (14.0%) which together formed 65.4 per cent of the total annual spore count.

Key-words- Aeromycoflora, *Aspergillus*, *Alternaria*.

INTRODUCTION

In the past few years aerobiology has received much attention due to its application in plant pathology, allergy, biodeterioration and various other aspects of microbiology. Aerobiological surveys coupled with clinical studies in different parts of the world have revealed that bioparticles are of paramount importance as causative factors of allergic disorders (Newmark, 1968; Hyde, 1972; Harvey, 1973; Chanda & Mandal 1978; Gravesen, 1979; Shivpuri, 1980, 1982; Singh & Gangal, 1986). Continuous monitoring of qualitative and quantitative composition of airspora is a prerequisite of any planned aerobiological programme required for an effective and efficient mode of diagnosis and therapeutic treatment of respiratory allergic disorders in human beings. The results of visual identification of fungal spores during March 1969 - February 1970 had already been reported (Vishnu Mittre & Khandelwal, 1973). The present paper embodies comprehensive and systematic study of aeromycoflora at Lucknow during 1970 - 1971 and the results thus obtained are correlated with the meteorological and clincal data.

MATERIAL AND METHOD

During 1969 - 1970, while studying the spore catches on the slides, a few problems were faced regarding the specific identification of certain fungi. Firstly, it was generally not clear to which group of fungi the spore belonged and also the viability of fungal hyphae was uncertain. Secondly, some very common fungi especially, small, inconspicuous and transparent ones remained unnoticed, or when noticed, their identification was limited only upto generic level. In order to overcome these difficulties the gravity petriplate method was also incorporated which is valued for the precision with which organisms can be identified in resulting cultures.

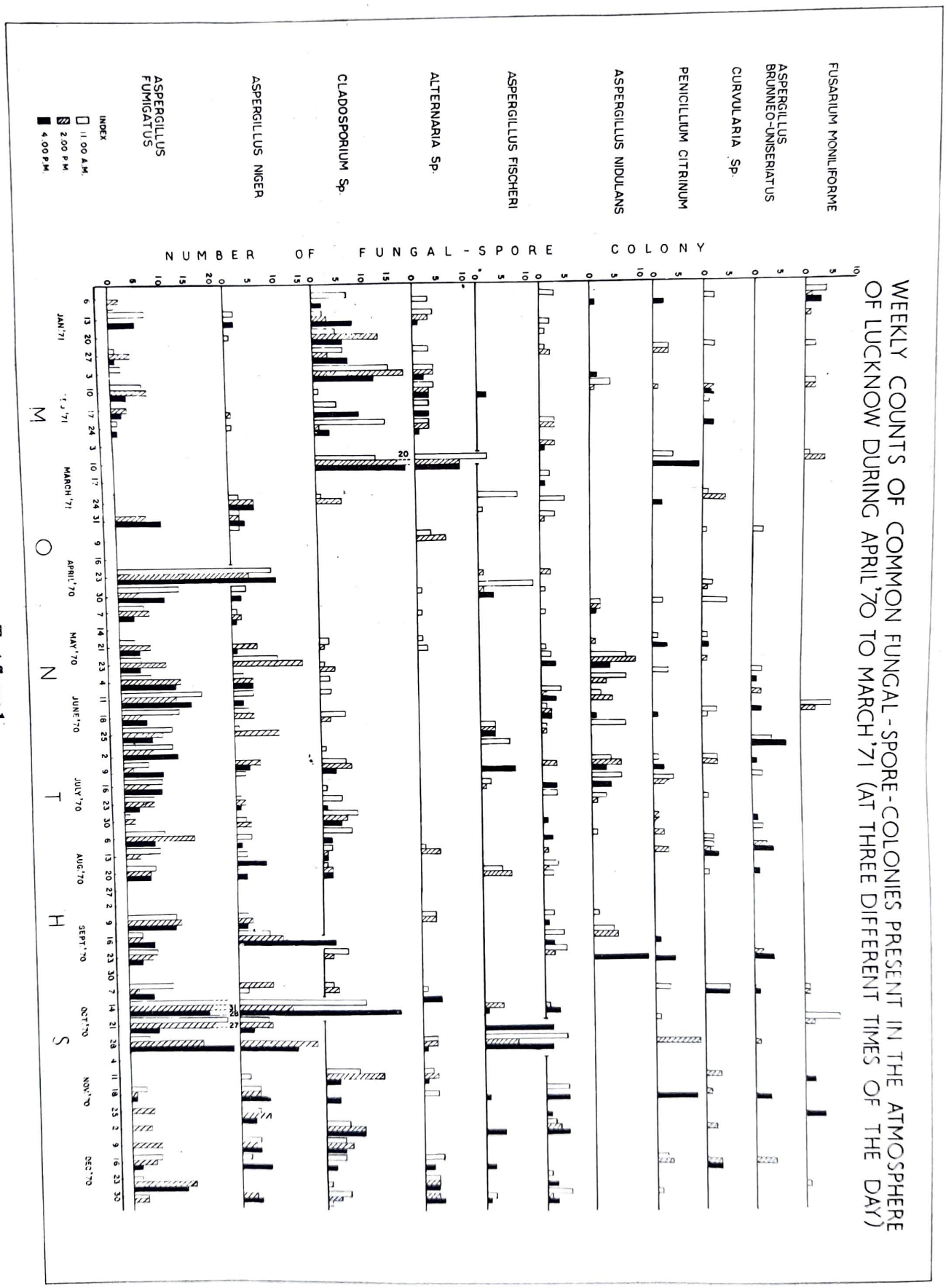
Two sets of petridishes (10 cm diameter) containing Czapek-Dox Agar medium were exposed weekly, three times (11 A.M., 2 P.M. and 4 P.M.) for 5 minutes on the terrace (9 m above the ground) of the Birbal Sahni Institute of Palaeobotany, Lucknow for the period of one year (April 1970 -March 1971) . The exposed petridishes were incubated at 28±1°C for 7-10 days and colonies developed were counted and identified. Simultaneously, the slide smeared with safranin stained glycerine jelly was also exposed to the atmosphere for 24 hours in an apparatus used for earlier studies at Lucknow (Lakhanpal & Nair, 1958). The exposed slides were mounted using 20x50 mm cover slip and fungal spores were identified and counted.

RESULTS

Seasonal distribution of fungal colonies

The total number of colonies (including mycelia sterilia) recorded on petriplates during the period of April 1970 - March 1971 were 2951 (Table 1). The maximum number of fungal colonies were recorded in the month of October (481/16.2% T.A.C.C.*) and minimum in the month of May

* T.A.C.C. = Total annual colony count
** T.A.S.C. = Total annual spore catch
*** T.M.S.C. = Total monthly spore catch



Text-figure 1

(158/5.3% T.A.C.C.). The spp. of *Aspergillus* were found dominating the aeromycoflora of Lucknow. As many as 20 spp. were recognised of which *Aspergillus fumigatus* (27.0%), *Aspergillus niger* (13.4%) together with *Cladosporium* spp.(12.0%) constituted 50% of the aeromycoflora. Six species of *Penicillium* and three species each of *Fusraium* and *Paecilomyces* were also identified.

Curvularia spp., *Aspergillus fumigatus, A. niger* and *A. flavus* were present throughout the year although their frequencies varied every month (Text-fig. 1). *Cladosporium* sp., *Aspergillus terreus* and *Penicillium citrinum* were found in all the months except in April. *Alternaria* spp. were recorded intermittently throughout the year. The maximum qualitative abundance of fungal colonies was found during the winter season and was constituted by thirteen species, viz., *Alternaria* spp., *Aspergillus versicolor, A. tubingensis, Chaetomium globosum, Fusarium merismoides, F. moniliforme, Helminthosporium* spp., *Paecilomyces fusisporus, P. stipitatum, P. vinaceum, P. brefeldianum, Rhizoctonia,* sp. and *Trichothecium roseum.*

The summer air-spora was constituted by six species viz., *Acrophialophora nainiana, Aspergillus nidulans* var. *latus, A. tamarii, A. striatus, Papulospora* sp. and *Trichoderma lignorum.* The rainy season air-spora was represented by *A. nidulans, A. brunneo-uniseriatus. A. carneus* and *Fusarium semitectum* only. *Achaetomium strumarium, Aspergillus fumigatus* var. *albus, Paecilomyces varioti* and *Penicillium funiculosum* were found during both winter and rainy season.

Seasonal distribution of fungal spores

During the period of one year (March 1970 - February 1971) twenty two types of fungal spores were identified on the slides among the total spore catch of 19979 (Table 2). The fungal spore catch during this period revealed both quantitative and qualitative increase over the spore catch in 1969-1970 (Vishnu Mittre & Khandelwal, 1973).

The maximum number of spores were recorded in the month of March (4985/24.9% T.A.S.C.,**) and minimum in the month of August (286/1.4% T.A.S.C.). The maximum spores of *Alternaria, Helminthosporium* and *Cladosporium* otherwise occurring throughout the year were found in March i.e. 2029, 698 and 196 T.M. S.C. *** respectively, with the peak for *Alternaria* on March 24, for *Helminthosporium* on March 10 and for *Cladosporium* on March 24 (Text fig. 2). The highest number of spores of *Aspergillus* type, *Nigrospora* and *Chaetomium* were recorded in November i.e. 313, 245 and 40 T.M.S.C. respectively. During rest of the year their spores were uniformly distributed in the atmosphere.

The spores of *Cercospora*, uredospores of *Puccinia* and *Tetraploa* were recorded throughout the year except for the absence of *Cerospora* in July. *Cercospora* had abundant spores in October (275) and the highest on October 17. The maximum uredospores of *Puccinia* occurred in March (1427) with the peak on April 9. *Tetraploa* had the highest number of spores in October (14). The highest number of spores of *Epicoccum* occurred in April (422) and the lowest in July (5) but were absent during August to October. The highest number of Teleutospores of *Puccinia* were recorded in May (49) and the lowest in November (1).

The spores of *Acrothecium* present during April to June and December to February, the highest number was in May (21). The spores of *Diplodia* were uniformly distributed in all the months except in March, April, July and September. The spores of *Torula* present only during April, May and December to February were evenly distributed. The spores of *Tilletia* caught during May and November to February had the highest number in November (7). *Zygodesmus* spores were caught in October and January, *Beltrania* In July (4). and *Botryodiplodia* in June (3).

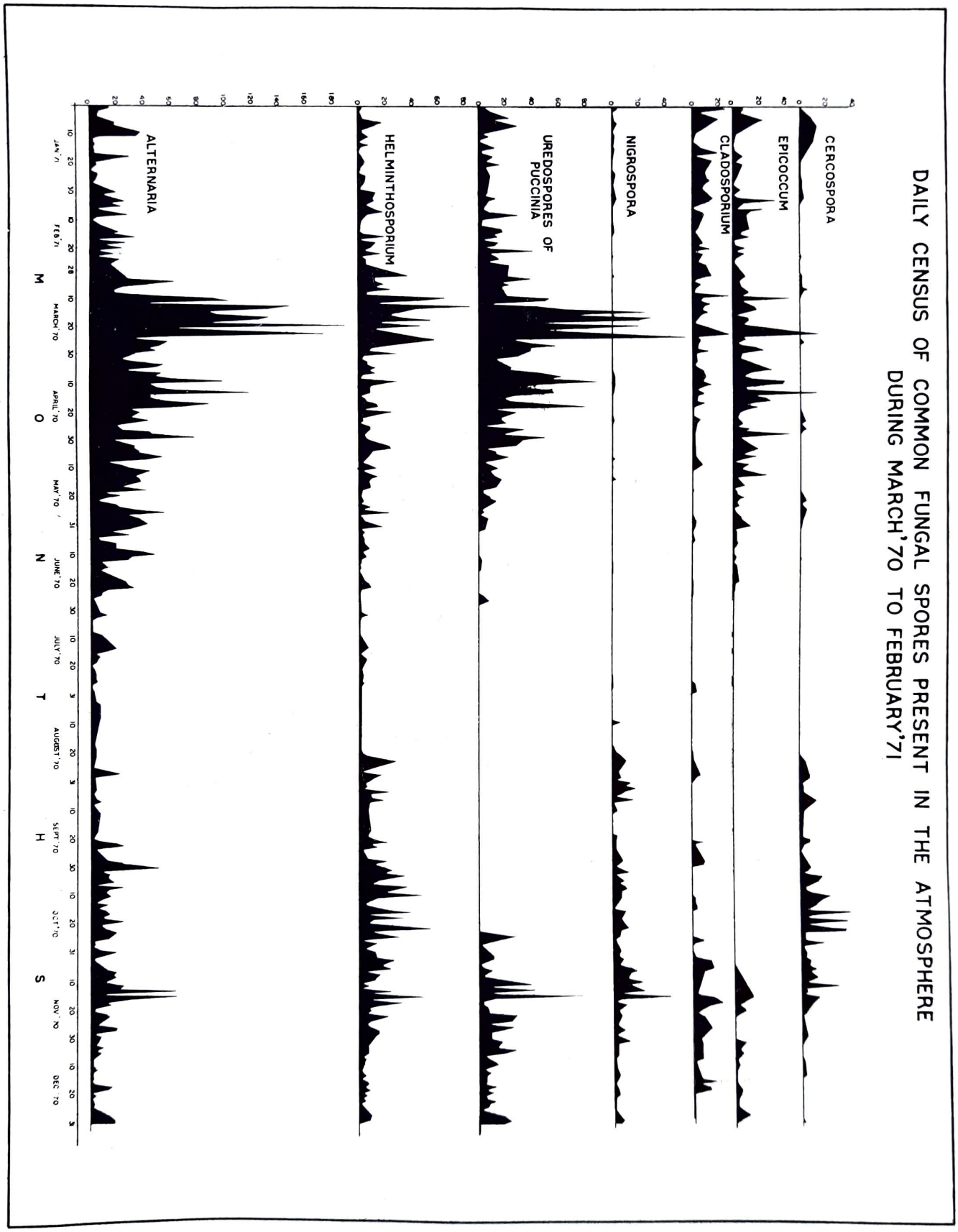
Correlation of fungal flora with meteorological factors

Seasonal variations were noted in the prevalence of fungal spores/colonies in Lucknow atmosphere which probably were due to variations in meteorological factors such as temperature, wind velocity and its direction, rainfall, photoperiod, relative humidity and clouds etc. These factors generally influence the production and dissemination of spores by affecting the time of onset and duration of sporulation.

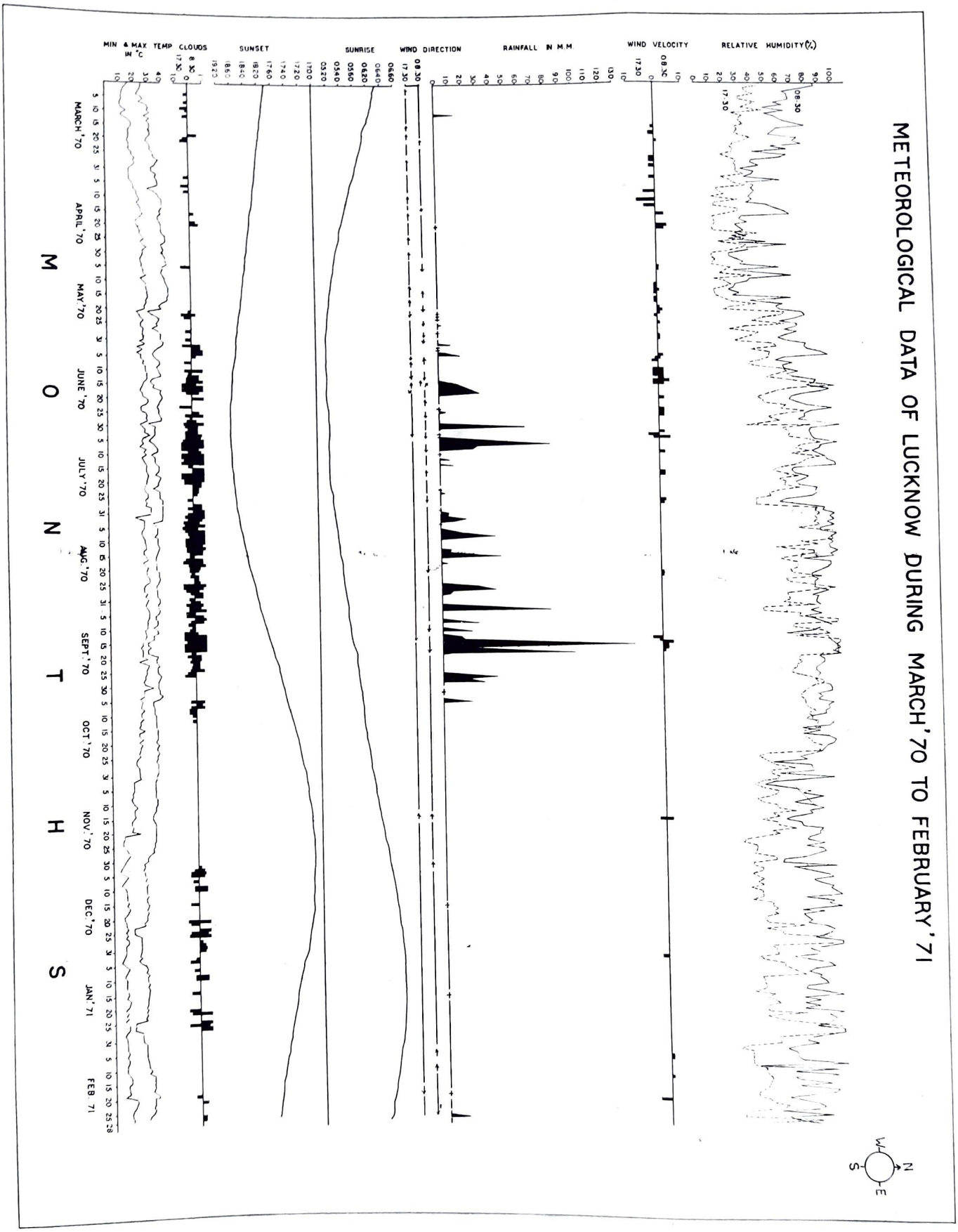
The maximum number and types of spores were caught on May 6 and August 24 and colonies mainly of *Aspergillus fumigatus* and *A niger* in the 3rd week of October. Large number of fungal spores, during spring and early summer (February to May), may be accounted for optimum temperature ranging from 22°C to 29°Cand relative humidity from 53% - 70% (Text-fig. 3). The wind direction was westerly and sometimes easterly too with the bright sunshine which must have influenced the shedding and dispersal of fungal spores during this period. During late summer and rainy season (June to September) the frequency of fungal spores was considerably reduced because most of the fungal spores were washed down by the rain. During late rainy season and winter (October - January) the bright sunshine, optimum temperature and relative humidity favoured the fungal growth resulting in a high number of fungal spores in atmospere although comparatively less than those during spring and early summer.

DISCUSSION AND CONCLUSION

The distribution of fungal spores revealed that during spring and early summer (Febraury to May) there were large number of fungal spores, during late summer and



Text-figure 2



Text-figure 3

Table 1. Colony counts of different fungi in each month during April, 1970 - March, 1971.

Taxa	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	Total	%	
1.	*Achaetomium strumarium*	-	4	1	-	-	-	-	1	2	3	-	-	11	0.37
2.	*Acrophialophora nainiana*	-	-	-	-	-	-	-	-	1	-	-	5	6	0.20
3.	*Acrostalagmus cinnabarinus*	-	-	-	-	-	-	5	-	-	-	5	5	15	0.50
4.	*Alternaria* spp.	10	4	-	-	5	6	9	9	20	14	33	47	157	5.32
5.	*Aspergillus brunneo-uniseriatus*	2	-	7	15	10	6	2	3	4	-	-	-	49	1.66
6.	*A. carneus*	2	-	1	6	-	-	-	-	-	-	-	-	9	0.30
7.	*A. fischeri*	*16*	-	9	18	10	-	57	1	9	-	2	9	131	4.43
8.	*A. flavipes*	1	-	-	-	2	-	5	-	-	5	-	2	15	0.50
9.	*A. flavus*	7	7	15	10	9	15	4	9	20	9	12	6	123	4.16
10.	*A. fumigatus*	123	44	117	84	54	58	188	9	55	21	24	21	798	27.00
11.	*A. fumigatus var albus*	1	-	-	-	-	-	-	2	-	-	-	-	3	0.10
12.	*A. japonicus*	-	-	-	-	-	5	11	-	-	-	3	-	19	0.64
13.	*A. nidulans*	-	26	24	27	1	21	-	-	-	1	6	-	106	3.59
14.	*A. nidulans var latus*	-	-	-	-	-	-	-	-	-	-	-	3	3	0.10
15.	*A. niger*	15	33	33	19	18	56	125	29	37	5	8	19	397	13.46
16.	*A. striatus*	-	-	2	-	-	-	-	-	-	-	-	-	2	0.06
17.	*A. sulphureus*	-	-	-	1	-	-	-	-	-	-	3-	-	4	0.13
18.	*A. sydowi*	-	-	-	1	-	-	-	-	-	2	-	-	3	0.10
19.	*A. tamarii*	-	1	1	1	-	-	-	-	-	-	-	-	3	0.10
20.	*A. terreus*	-	7	6	11	6	7	9	11	36	9	6	6	114	3.86
21.	*A. tubingensis*	-	-	-	-	-	-	-	-	-	-	2	-	2	0.06
22.	*A. ustus*	1	-	-	2	3	-	5	7	7	-	-	-	25	0.85
23.	*A. variecolor*	-	-	6	-	-	-	-	-	1	-	-	-	7	0.23
24.	*A. versicolor*	-	-	-	-	-	-	-	-	1	3	-	-	4	0.13
25.	*Chaetomium globosum*	-	-	-	-	-	-	-	3	2	-	6	5	16	0.54
26.	*Cladosporium* spp.	-	7	11	37	17	10	6	25	51	61	76	56	357	12.09
27.	*Curvularia* spp.	4	8	4	7	9	7	10	4	8	4	7	5	77	2.60
28.	*Dactylium fusarioides*	-	-	-	3	-	-	-	-	2	4	6	5	20	0.67
29.	*Fusarium merismoides*	-	2	-	-	-	2	-	-	-	-	12	10	26	0.88
30.	*F. moniliforme*	-	-	9	-	-	-	11	6	1	10	4	5	46	1.55
31.	*F. semitectum*	-	-	-	1	7	-	-	-	-	2	-	-	10	0.33
32.	*Helminthosporium* spp.	-	-	-	-	-	-	-	-	3	4	-	-	7	0.23
33.	*Mucor* spp.	1-	-	-	-	1	-	-	-	-	-	4	-	6	0.20
34.	*Paecilomyces fusisporus*	-	-	-	-	-	-	-	-	2	4	-	-	6	0.20
35.	*P. marquandii*	-	-	-	-	-	-	-	1	-	4	-	-	5	0.16
36.	P. varioti	1	1	-	1	-	-	-	-	6	4	-	-	13	0.44
37.	*Papulospora* spp.	4	-	-	-	-	-	-	-	-	-	-	-	4	0.13
38.	*Penicillium brefeldianum*	-	-	-	-	-	-	-	9	-	-	-	-	9	0.30
39.	*P. citrinum*	-	6	4	11	5	5	13	8	17	12	1	15	97	3.28
40.	*P. funiculosum*	-	-	3	-	-	-	-	-	5	3	-	-	11	0.37
41.	*P. pallidum*	-	-	-	-	-	-	-	-	-	1	-	-	1	0.03
42.	*P. stipitatum*	-	-	-	-	-	-	-	-	-	11	-	-	11	0.37
43.	*P. vinaceum*	-	-	-	-	-	-	-	-	-	4	-	-	4	0.13
44.	*Rhizoctonia* spp.	-	-	-	-	-	-	-	-	-	5	-	-	5	0.16
45.	*Rhizoplus* spp.	-	-	-	1	-	2	-	-	10	-	-	-	13	0.44
46.	*Trichoderma lignorum*	1	-	1	2	-	-	-	-	1	-	-	-	5	0.16
47.	*Trichothecium roseum*	-	-	-	-	-	-	-	5	-	5	-	3	13	0.44
48.	Mycelia sterilia	11	8	12	8	12	5	21	22	20	23	22	19	183	6.20
	Total	200	158	266	266	169	205	481	164	321	233	242	246	2951	
	%	6.77	5.35	9.01	9.01	5.72	6.94	16.29	5.55	10.87	7.89	8.20	8.33		

Table 2. Spore counts of different fungi in each month during March, 1970 - February, 1971

Taxa	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	To tal	%
1. *Acrothecium* spp.	-	12	21	2	-	-	-	-	-	17	6	15	73	0.36
2. *Alternaria* spp.	2029	1386	876	417	106	52	164	334	404	145	256	394	6563	32.87
3. *Aspergillus* type	118	169	162	50	38	19	54	102	313	192	208	122	1547	7.74
4. *Beltrania* spp.	-	-	-	-	4	-	-	-	-	-	-	-	4	0.02
5. *Botryodiplodia* spp.	-	-	-	3	-	-	-	-	-	-	-	-	3	0.01
6. Cercospora spp.	13	3	1	2	-	21	70	275	113	5	43	7	553	2.76
7. *Chaetomium* spp.	5	3	38	27	25	6	2	16	40	24	3	4	193	0.96
8. *Cladosporium* spp.	196	103	15	6	3	18	27	21	135	66	146	128	864	4.32
9. *Curvularia* spp.	6	21	35	9	15	24	92	77	88	23	36	33	459	2.29
10. *Diplodia* spp.	-	-	1	3	-	3	-	7	4	1	3	2	24	0.19
11. *Epicoccum* spp.	318	422	175	34	5	-	-	-	34	59	94	147	1288	6.44
12. *Fusarium* spp.	1	3	7	10	4	2	11	31	12	18	4	6	109	0.54
13. *Helminthosporium* spp.	698	212	207	78	60	82	205	483	375	131	119	156	2806	14.04
14. *Nigrospora* spp.	3	7	5	2	4	52	109	118	245	58	24	17	644	3.22
15. *Puccinia* spp. (teleutospore)	-	27	49	2	-	-	-	-	1	-	4	4	87	0.43
16. *Puccinia* spp. (uredospore)	1427	973	229	19	3	-	3	28	361	246	159	278	3726	18.64
17. Smut	101	71	25	14	13	2	6	2	59	34	23	52	402	2.01
18. *Tetraploa* spp.	2	4	2	2	-	2	2	14	10	5	12	2	57	0.28
19. *Tilletia* spp.	-	-	3	-	-	-	-	-	7	1	1	1	13	0.06
20. *Torula* spp.	-	8	1	-	-	-	-	-	-	2	3	3	17	0.08
21. *Zygodesmus* spp.	-	-	-	-	-	-	-	3	-	-	2	-	5	0.02
22. 2-4 celled brown coloured spores	52	172	123	22	5	1	1	7	4	15	19	39	460	2.30
23. Unidentified	16	7	10	4	3	2	3	-	9	4	2	22	82	0.41
Total	4985	3603	1985	706	288	286	749	1518	2214	1046	1167	1432	19979	
%	24.97	18.04	9.94	3.53	1.44	1.43	3.75	7.50	11.09	5.23	5.84	7.09		

rainy season (June to September) the number was considerably reduced and during late rainy season and winter (October to January) there was again high number of fungal spores in the atmosphere. Most of the fungal spores were present throughout the year showing diurnal and seasonal variations.

The different type of composition of aeromycoflora have been obtained by employing visual identification method and culture plate method. The former method revealed the spore peak in the month of March constituting about 25 per cent of the total annual spore catch and then followed by April (18.0% T.A.S.C.) and November (11.0% T.A.S.C.). The dominant genus was *Alternaria* (32.8% T.M.S.C.) followed by uredospores of *Puccinia* (18.6% T.M.S.C.) and *Helminthosporium* (14.0% T.M.S.C.) which together formed 65.4% of the total annual spore count. While in the latter method the maximum number of colonies were counted in the month of October (16.2% T.A.C.C.) and then in December (10.8% T.A.C.C.).

The petriplate method recorded the dominance of *Aspergillus* having 20 species which alone constituted about 60 per cent of the total annual colony count. Wadhwani (1979) has also reported the numerical dominance of *Aspergillus, Penicillum, Alternaria, Cladosporium* and *Fusarium* from the area of Lucknow University. The highest prevalence of *Aspergillus* has been observed at Kanpur too (Rajan *et al.*, 1952). The data collected from different aerial surveys at other places in India has also exhibited *Asoergukkys, Penicillium, Mucor, Cladosporium, Alternaria* and *Nigrospora* as major constituents of aeromycoflora.

The fungi such as *Achaetomium strumarium, Acrophialophora nainiana, Acrostalamus cinnabrinus, Dactylum fusarioides, Paecilomyces varioti, P. fusisporus, P. marquandii, Papulospora* spp., *Rhizoctonia* spp., and *Trichoderma lignorum* have been recorded in good numbers from the petridishes but remained unaccounted on the slides. Some genera such as *Aspergillus, Pencillum, Paecilomyces* and *Mucor* were also identified precisely by*their cultures which otherwise would have remained unnoticed or grouped under *'Aspergillus* type'. The spors of some fungi such as *Puccinia, Epicoccum, Nigrosopora, Cercospora, Acrothecium, Tertrapoloa, Zygodesmus, Beltrania, Botryodiplodia, Tilletia* and smut spores have been encountered only on the slides but were not obsorved in petidishes. Among these fungi there wera a few taxa which require different nutrient media for their growth and development and therefore remained undeveloped on the

medium used for present studies. Agarwal *et al.* (1969) have also emphasized upon the simultaneous use of both the methods for precise identification of aerospora. They pointed out the limitations of both the methods. The culture plate method cannot be used for continuous sampling and is restricted only to viable and cultivable particles. On the other hand, the slide, exposure method gives theoretically zero catch of larger particles in still air and shows great change in efficiency with wind speed. However, both the methods are mainly based on natural deposition process and are usually difficult to translate into volumetric results which are generally preferred by allergist to know the concentration of viable and non-viable particles present in a given volume of air.

In India, the clinical investigations of aerial biopollutants for the treatment of patients of respiratory allergy are carried out at four main centres, viz., S.M.S. Medical College, Jaipur; V.P. Chest Institute, Delhi; Bose Institute in collaboration with the School of Tropical Medicine, Calcutta and K.G. Medical College, Lucknow. The clinical investigations carried out at Lucknow have proved the allergenicity of many fungal spores and pollen grains present in the air of Lucknow (Agnihotri & Singh, 1971; Khandelwal 1974; Jamil *et. al.*, 1981 - 1986). The significant aeroallergens are *Aspergillus, Mucor, Alternaria, Penicillium, Rhizopus, Curvularia* and *Phoma*. Besides the cutaneous reactivity of these allergens, the preponderance of *Candida, Aspergillus, Penicillum, Mucor, Curvularia, Helminthosporium, Alternaria, Paecilomyces* and *Trichoderma lignorum* have also been reported in the respiratory tract of many allergic patients (Singh *et. al.*, 1981).

Recently, Wadhwani, *et. al.* (1986) have reported positive allergenic reaction for fifteen species of *Aspergillus* and two smuts from air in and around Lucknow. Jamil *et al.* (1986) while studying four hundred cases of allergic disorders found six species of pollen and two of fungi abundantly prevalent in the air of Lucknow. Singh and Gangal (1986) have also recognised the priorities for future aerobiological researches in India.

Thus, the botanical aspect of aerobiological researches carried out at Lucknow has provided an explanation for the clinical observations as to why local patients get their symptoms or aggravation specifically during spring and early summer season and in late rainy and winter season or all throughout the year. The studies on atmosperic pollen grains have also exhibited their higher prevalence during these two periods. Therefore, it appears that both pollen grains and fungal spores which attain their peaks during these two periods are responsible jointly or seperately for aggravation of symptoms.

ACKNOWLEDGEMENTS

The author expresses her gratitude to the Late Dr. Vishnu Mittre, Ex-Head, Quaternary Biogeography & Archaeobotany, Birbal Sahni Institute of Palaeobotany and to Dr. S.C. Agarwal, Central Drug Research Institute, Lucknow for their keen interest and help during the course of investigation.

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