

Pathogenic and non-pathogenic mycoflora in the air and phylloplane of *Triticum aestivum* L.

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Abstract

Aerobiological investigations were carried out over wheat (*Triticum aestivum* L.) fields near Barrackpore, West Bengal, India for two consecutive crop seasons. In 1990–91, except for the very seedling stage there was a more or less uniform spore count up to the maximum vegetative growth stage and from flowering onwards, a gradual increase in total cfus was observed with the peak (331 CFUs) during the harvesting stage. In the second season, similar results were recorded where peak spore load (2064 CFUs) in the air was during harvesting operation followed by a sudden decline in the empty field. *Cladosporium* spp. dominated both the seasons mainly up to February. *Penicillium*, *Curvularia*, *Aspergillus* and *Cephalosporium* were the most frequent non-pathogenic fungal types. Among the pathogenic fungi, *Alternaria* and *Drechslera* were recorded to be the most virulent to wheat crop, after inflorescence to later stages in both the seasons causing heavy leaf damage producing necrotic spots during which peak cfus were found. *Fusarium* was frequently found showing the peak during vegetative growth period. *Sclerotium* was more common in the second season than in the first season. High occurrence of *Nigrospora* was recorded in the earlier stages. 'Sterile forms' were found regularly. The phylloplane studies revealed that *Cladosporium* was the most dominant flora contributing 40–66% up to February in winter; gradually disappeared from the leaf surface with the onset of summer. *Aspergillus* and *Penicillium* showed the reverse picture. While *Curvularia* was isolated in high amount during later stages; *Nigrospora* mainly during the vegetative growth stages. *Alternaria* (caused Alternaria blight) and *Drechslera* were significantly isolated during harvesting (28.08%) and at late 'grain maturation' stage (80.10%), respectively. *Sclerotium* and 'Sterile forms' were recorded regularly unlike *Fusarium* and *Helminthosporium*. Copyright © 1996 Elsevier Science Ireland Ltd.

Keywords: Aerobiology; Aeromycoflora; Phylloplane flora; Seasonal periodicity; *Triticum aestivum* L.

1. Introduction

Attention on aeromycoflora studies over cities and towns have been centered by a number of workers (Papavassiliou and Bartzokas, 1975; Calvo et al., 1980; Youssef and Karam El-Din, 1988; Satheesh et al., 1993) from the very beginning (Cunningham, 1873) throughout the world and over other environments (Burge et al., 1978; Pandit and Singh, 1992). A number of reports are also available about the airspora studies over various crop fields (Singh and Singh, 1992; Singh et al.,

1992; Uddin and Chakraverty, 1995). Scanty reports (Sharma and Gupta, 1985; Chandel and Chandel, 1993) could be obtained about the airspora and phylloplane flora of cultivated crops in India. In the crop fields, crop plant itself is the main source and hosts many of the fungal flora which account for the airspora. The pathogenic fungi coming from the source are carried away through the air infecting the neighbouring plants or may be transported long distances before they are deposited (Chanda, 1992), having the impact of infection to other plants. Phylloplane studies are, therefore, essential along with the airspora studies in the crop fields concerned. Hence, an attempt has been made to get an insight about the airspora and phylloplane flora

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of wheat crop in West Bengal, India; wheat being the second cereal crop in this state.

2. Materials and methods

2.1. Air monitoring

Aerobiological investigations were initiated for two consecutive crop seasons over wheat (*Triticum aestivum* L.) fields occupying 4 acres of land at Barbagan-Dangadighila area, about 3 km from Barrackpore, West Bengal using the gravimetric culture plate exposure technique (Chandel and Chandel, 1993; Uddin and Chakraverty, 1994). Two per cent malt extract agar (MEA 2%) fortified with streptomycin (40 µg/ml) was used as the culture medium. The study was started on 25 November, 1990 for the first season and on 18 December, 1991 for the second season during seedling stages and continued up to 10 March, 1991 and 30 March, 1992 during harvesting stage and harvesting operation, respectively. A set (5 petri dishes) of petri plates was exposed at 13-day intervals for 3 min between 1200 h and 1400 h (IST). The plates were distributed in the way so as to cover all the possible corners of the field including the central position. Starting from the ground level the height of exposure was gradually lifted as the plants increased in height up to the maximum (81 cm) height reached. After harvesting, another set of exposures were taken in the field when there was no standing crop. The exposed plates were immediately brought to the laboratory and incubated at $28 \pm 1^\circ\text{C}$ for 10 days after which the total CFUs (colony forming units) were counted and identifications were made.

2.2. Recording of meteorological data

The daily records of maximum and minimum temperature, relative humidity and rainfall were available from Dum Dum Station, of the Regional Meteorological Office, Alipore, Calcutta, a place nearest to the crop field. The mean of 14 days temperature and relative humidity were calculated.

2.3. Phylloplane study

The fresh green vegetative leaves as well as the diseased leaves were collected simultaneously for phylloplane studies with the exposure procedure during the second season starting from the early vegetative growth stage (31 December 1991) till harvesting (30 March 1992). The surface sterilised leaf disc method (Ruscoe, 1971; Vardavakis, 1988) was applied with some modifications. The leaves were cut into pieces

with sterile scissors measuring $5 \times 5 \text{ mm}^2$ area i.e. 25 mm^2 leaf area. After having washed once with sterile distilled water, four such pieces were inoculated in a petri dish containing MEA (2%) plus streptomycin (40 µg/ml). The inoculated plates were incubated at $28 \pm 1^\circ\text{C}$ for 10 days and the fungal isolates were identified.

2.4. Statistical analysis

In this paper, the statistical analyses of the data on airspora have been done using the technique of two-way analysis of variance. Thus, suppose $\{x_{it}, i = 1, 2, \dots, k, t = 0 \text{ (1990–91) and } 1 \text{ (1991–92)}\}$ is the data on counts of spora for a particular species observed in the i th stage of the t th year, there being $\dots k$ stages and 2 years. We postulate $x_{it} = \mu + a_i + b_t + c_{it} + \epsilon_{it}$, where μ , a_i , b_t and c_{it} denote the constant general effect, the stage effect, the time (year) effect and the interaction effect on the count of spora and ϵ_{it} denote the pure random effect. The values of a_i , b_t , c_{it} and ϵ_{it} are assumed to be independently normally distributed with zero means and variances σ_a^2 , σ_b^2 , σ_{ab}^2 and σ_ϵ^2 , respectively. The two null hypotheses of interest are thus:

H_{01} : $\sigma_a^2 = 0$ and H_{02} : $\sigma_b^2 = 0$ which corresponds to the assumption of absence of any stage effect and time effect, respectively.

For testing H_{01} , the F -statistic defined as

$$F_a = \frac{\text{MSA}}{\text{MSE}}$$

is used which under the null hypothesis follows $F(k-1, k-1)$, where

$$\text{MSA} = k \sum_{i=1}^k (\bar{x}_{i.} - \bar{x})^2$$

and

$$\text{MSE} = \frac{1}{k-1} \sum_{i=1}^k \epsilon_i \epsilon_t (x_{it} - \bar{x}_{i.} - \bar{x}_{.t} + \bar{x})^2, \bar{x}_{i.}, \bar{x}_{.t}, \bar{x}$$

being the two marginal means and the overall mean, respectively.

For testing H_{02} , similarly, the F -statistic

$$F_b = \frac{\text{MSB}}{\text{MSE}}$$

is used where

$$\text{MSB} = \sum_{t=0}^1 (\bar{x}_{.t} - \bar{x})^2,$$

which under the null hypothesis follows $F(1, k-1)$ in the present case. If the computed value of F_a or F_b exceed the corresponding critical value at a given level of significance, the corresponding null hypothesis is rejected.

3. Results

3.1. Air survey

The total CFUs recovered during the different growth stages for the two seasons of wheat are plotted in Figs. 1 and 2 along with the climatic factors. In 1990–91, except for the seedling stage, there was a gradual increase in spore concentration from the early vegetative growth stage with peak spore count (331 CFUs) at harvest time. In the second season (1991–92), except for the early 'vegetative growth' stage when a peak was observed, spore concentration increased more or less gradually towards latter phases of crop growth showing the highest peak (2064 CFUs) during harvesting operation. An abrupt fall was noticed in the field after harvest while there was no standing crop. So far as the climatic conditions are concerned, from the very beginning, the climate was cold up to February with low temperature, minimum relative humidity and with infrequent rainfall. It was observed that with the onset of summer and consequently with the ageing of the crop plants, the spore level increased significantly.

The statistical analysis for total CFUs indicated that the null hypothesis and the distribution of number of spores over stages are identical for both years could not be rejected as the observed F value (0.43) of the analysis

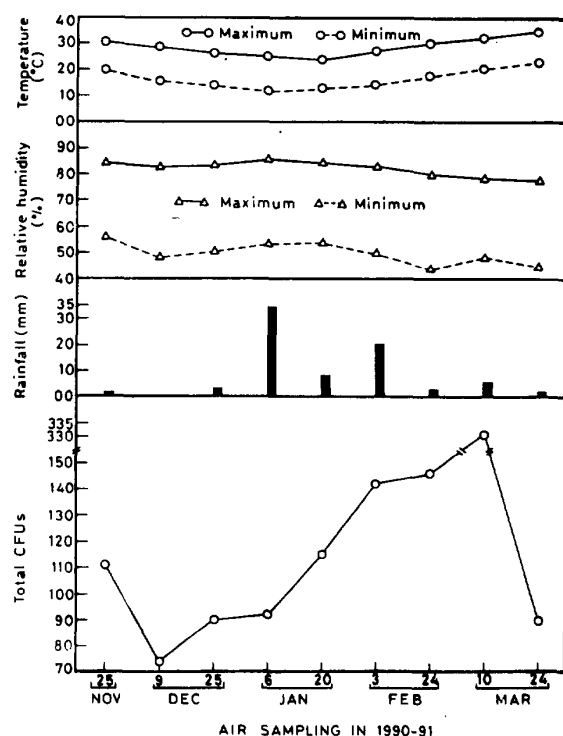


Fig. 1. Total fungal count in air, along with the meteorological variables in 1990–91.

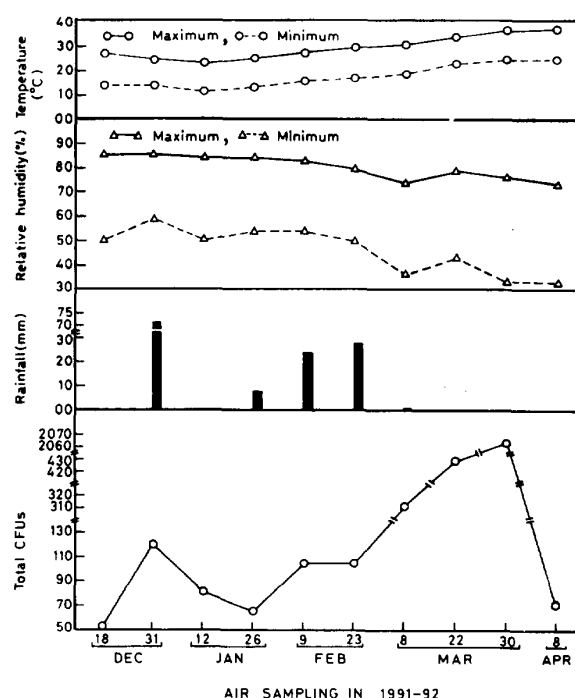


Fig. 2. Total fungal count in air, along with the meteorological variables in 1991–92.

of variance exercise was found to be smaller than the corresponding critical value at $F_{0.05}(5.32)$ for df 1,8.

For each year the number of spores at different stages was observed to vary across stages. In fact, the statistical analysis confirmed this as the null hypothesis that the number of spores at different stages were identical got rejected at 1% level of significance since the observed F value (7.66) was greater than the corresponding critical F value (6.03) for 8,8 degree of freedom.

The percentage contribution of different fungi recovered during the first season (1990–91) was represented in Table 1 along with the age and growth stages of the crop. *Cladosporium* spp. represented by *C. herbarum* and *C. cladosporioides* were recorded to be the most dominant type throughout the season showing the highest peak (33.09%) in early February, except for early March when least (2.41%) amount was observed. Non-pathogenic *Aspergillus* mainly recorded from the air up to 'vegetative growth' stage (within around 20%) and from flowering onwards either it was totally absent or present in negligible amount. *Penicillium* spp. were recorded throughout with two peaks, one at vegetative growth stage (18.88%) and the other at 'grain formation' stage (14.78%). Regular occurrence of *Curvularia* and *Cephalosporium* (except for one sample) was noticed. *Curvularia* spp. represented by *C. lunata* and *C. geniculata* were found mainly in the earlier stages show-

Table 1
Total CFUs (colony forming units) and percentage incidence of fungi in the air in wheat crop during 1990–1991

Date of exposure (1990–91)		Nov. 25	Dec. 9	Dec. 25	Jan. 6	Jan. 20	Feb. 3	Feb. 24	Mar. 10	Mar. 24
Age of the crop (days)		7	21	37	49	63	77	98	112	Empty field
Growth stages		Seedling	Early vegetative growth	Vegetative growth	Max. vegetative growth	Flowering	Fruit formation	Grain Maturation	Harvesting	—
Total CFUs	Genera	111	74	90	92	115	142	146	331	90
Serial No.					% Occurrence					
1.	<i>Cladosporium</i> spp.	25.22	12.16	22.22	28.26	25.21	33.09	8.21	2.41	20.00
2.	<i>Aspergillus</i> spp.	14.41	20.27	20.00	4.34	1.73	4.92	—	—	4.44
3.	<i>Penicillium</i> spp.	8.10	4.05	18.88	5.43	9.56	14.78	1.36	3.92	10.00
4.	<i>Curularia</i> spp.	18.01	12.16	1.11	10.86	4.34	0.70	0.68	1.81	8.88
5.	<i>Nigrospora sphaerica</i>	—	6.75	6.66	2.17	1.73	0.70	0.68	0.30	—
6.	<i>Alternaria</i> spp.	—	—	—	11.95	13.04	0.70	15.75	14.80	11.11
7.	<i>Helminthosporium</i> spp.	3.60	1.35	3.33	2.17	—	—	2.05	0.30	2.22
8.	<i>Drechslera</i> sp.	—	—	—	—	1.73	—	18.49	41.99	2.22
9.	<i>Fusarium</i> spp.	5.40	—	3.33	10.86	6.95	4.22	1.36	2.71	4.44
10.	<i>Sclerotium rolfsii</i>	—	—	—	2.17	—	—	4.10	—	3.33
11.	<i>Stenphylium</i> spp.	—	—	—	2.17	1.73	—	3.42	1.20	—
12.	<i>Epicoccum purpurascens</i>	—	—	—	2.17	7.82	—	2.05	0.60	—
13.	<i>Humicola fuscoatra</i>	4.50	1.35	1.11	2.17	3.47	3.52	—	—	1.11
14.	<i>Cephalosporium</i> spp.	2.70	—	2.22	5.43	12.17	2.81	8.21	0.90	6.66
15.	<i>Trichoderma lignorum</i>	4.50	—	1.11	—	—	1.40	—	—	—
16.	<i>Chaetomium</i> spp.	—	—	—	—	—	5.63	—	0.30	2.22
17.	<i>Stachybotrys</i> spp.	—	—	—	—	1.73	0.70	0.68	1.51	—
18.	<i>Rhizopus</i> spp.	—	9.45	2.22	—	—	—	—	—	—
19.	<i>Mucor</i> spp.	—	8.10	3.33	—	—	—	—	—	—
20.	<i>Dendrographium</i> sp.	—	—	—	—	—	—	0.68	0.30	—
21.	Miscellaneous	4.50	—	4.44	2.17	1.73	3.52	19.85	15.40	6.66
22.	Sterile Forms	9.00	24.32	10.00	7.60	6.95	23.23	12.32	11.48	16.66

ing peak (18.01%) at seedling time. Except for the very seedling stage and in the empty field, *Nigrospora sphaerica* was present regularly. Among the other non-pathogenic fungi, *Humicola fuscoatra* was recorded till 'grain formation' stage; while *Epicoccum purpurascens*, *Stachybotrys* and *Chaetomium* were recorded in late stages. *Rhizopus* and *Mucor* were only found during earlier vegetative growth stages. *Trichoderma* and *Dendrographium* were found occasionally. *Beltrania*, *Brachysporium* and yeasts appeared only once in the whole sampling period. Significant number of 'sterile forms' were present throughout the crop season.

Among the plant pathogenic fungi, *Alternaria* spp. represented by *A. tenuissima*, *A. humicola* and *A. tenuis*, *Drechslera*, *Helminthosporium*, *Fusarium* and *Sclerotium* were the main types. *Alternaria* was first isolated during the maximum vegetative growth stage and was found to increase at later phases of crop growth showing peak (15.75%) during 'grain maturation' stage. *Drechslera* was isolated first from flowering stage and peak level (41.99%) was reached at harvest time. *Helminthosporium* was found in early as well as in later stages in low concentrations. Except for one sample, i.e. during early vegetative growth stage, *Fusarium* spp. were a common constituent in the airspora with peak (10.86%) during maximum vegetative growth stage. *Sclerotium rolfii* was occasionally found to be present.

Table 2 represents the results of the crop season 1991–92. *Cladosporium* dominated up to 'grain maturation' stage (23.45 to 55.83% contribution) with peak concentration (55.83%) in late December, during early vegetative growth stage; decreased gradually and even disappeared in the field when there was no standing crop. The occurrence and percentage frequency of *Curvularia* was more in comparison to *Aspergillus* and *Penicillium*. Except for one sample *Curvularia* was regularly isolated showing peak (18.51%) in early January. *Aspergillus* and *Penicillium* were frequently found to be present. *Nigrospora* spp. represented by *N. sphaerica* and *N. oryzae* mainly recorded so long the vegetative growth was continued. *Epicoccum purpurascens* was restricted only in late winter. A fair number of *Cephalosporium* was observed up to the 'grain maturation' stage, after that it was declined. *Spicaria* was found occasionally; while *Papularia sphaerosperma* was recorded mostly in the earlier stages, *Graphium* in late stages. *Rhizopus* was found in seedling and in high amount in the empty field. Other saprobic forms like *Humicola*, *Verticillium*, *Stachybotrys*, *Chaetomium*, *Geotrichum candidum*, *Diplodia*, *Tetracoccoporus paxianum*, *Zygosporium oschegides*, *Torula herbarum*, *Cunninghamella*, *Mucor*, *Sporocybe*, *Emerellopsis*, *Heterocephalum*, *Stilbella*, *Brachysporium*, *Acremonium*, *Heterosporium*, *T. lignorum* and some yeasts were recorded only once or twice from the exposed plates. 'Sterile forms' were the common constituents in the wheat airspora.

Alternaria and *Drechslera* were the most virulent wheat pathogens, followed by *Fusarium*, *Sclerotium*, *Stemphylium*, *Helminthosporium* and *Rhizoctonia*. *Alternaria* spp. represented mainly by *A. tenuissima*, *A. humicola*, *A. tenuis* and *A. brassicae* were isolated regularly, except for the maximum vegetative growth stage. There was a clear trend of increase in *Alternaria* concentration with the ageing of the crop plants reaching peak (85.27%) during harvesting. A fair amount of *Alternaria* was still present in the field after harvest. *Drechslera* was first recorded during inflorescence and the peak (20.57%) was observed in late grain maturation stage; decreasing gradually afterwards. Except for the seedling stage *Sclerotium* was continuously found to be present. *Fusarium* with peak (12.34%) in early January was more commonly isolated unlike *Stemphylium*, *Rhizoctonia*, *Helminthosporium*, *Pestalotia truncata* and *Cordana musae*. While *Stemphylium* and *Rhizoctonia* were recorded from the later part of crop growth; *Helminthosporium*, *Pestalotia truncata* and *Cordana musae* were recorded only once during the seedling, flowering and harvesting stages, respectively.

The statistical analysis indicated that the null hypothesis and the distribution of number of spores over stages are identical for both years and across the stages could not be rejected as the observed *F* value of *Cladosporium* (1.27 and 1.59), *Aspergillus* (4.36 and 1.18), *Penicillium* (2.88 and 0.036), *Curvularia* (0.004 and 0.362), *Nigrospora* (0 and 0.96), *Alternaria* (1.37 and 2.10), *Fusarium* (2.015 and 0.464) and *Cephalosporium* (0.34 and 1.617) were found to be smaller than the corresponding critical value at $F_{0.05}$ 5.32 and 3.44 for degree of freedom 1,8 and 8,8, respectively. Thus, in these cases time and growth stages affected the spore count in the same way in both the seasons.

In case of *Drechslera*, the null hypothesis that the distribution of spores for the two years could not be rejected as the observed *F*(0.0891) was found to be smaller than the corresponding critical value at $F_{0.05}$ (7.71) for *df* 1,4; but the number of spores observed at different stages were identical was rejected as the calculated *F* value (7.717) was found to be greater than the critical value at $F_{0.05}$ (6.39) for 4,4 *df*.

The analysis for 'sterile forms' indicated that the null hypothesis that the number over stages are identical for the two years was got rejected as the observed *F*(6.17) was found greater than the critical value at $F_{0.05}$ (5.32) for 1,8 *df*. And for each year, these were also found to vary across stages and statistical analysis confirmed this as the null hypothesis that the number of sterile forms at different stages were identical got rejected at 1% level of significance since the observed *F* value (12.16) was greater than the corresponding critical value (6.03) for 8,8 degree of freedom.

Table 2
Total CFUs (colony forming units) and percentage incidence of fungi in the air in wheat crop during 1991–92

Date of exposure (1991–92)		Dec. 18	Dec. 31	Jan. 12	Jan. 26	Feb. 9	Feb. 23	Mar. 8	Mar. 22	Mar. 30	Apr. 8
Age of the crop (days)		7	20	32	46	60	74	88	102	110	—
Growth stages		Seedling	Early vegetative growth	Vegetative growth	Max. vegetative growth	Flowering	Grain maturation	Late grain maturation	Pre-harvesting	During harvesting	Empty field
Total CFUs	Serial No.	51	120	81	65	104	104	311	428	2064	71
		% Occurrence									
1.	<i>Cladosporium</i> spp.	31.37	55.83	23.45	53.84	45.19	43.26	11.25	1.16	1.79	—
2.	<i>Aspergillus</i> spp.	1.96	5.83	1.23	7.69	2.88	—	—	0.46	4.15	2.81
3.	<i>Penicillium</i> spp.	3.92	5.00	—	1.53	1.92	—	0.96	1.16	1.40	7.04
4.	<i>Curvularia</i> spp.	3.92	2.50	18.51	7.69	—	1.92	6.43	2.10	2.32	9.85
5.	<i>Nigrospora</i> spp.	3.92	2.50	1.23	3.07	—	0.96	2.89	—	0.04	—
6.	<i>Alternaria</i> spp.	5.88	0.83	7.40	—	9.61	4.80	24.75	54.67	85.27	18.30
7.	<i>Helminthosporium</i> sp.	3.92	—	—	—	—	—	—	—	—	1.40
8.	<i>Drechslera</i> sp.	—	—	—	—	0.96	—	20.57	18.69	1.69	—
9.	<i>Fusarium</i> spp.	3.92	3.33	12.34	—	5.76	—	0.64	—	0.29	—
10.	<i>Sclerotium rolfsii</i>	—	3.33	1.23	3.07	2.88	2.88	3.85	1.86	0.29	—
11.	<i>Epicoccum purpurascens</i>	—	—	4.93	1.53	5.76	6.73	2.25	—	—	—
12.	<i>Humicola</i> spp.	1.96	2.50	—	—	—	—	—	—	—	—
13.	<i>Cephalosporium</i> spp.	9.80	3.33	2.46	9.23	5.76	7.69	1.92	0.93	0.33	—
14.	<i>Chaetomium</i> sp.	—	0.83	—	—	—	—	0.64	—	—	—
15.	<i>Stachybotrys atra</i>	—	—	2.46	—	0.96	—	—	—	—	—
16.	<i>Rhizopus</i>	5.88	—	—	—	—	—	—	—	—	29.57
17.	<i>Graphium</i> spp.	—	—	—	—	2.88	3.84	0.96	0.93	—	—

Table 2 (continued)

Date of exposure (1991–92)	Dec. 18	Dec. 31	Jan. 12	Jan. 26	Feb. 9	Feb. 23	Mar. 8	Mar. 22	Mar. 30	Apr. 8
Age of the crop (days)	7	20	32	46	60	74	88	102	110	—
Growth stages	Seedling	Early vegetative growth	Vegetative growth	Max. vegetative growth	Flowering	Grain maturation	Late grain maturation	Pre-harvesting	During harvesting	Empty field
Total CFUs	51	120	81	65	104	104	311	428	2064	71
Serial No.	Genera	% Occurrence								
18.	<i>Papularia</i>	—	2.50	2.46	—	1.92	—	—	—	—
19.	<i>sphaerosperma</i>	—	—	—	—	—	—	—	—	5.63
20.	<i>Mucor</i> spp.	—	—	—	—	0.96	—	—	—	9.85
21.	<i>Spicaria</i>	1.96	—	—	—	2.88	—	0.46	0.04	—
22.	<i>Torula herbarum</i>	—	—	2.46	—	—	—	—	0.14	—
23.	<i>Brachysporium</i>	—	—	1.23	—	—	0.96	0.23	—	—
24.	sp.	—	—	—	—	—	—	—	—	—
25.	<i>Stemphylium</i> sp.	—	—	—	—	—	0.32	0.46	0.33	—
26.	<i>Rhizoctonia</i>	—	—	—	0.96	—	1.60	2.80	—	—
27.	<i>solani</i>	—	—	—	—	—	—	—	—	—
28.	Yeast	—	—	3.70	—	—	2.89	0.46	0.29	1.40
29.	Miscellaneous	7.85	6.66	4.94	3.07	5.76	1.60	2.81	0.72	7.04
30.	Sterile forms	13.72	5.00	9.87	6.15	5.76	15.43	10.74	0.82	7.04

Table 3
Phylloplane mycoflora of wheat and their relative incidence

Date of leaf sampling		Dec. 31 (1991)	Jan. 12 (1992)	Jan. 26	Feb. 9	Feb. 9	Feb. 23	Feb. 23	Mar. 8	Mar. 22	Mar. 30
Crop growth stage		Early vegetative growth	Vegetative growth	Max. vegetative growth	Inflorescence		Grain maturation		Late grain maturation	Ripening	Harvesting
Nature of leaves [Area covered (mm ²)]		Fresh green vegetative (300)	Green vegetative (300)	Green vegetative (300)	Green vegetative (200)	Lower yellow (100)	Upper grain vegetative (200)	Lower 1/2 senescent (100)	Half senescent (300)	Senescent (300)	Senescent dry (300)
Serial No.	Genera	% Occurrence of recovered genera									
1.	<i>Cladosporium</i> spp.	49.26	39.62	52.23	65.06	60.31	66.32	53.03	0.51	3.27	0.85
2.	<i>Curularia</i> spp.	4.34	—	8.95	—	12.69	2.04	1.51	3.06	25.00	18.29
3.	<i>Nigrospora</i> spp.	15.94	9.43	11.94	1.20	—	4.08	1.51	—	—	7.65
4.	<i>Epicoccum purpurascens</i>	2.89	5.66	4.47	4.81	9.52	6.12	12.12	—	—	—
5.	<i>Cephalosporium</i> spp.	1.44	—	5.97	3.61	—	—	—	0.51	—	—
6.	Yeast	1.44	5.66	1.49	—	—	—	—	—	2.45	2.55
7.	<i>Aspergillus</i> spp.	—	—	—	—	—	—	—	—	0.40	1.70
8.	<i>Penicillium</i> spp.	—	—	—	1.20	1.58	—	—	2.55	2.45	3.82
9.	<i>Alternaria</i> spp.	2.89	11.32	1.49	8.43	1.58	2.04	3.03	4.08	22.13	28.08
10.	<i>Drechslera</i> spp.	—	—	—	—	—	4.08	19.69	80.10	32.37	16.17
11.	<i>Fusarium</i> spp.	—	1.88	—	1.20	—	—	—	—	—	—
12.	<i>Sclerotium rolfsii</i>	1.44	3.77	1.49	1.20	4.76	—	—	1.02	1.22	4.67
13.	<i>Helminthosporium oryzae</i>	—	—	—	—	—	—	—	0.51	—	—
14.	<i>Rhizopus oryzae</i>	—	—	—	—	—	2.04	—	—	—	2.97
15.	<i>Sordaria fimicola</i>	2.89	3.77	1.49	—	—	—	—	—	—	—
16.	<i>Papularia sphaerosperma</i>	2.89	3.77	—	—	—	—	—	—	—	—

Table 3 (continued)

Date of leaf sampling		Dec. 31 (1991)	Jan. 12 (1992)	Jan. 26	Feb. 9	Feb. 9	Feb. 23	Feb. 23	Mar. 8	Mar. 22	Mar. 30
Crop growth stage		Early vegeta- tive growth	Vegetative growth	Max. vegeta- tive growth	Inflorescence	Grain maturation		Late grain maturation	Ripening	Harvesting	
Nature of leaves [Area covered (mm ³)]		Fresh green vegetative (300)	Green vegeta- tive (300)	Green vegeta- tive (300)	Green vegeta- tive (200)	Lower yellow (100)	Upper grain vegetative (200)	Lower 1/2 senescent (100)	Half senes- cent (300)	Senescent (300)	Senescent dry (300)
Serial No.	Genera	% Occurrence of recovered genera									
17.	<i>Brachysporium</i> sp.	—	—	—	—	—	—	—	—	0.40	2.97
18.	<i>Rhizoctonia</i> <i>solanii</i>	1.44	—	—	—	—	—	—	—	—	—
19.	<i>Botrytis cinerea</i>	2.89	—	—	—	—	—	—	—	—	—
20.	<i>Arthrotrichys</i> <i>superba</i>	—	—	—	—	1.58	—	—	—	—	—
21.	<i>Mucor</i> sp.	—	—	—	—	—	—	1.51	—	—	—
22.	<i>Stemphylium</i> sp.	—	—	—	—	—	—	—	0.40	—	—
23.	<i>Spicaria</i> sp.	—	—	—	—	—	—	—	—	—	0.42
24.	<i>Acrononium</i> sp.	—	—	—	—	—	—	—	—	—	0.42
25.	Unidentified Types	2.89	—	1.49	1.20	3.17	1.02	1.51	3.06	—	1.27
26.	Sterile Forms	7.24	15.09	8.95	12.04	4.76	12.24	6.06	4.59	9.83	8.08
Total colony count		69	53	67	83	63	98	66	196	244	235

3.2. *Phylloplane mycoflora*

The phylloplane studies (Table 3) revealed that total number of cfus were more or less constant up to the maximum vegetative growth showing a same amount of fungal colonisation on leaves. Fungal colonisation gradually rose from inflorescence with the ageing of the plants reaching peak (244 CFUS) spore production at pre-harvesting stage. *Cladosporium* was the dominant leaf surface fungus contributing 40–66% to the total flora up to late February and with the onset of summer, i.e. in March, it declined abruptly. On the contrary, *Aspergillus* and *Penicillium* were absent totally in winter which colonised the leaves in summer. *Nigrospora* was mainly recovered from fresh green leaves during vegetative growth stages and during harvesting stage. Predominance of *Epicoccum purpurascens* was restricted in winter season. *Curvularia* was isolated in huge number (25.00% during ripening and 18.29% during harvesting) from leaves at senescence stage. Some 'sterile forms' were found throughout the period. Yeasts were found in early and late stages. *Cephalosporium* and *Rhizopus* were occasionally found. *Alternaria* spp. (dominated by *A. humicola*, and *A. tenuissima*), although present on leaf surface from the very early vegetative growth, it was heavily isolated in later stages with peak (28.08%) during harvesting while massive infection of leaves was found. *Drechslera* infected the plants heavily from grain maturation with peak spore production (80.10%) at late grain maturation stage and was found to decrease afterwards. Among the other pathogens, *Sclerotium* was recovered regularly except for the 'grain maturation' stage, unlike *Helminthosporium* and *Fusarium* which were recorded to be present on leaf surface only very rarely.

4. Discussion

The whole calendar year can broadly be divided into three major seasons (Uddin and Chakraverty, 1995) viz. summer (March to June), rainy (July to October) and winter (November to February) with their distinct temperature, relative humidity and rainfall range (Figs. 1 and 2). In West Bengal, the wheat seeds are sown in November and are harvested in March, i.e. the crop season covers the whole winter and early summer. The more or less uniformity in spore concentration from seedling to the maximum vegetative growth in both the seasons, and from flowering onwards the gradual increase in spore count with peak during harvesting agree with the observation made on other crop (Uddin and Chakraverty, 1995). The excessive rise in spore load in air during harvesting operation in the second season was due to the human activity over the full senescent wheat plants. Singh and Singh (1992) and Uddin and

Chakraverty (1994) observed similar results studied during the mechanical disturbances of source materials.

Cladosporium spp. dominated the air throughout the winter period, declined gradually with the onset of summer. A comparatively lower occurrence of *Aspergillus* and *Penicillium* especially in the second season was found, which were most predominant during monsoon (Agashe et al., 1992; Uddin and Chakraverty, 1995). No climatic effect was observed in cases like *Curvularia*, *Nigrospora* and 'sterile forms'. Raha and Bhattacharya (1992) reported the presence of *Curvularia* throughout the year in outdoor environment during their aeromycoflora studies at Santiniketan. The occurrence of *Epicoccum* was restricted in mid- or late winter. The seasonality of *Epicoccum* was also reported by Pandit and Singh (1992). It was found that saprophytic *Aspergillus*, *Penicillium*, *Cladosporium* [although some species were reported pathogenic (Morgan-Jones and McKemy, 1992)] and *Epicoccum* were mostly fluctuated with the meteorological variables; host specificity was the main factor for pathogenic types provided the environmental conditions were favourable. While *Helminthosporium oryzae* was recorded to be the most virulent fungal genera reducing crop yield for 'Aman' variety of rice (Uddin and Chakraverty, 1995), *Alternaria solani* for potato (unpublished data; Haware, 1971), *A. brassicicola* for mustard (unpublished data) causing severe crop damages; *Alternaria* spp. represented by mainly *A. humicola* and *A. tenuissima* were the most virulent to wheat crop. Earlier, Prasada and Prabhu (1962) reported the leaf blight disease of wheat by *Alternaria trititica*. *Alternaria* spp. were isolated from vegetative growth in 1990–91 in air and from seedling in 1991–92 which were found to infect the plants from flowering with gradual increase afterwards and consequent leaf damage at later stages while peak spore load was observed (Tables 1 and 2). Another virulent genera, *Drechslera* showed its peak viz. 41.99% and 20.57% at later stages in the first and second crop season, respectively. Thus it also showed the aggressiveness towards the late phases of crop growth. Similarly, Uddin and Chakraverty (1995) reported that in paddy, a high amount of *Helminthosporium oryzae* and *Cladosporium* was present during later phases of growth stages. While *Sclerotium* was regularly isolated in the second season, *Fusarium* was frequently found in air with peak in early January in both seasons. The 'sterile forms' found in wheat airspora were partly due to some obligate basidiomycetous parasites viz. rusts and smut as these spores were caught with the gravity slide method in the same area.

The amount and type of colonisation on leaves was related with numerous factors (Chakraverty and Uddin, 1995). The wheat leaves are long, slender, striped, hairy and the surface texture is rough. There was a more or less constant amount of colonisation as revealed by

total cfus up to maximum vegetative growth stages and from flowering to later stages of senescence, spore production gradually raised with peak during pre-harvesting stage. These showed a similarity to some extent with the airspora. The dominant genera recorded in the phylloplane studies were *Cladosporium*, *Alternaria*, *Drechslera*, *Curvularia*, *Nigrospora*, *Sclerotium*, *Epicoccum* and 'sterile forms'. The pattern of prevalence of fungi was similar with the airspora in cases of *Cladosporium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Curvularia*, *Epicoccum*, *Drechslera* and 'sterile forms'. *Cladosporium* was the most common and dominant flora up to February in winter both in air and phylloplane. *Cladosporium* was reported to be present on leaves of other plants (Pugh and Mulder, 1971; Abdel-Wahab, 1975; Kuter, 1986). Abdel-Hafez (1981) also reported that this genus was present in 100% of the leaf samples.

In the phyllosphere studies of wheat plants (Abdel-Hafez, 1981), the most frequent genera recorded were *Cladosporium*, *Aspergillus*, *Alternaria* and *Penicillium* followed by *Rhizopus*, *Drechslera*, *Fusarium*, *Epicoccum* and *Curvularia*, and in the phylloplane the most frequent fungi were *Cladosporium herbarum*, *A. niger*, *A. ochraceus*, *Alternaria alternata* and *A. Fragmospora*. In our studies, *Aspergillus*, *Penicillium*, *Rhizopus* and *Fusarium* were not frequently isolated due to the variable environmental conditions. Mazen et al. (1984) isolated *Aspergillus*, *Alternaria alternata*, *Cladosporium herbarum* and *Fusarium oxysporum* during their studies on wheat grains and their lemmas and paleas. *Alternaria* was reported to be present on a number of host plants throughout the world (Pugh and Mulder, 1971; Abdel-Wahab, 1975; McLean and Sutton, 1992). Except for the 'grain maturation' stage, *Sclerotium* was isolated from leaves throughout and was not so severe as reported in other plants (Bowen et al., 1992).

Previously, Dickinson (1967) reported that at the onset of senescence, colonisation proceeded rapidly with *Cladosporium*, *Aureobasidium* and Actinomycetes becoming dominant on the yellow leaves and during senescence *Alternaria* and *Stemphylium* increased in frequency with *Cladosporium* dominated on newly dead leaves. In the present study, up to the 'grain maturation' stage, both lower yellow and upper fresh green leaves were colonised mainly by *Cladosporium* followed by *Epicoccum*, *Nigrospora*, *Sclerotium* and *Alternaria*. From late 'grain maturation' onwards *Cladosporium* was gradually disappeared from leaves and transposed by *Drechslera*, high amount of *Alternaria*, *Curvularia* and even *Aspergillus* and *Penicillium*.

It can therefore be concluded that the airspora of wheat was rich in both pathogenic and non-pathogenic fungi. Their concentration was found to fluctuate according to the growth stages of the crop and with the meteorological variables (as recorded for some types,

e.g. *Cladosporium*). There was generally a similarity in the quality and quantity of fungal flora in the air and phylloplane. A clear succession of fungal colonisation was recorded on leaves of wheat. The non-pathogenic fungal types were heavily present during earlier stages and during later part of crop growth saprophytes were disappeared, but pathogenic types were flourished on leaves and caused the disease incidence remarkably at these stages. Spores coming out heavily from the senescence plants at harvesting time may probably have some allergenic effects to the people working during harvesting operation.

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