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Air sampling was carried out for a continuous period of two years (From April 2014 to March 2016) in a paddy field of Birbhum district, rural Bengal, India, using Rotorod Air Sampler with a rotating speed of 2500 rpm. The data obtained from the two years survey showed that January (439.75 spores/ m^3) was the month of highest spore concentration in air followed by December (417.98 spores/ m^3) in 2014-2015. But in 2015-2016 February (468.06 spores/m^3) was the month of highest spore concentration followed by January (450.64 spores/m^3). Seasonal variation of the fungal spores showed that the highest concentration was occurred in winter season followed by rainy and summer season. Variation in concentration of fungal spores in three different seasons may be due to change in temperature, rainfall, relative humidity and wind speed. A total of 23 different fungal spore types were identified during this sampling, among them *Alternaria* sp. showed the highest percentage of occurrence followed by *Curvularia* sp., *Helminthosporium* sp.,*Cladosporium* sp., *Nigrospora* sp., *Aspergillus fumigatus* and *Aspergillus niger*.

Key words: Airborne, atmosphere, fungal pathogens, glycerine jelly, meteorological factors

INTRODUCTION

Aeromycology is a very important branch of Aerobiology which includes the study of airborne fungal spores and their impact on both plants and human health. So, the knowledge about the prevalence of different fungal spore types in the atmosphere of a particular region can help us to forecast various types of fungal diseases of plants in that area. Thus, aeromycological studies can be used to develop an efficient forecasting system for a particular disease to avoid severe crop loss (Devi and Singh, 2007). Aeromycological studies for crop fields have been carried out by several workers in different parts of India (Debnath and Baruah, 2008; Deka and Devi, 2012; Kame and Pande, 2007).

Birbhum district of West Bengal, India is known as Land of Red Soil as it is a part of red lateritic belt. Birbhum is primarily an agricultural district with around 75% of the population being dependent on agriculture and 3,329.05 km2 (1,285.35 sq mi) of land is used for agricultural purposes. So, the aeromycological survey of the paddy field in Birbhum district and the airborne fungal diversity study will make a holistic approach for farmers from agricultural point of view.

MATERIALS AND METHODS

A continuous two years (From April 2014 to March 2016) air sampling was carried out at Mohulla paddy field (with an area of approximately 350 ha), a rural area under Birbhum district of Eastern India situated about 23km away from the Rampurhat town, by using Rotorod Air Sampler with a rotating speed of 2500 rpm for a regular interval of 7 days. Three distinct seasons are recognized in West Bengal i.e. Summer (March-June), Rainy (July-October) and Winter (November-February) with the production of *Boro*, *Aus* and *Aman* rice respectively.During sampling the rotating arms of the sampler was coated with sticky cellotape along the full length facing the wind and the surface of the cellotape was also coated with safranin stained glycerine jelly as a mountant. The sampler was placed at desired site and sampling was done for 30 minutes. After sampling slides were prepared

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and scanned, the fungal spores were identified up to generic level with the help of published literature and reference slides (Baishya *et al.* 2007; Nagmani *et al.* 2006). The counts were converted to concentration/ m^3 air by multiplying with an appropriate conversion factor (suggested by British Aerobiological Federation, 1995).

Plate exposure method once in a week was followed for enumeration of airspora. Petriplates (9 cm) were exposed in the rice field for 15 minutes once a week, by using PDA (Potato Dextrose Agar) medium with streptomycin (to resist the bacterial growth) and the Petriplates were incubated at 28^0 C for 5 days (Shobha *et al.* 2012). The colonies were identified based on their colony colour, shape and other morphological features of mycelia and spores available from literature (Fig.5**).**

Meteorological data i.e temperature, rainfall, relative humidity and wind speed of the sampling site was also recorded from nearest weather station (Sriniketan, Bolpur) to correlate the data with the occurrence and concentration of the aeromycoflora in the atmosphere **(** Table 1**).**

Calculation for Conversion Factor

When a rod rotates at high speed, the arms are thrown out. So, the radius of the arm in static (r1= 3.5 cm.) and rotating (r2 = 4 cm.) conditions must be estimated. If b is the width of the rod, l is the length of the strips, the volume swept/ revolution is :

$v = 2\pi lb\ (r1+ r2) / 2 = 2 \times 3.14 \times 5.5 \times 0.2 \times 3.75 =$ 25.91 cu. cm

The volume sampled by two arms/ min:

$V= 2 \times v \times 2500 = 129550$ cu.cm

Area of the strips counted (a) = Field width × strip width × total number of traverses

$= 325 \times 10^{-4} \times 0.2 \times (2 \times 20) = 325 \times 10^{-4} \times 0.2 \times 40 =$ 0.26 sq. cm.

Total strip area (A) = $2 \times l \times b = 2 \times 5.5 \times 0.2$ sq. cm = 2.2 sq. cm.

The concentration can be calculated as:

C = Number of spores counted (N) × A / a × V × time of exposure (T)

$= N/T \times A/ a \times V$

$= N/T \times 2.2 / 33683 = N/T \times 0.00006531$

$= N/T \times 6.531 \times 10^{-5}$ / cu. Cm. $= N/T \times 6.531 \times 10^{-5} \times 10^6 / m^3 = N/ T \times 65.31 / m^3$

Using this formula the concentration of fungal spores per m^3 was calculated.

RESULTS AND DISCUSSION

A total of 23 different fungal spore types were identified during this sampling and the variation of their monthly concentration was also recorded (Table 2 ; Fig.1). The total number of fungal spores encountered was 4190.73spores/m^3 in the year 2014-2015 and 4554.28 spores/m^3 in the year 2015-2016. It has been noticed that both the year the most dominant type of fungus was *Alternaria* sp.(15.92%),followed by *Curvularia* sp.(9.06%), *Helminthosporium* sp. (8.56%), *Cladosporium* sp.(8.51%), *Nigrospora* sp. (7.60%), *Aspergillus fumigatus* (6.60%), *Aspergillus niger* (6.66%). Other frequently dominant types recorded spores were *Pyricularia oryzae* (4.69%), *Fusarium* sp. (4.09%), smut spores (2.45%), rust spores (2.83%). The fungal spores which contributed less than 2% of the airspora were *Penecillium* sp. (1.72%), *Drechslera* sp. (1.31%), uredospores (1.21%), *Bispora* sp. (1.16%), *Rhizophus* sp. (1.11%), *Trichoderma* sp. (1.10%), *Chaetomium* sp.(1.03%). Remaining fungal spore types such as *Lophiostoma* sp., *Pithomyces* sp., *Tetraploa* sp., *Periconia* sp., *Torula* sp., were recorded in very lower concentration in the atmosphere of the rice field. Among the total fungal spores trapped about 10.66% remain as unidentified (Table 4; Fig.3,4).Fungal colony and spore morphological micrographs were also prepared for easy visual identification of the studied genera (Fig.5,6).

The data obtained from the two years continuous survey showed that January (439.75 spores/ m^3) was the month of highest spore concentration followed by December (417.98 spores/ m^3) in 2014-2015. But in 2015-2016 February (468.06 spores/ m^3) was the month of highest spore concentration followed by January (450.64 spores/m^3). Seasonal variation of the fungal spores showed that the highest concentration was occurred in winter season (1532.61 spores/m^3) followed by rainy (1435.74 spores/m^3) and summer season (1404.17 spores/ m^3) (Table 3 ; Fig.2). In both the years the month of August and September in rainy season showed the highest peak of concentration. But in summer season June has the highest concentration for first year and May for the second year.

The present aeromycological investigation of the paddy field of Mahulla village of Birbhum district of West Bengal in Eastern India reflects that the atmosphere of the survey area contain a large num-

Table 1 : Meteorological data of survey area from 2014-2016

Months	2014-2015					2015-2016				
	Max. Temp. (C^0)	Min. Temp ((C^0)	Rainfall (mm.)	R.H (%)	Avg. Wind speed / hr	Max. Temp. (C^0)	Min. Temp ((C^0)	Rainfall (mm.)	R.H (%)	Avg. Wind speed / hr.
April	39.5	24.95	0.16	86	4	35.28	24.64	91.2	83.75	3
May	37.4	26.52	80.7	88	3	36.96	28	69.8	86.75	3
June	35.4	27.18	148.8	93.25	4	34.72	26.88	304	89.2	4
July	32.9	26.95	429.5	96	8	33.04	25.76	696.56	94.2	7
August	34.1	26.48	276.2	95	6	34.16	27.44	331.97	95.7	8
September	34.2	25.88	128.6	94	6	34.72	26.87	171.2	94.3	7
October	33.8	23.5	5.8	87.75	7	33.6	24.65	21.08	88.65	6
November	32.1	16.14	0	80.6	4	31.36	20.16	0	81.5	5
December	26.5	12	0	86.25	5	26.88	16.8	0	85.45	4
January	25.2	14.56	5.6	84.25	2	26.32	14	0.51	82.7	3
February	30.24	17.92	9.3	84.5	3	30.8	20.15	104.14	81.5	2
March	33.6	21.28	30.4	83.75	5	34.72	23.52	37.85	81.75	2

Table 2 : Monthly concentration (spores/ m^3) of the spore types during the years 2014-2015(Upper number) and 2015-2016 (Lower number)

Spore types	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Total
Alternaria sp.	100.14	80.55	32.66	17.42	15.24	41.36	34.83	60.96	82.73	65.31	76.19	69.66	677.05
	78.37	54.43	74.02	41.36	53.25	58.78	21.77	63.13	60.96	91.43	71.84	45.72	715.06
Aspergillus	0	34.83	41.36	54.43	37.01	17.42	0	0	39.19	30.48	45.74	6.53	306.96
fumigatus	0	50.07	45.72	37.01	10.88	0	0	21.77	26.12	34.83	41.36	0	267.77
Aspergillus niger	17.42	0	37.01	47.89	41.36	26.12	13.06	0	32.66	50.07	0	0	265.6
	26.12	37.01	56.60	28.30	45.72	32.66	17.42	0	15.24	39.19	0	19.6	317.84
Bispora sp.	0	4.35	0	13.06	26.12	0	0	0	0	0	0	0	43.54
	0	6.53	0	15.24	21.77	0	0	6.53	0	0	8.71	0	58.78
Chaetomium sp.	0	0	19.59	6.53	0	17.42	0	0	0	0	0	8.71	52.23
	10.88	0	0	13.06	0	6.53	0	0	4.35	0	0	2.18	37.01
Cladosporium	34.83	21.77	0	32.66	23.95	15.24	39.19	28.3	45.72	76.2	65.31	0	383.15
sp.	39.19	47.89	19.60	0	0	26.12	37.01	56.60	32.66	41.36	34.83	23.95	359.21
Curvularia sp.	28.3	43.54	54.43	37.01	47.89	56.60	30.47	23.95	34.83	0	0	17.42	374.44
	0	63.13	41.36	32.66	0	45.72	34.83	50.07	39.19	43.54	30.48	37.01	417.98
Drechslera	15.24	0	0	0	6.53	0	8.71	6.53	0	0	0	4.35	41.36
sp.	13.06	0	17.42	0	13.06	0	4.35	8.71	0	10.88	0	6.53	74.02
Fusarium sp.	13.06	0	15.24	0	17.42	23.95	6.53	0	17.42	21.7	15.24	26.12	163.28
	15.24	21.7	0	0	26.12	19.59	10.88	0	13.06	32.66	23.94	30.48	195.93
Helmintho-	6.53	23.95	50.07	15.24	32.66	28.30	45.72	54.43	39.19	30.48	0	10.88	337.44
sporium sp.	26.12	0	0	19.59	39.19	69.66	82.73	65.31	50.07	0	43.54	17.42	413.63
Lophiostoma	0	0	6.53	0	2.18	0	0	0	0	2.18	4.35	0	15.24
sp.	0	8.71	4.35	0	0	10.88	0	0	0	0	6.53	4.35	34.83
Nigrospora sp.	0	56.60	47.89	32.66	60.96	41.36	0	17.42	34.83	0	0	30.78	322.20
	0	65.31	43.54	41.36	56.60	30.48	0	0	0	37.01	43.54	23.94	341.78
Penicillium sp.	0	8.71	6.53	0	10.88	13.06	2.18	0	15.24	0	0	21.7	78.37
	23.94	0	0	6.53	0	0	8.71	0	19.59	0	0	13.06	71.84
Periconia sp.	6.53	0	0	0	4.35	0	6.53	0	10.88	2.18	0	0	30.48
	0	10.88	6.53	0	0	0	4.35	0	0	8.71	4.35	0	34.83
Pithomyces sp.	4.53	0	0	0	6.53	4.35	0	0	4.35	0	0	17.42	37.01
	0	6.53	0	4.35	0	0	0	0	0	0	0	10.88	21.7
Pyricularia	0	0	19.59	0	0	41.36	37.01	0	0	65.31	50.07	45.72	259.06
oryzae	0	0	0	0	21.7	45.72	34.83	0	0	0	28.30	15.24	145.86
Rhizopus sp.	0	10.88	2.18	0	8.71	6.53	0	10.88	0	0	0	0	39.19
	0	0	0	6.53	17.42	0	0	0	0	19.59	10.88	4.35	58.78
Rust spores	6.53	0	0	0	26.12	10.88	19.59	0	0	17.42	15.24	0	95.78
	13.06	6.53	0	0	19.59	15.24	32.66	0	0	4.35	17.42	10.88	119.73
Smut spores	0	0	0	0	4.53	0	8.71	15.24	0	37.01	32.66	15.24	113.20
	0	10.88	15.24	0	0	10.88	17.42	6.53	0	13.06	37.01	23.95	134.97
Tetraploa sp	10.88	0	0	0	6.53	2.18	0	0	8.71	0	0	0	28.30
	0	4.35	0	0	0	6.53	0	0	13.06	0	0	10.88	34.83
Torula sp	0	0	4.53	0	13.06	0	4.53	0	0	15.24	0	0	32.66
	0	13.06	8.71	0	10.88	8.71	6.53	0	0	0	0	0	47.89
Trichoderma	0	0	6.53	0	0	0	8.71	13.06	0	0	0	4.53	34.95
sp.	0	0	17.42	8.71	0	0	13.06	0	0	10.88	0	15.24	65.31
Uredospores	6.53	0	2.18	0	4.35	0	0	10.88	0	0	17.42	8.71	47.89
	8.71	0	0	0	0	0	0	0	0	21.7	15.24	13.06	58.78
Unidentified	32.66	37.01	26.12	21.7	30.48	23.95	39.19	45.72	52.25	26.12	43.54	30.48	409.28
	50.07	21.7	45.72	65.31	41.36	34.83	39.19	54.43	30.48	41.36	50.07	52.25	526.83
Total	283.01	322.19	372.26	278.65	426.69	370.09	311.31	287.36	417.98	439.75	363.56	317.84	4190.73
	306.96	428.87	396.21	320.02	376.62	422.34	365.74	333.08	304.78	450.64	468.06	380.98	4554.28

Table 3 : Average seasonal variation in spore concentration (spores /m3)for two years

Season	2014-2015	2015-2016	Average
Summer (Mar- Jun)	1295.32	1513.02	1404.17
Rainy (Jul-Oct)	1386.75	1484.71	1435.73
Winter (Nov-Feb)	1508.66	1556.55	1532.61

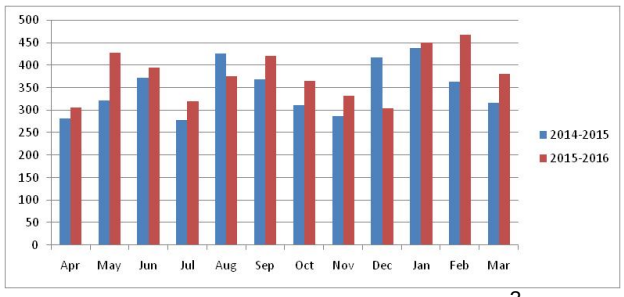

Fig. 1 : Monthly variation in concentration (spores/ m^3) of different spore types

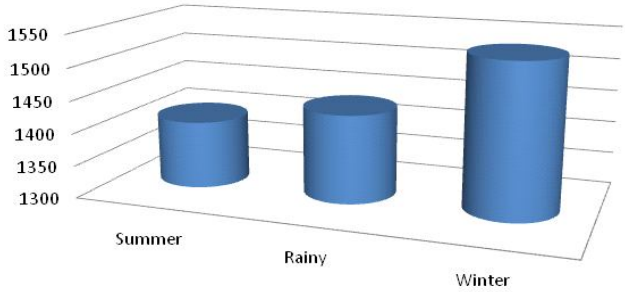

Fig. 2 : Average seasonal variation in spore concentration for two years

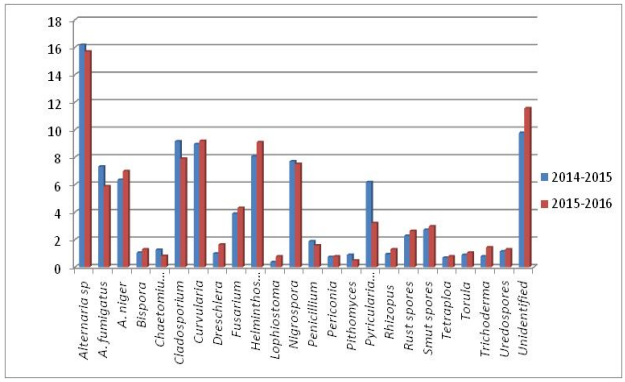

Fig. 3 : Percentage contribution of the individual fungal spore types for the two consecutive years

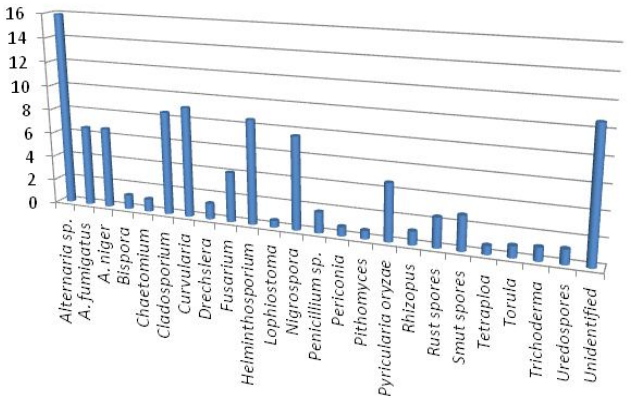

Fig. 4 : Average percentage contribution of the individual fungal spore types

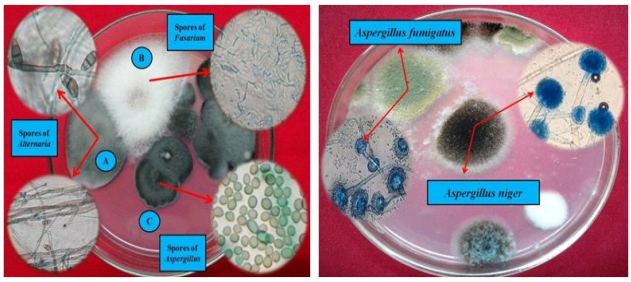

Fig. 5 : Petriplates showing fungal colonies after sampling

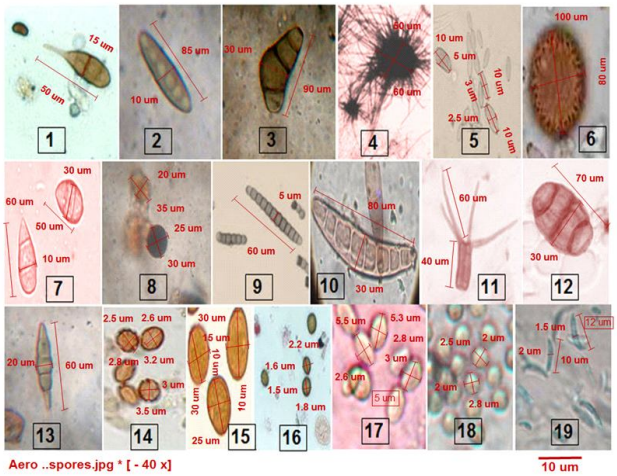

Fig. 6 : Airborne fungal spores trapped during sampling in paddy field

1. *Alternaria* sp. 2. *Helminthosporium* sp. 3. *Curvularia* sp 4. *Chaetomium* sp. 5. *Cladosporium* sp. 6. *Periconia* sp. 7. *Pyricularia oryzae* 8. *Nigrospora* sp. 9. *Torula* sp. 10. *Drechslera* sp. 11. *Tetraploa* sp. 12. *Pithomyces* sp. 13. *Lophiostoma* sp. 14. *Rhizopus* sp. spores 15. Uredospores 16. Smut spores 17. *Tricorderma* sp. spores 18. *Aspergillus* sp. spores 19. *Fusarium* spores

ber of airborne fungal pathogens and they were present throughout the year in different concentration. Among the 23 different fungal spore types *Alternaria* sp. shows the highest percentage of occurrence followed by *Curvularia* sp., *Helminthosporium* sp., *Cladosporium* sp., *Nigrospora* sp., *Aspergillus fumigatus* and *Aspergillus niger* . The cause of high concentration of *Alternaria* sp. in the air of paddy field can be explained as they have a wide host range and their spores may travel through a long distance transport. Among the other dominant

Table 4 : Percentage (%) contribution of the individual fungal spore types

Spore types	2014-2015	2015-2016	Average
Alternaria sp.	16.16	15.68	15.92
Aspergillus fumigatus	7.32	5.88	6.60
Aspergillus niger	6.34	6.98	6.66
Bispora sp.	1.04	1.29	1.16
Chaetomium sp.	1.25	0.81	1.03
Cladosporium sp.	9.14	7.89	8.51
Curvularia sp.	8.94	9.18	9.06
Drechslera sp.	0.99	1.63	1.31
Fusarium sp.	3.89	4.3	4.09
Helminthosporium sp.	8.05	9.08	8.56
Lophiostoma sp.	0.36	0.77	0.56
Nigrospora sp.	7.69	7.5	7.6
Penicillium sp.	1.87	1.58	1.72
Periconia sp.	0.73	0.77	0.75
Pithomyces sp.	0.88	0.48	0.68
Pyricularia oryzae	6.18	3.2	4.69
Rhizopus sp.	0.94	1.29	1.11
Rust spores	2.28	2.63	2.45
Smut spores	2.70	2.96	2.83
Tetraploa sp.	0.68	0.77	0.72
Torula sp.	0.88	1.05	0.96
Trichoderma sp.	0.78	1.43	1.10
Uredospores	1.14	1.29	1.21
Unidentified	9.77	11.56	10.66

fungi *Curvularia* sp, *Helminthosporium* sp., *Nigrospora* sp, *Fusarium* sp, *Pyricularia oryzae* are detected as the pathogens of rice (Devi *et al.* 2009). *Cladosporium* sp. is saprophytic in nature and they occur in dead organic matter of rice field. *Cladosporium* sp. is reported as the most dominant airborne fungus of crop fields by earlier workers (Deka and Devi, 2012). Other important fungi like *Aspergillus fumigatus, A. niger, Penicillium* sp. and *Rhizopus* sp. have already been reported as important aeroallergens (Khan and Karuppayil, 2012).

The occurrence of different fungal pathogen in air is highly influenced by meteorological factors. Variation in concentration of fungal spores in three different seasons may be due to change in temperature, rainfall and relative humidity (Khare and Tiwari, 2016; Rana *et al.* 2014). So, the result obtained from this investigation give a clear idea about the dominant airborne crop field fungus and their concentration in air in different seasons.

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