	Nature Environment and An International Quarterly S
Orig	inal Research Paper

Nature Environment and Pollution Technology An International Quarterly Scientific Journal

No. 3

pp. 473-479

2009

Environmental Monitoring of Airborne Fungal Biopollutants Over Brinjal Fields

Vol. 8

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Key Words: Aeroallergens Fungal biopollutants Kharif season Brinjal fields

ABSTRACT

The present investigation was undertaken to understand the incidence of different fungal biopollutants in atmosphere over brinjal field during Kharif season by operating Rotorod sampler and Petri plate exposer method. Altogether, 48 types of biopollutants were identified of which 43 belonging to fungal spore type and remaining 5 types belonging to group 'other types'. Out of the total 43 fungal spore types, 25 belonged to Deuteromycotina, 13 to Ascomycotina, 2 to Mastigomycotina, 2 to Zygomycotina, and 1 to Basidiomycotina. *Cladosporium, Alternaria, Curvularia*, Aspergilli, *Nigrospora* and *Epicoccum* were the dominant spore types. From Petri plate exposer method, 431 colonies were isolated, which were assigned to 16 genera with 13 genera of Deuteromycotina (75.55%), 3 to Mycelia Sterila (14.27%), 2 to Zygomycotina (8.34%), and 1 to Ascomycotina (1.85%). The relationship among incidence of these biopollutants in the air, changes in the meteorological conditions and the results are discussed.

INTRODUCTION

India is an agricultural country with one-third population depending on agriculture sector directly or indirectly. Agriculture continues to be the mainstay of the Indian economy. Vegetables play a vital role in the nutritional security of the Indian population and financial economy of the majority of small marginal farmers. The demand of vegetables has been increasing fast in the urban and rural areas with a gradual rise in standard of living coupled with development of communication and transport facilities. Vegetables are rich source of nutrients especially vitamins and minerals. They can play a significant role in food and nutrition security as well as in poverty alleviation. Malnutrition is a serious problem, which is attributed to an imbalanced diet.

Brinjal (*Solanum melongena* Linn.) is an important vegetable crop. It is nutritive and provides 24 kcal of energy, 4% carbohydrates, 1.4g protein and 0.3g fat per 100g of edible portion (Kanthaswamy et al. 2003). Brinjal crop is subjected to various diseases caused by fungi, bacteria, viruses and mycoplasma, which reduce the yield and production. These fungal diseases can be considered important from the point of view of epidemiology and management. The present paper reports the fungal spore dispersal pattern in the environment over brinjal field and also comparison of the spore concentration with the meteorological conditions.

MATERIALS AND METHODS

Environmental monitoring was carried out by operating Rotorod air sampler (Perkins 1957 modified by Harrington 1959) for one hour in the evening between 5 pm and 6 pm twice in a week at a constant height of 4.5 feet above the ground level. Brinjal (*Solanum melongena*) was grown in a test field at Vaduj in Satara district of Maharashtra. In an another set of experiment, PDA Petri plates of 9 cm

diameter were exposed horizontally once a week for 10 minutes between 5 pm and 6 pm at sampling site. The culture plates containing PDA with 40 unit streptomycin/mL medium were exposed above the test field. Exposed plates were incubated at $28 \pm 2^{\circ}$ C for 7 to 15 days. The colonies developed were examined and counted regularly. The colonies which could not sporulate after the treatment of induced sporulation were placed under 'sterile mycelium group'. The studies were conducted for kharif season (June 15, 2007 to September 25, 2007). The daily meteorological data of temperature, relative humidity and rainfall were maintained throughout the study period (Table 3).

The spore number trapped in the sampler was expressed as number of spores per cubic meter of air. For estimating the spore types and their concentration number and percentage contribution, slides were scanned. The identification of different spore types was based mainly on comparative spore morphology and spore description and was subsequently confirmed by relevant literature (Ellis & Ellis 1985, Barnett & Hunter 1972, Tilak 1989). The spores, which could not be identified due to their obscure nature or even otherwise, were placed under unidentified type. The identification of spore types was based on microscopic characters. Identification of fungal genera from PDA Petri plates was done on colony characteristics, growth and morphology.

RESULTS AND DISCUSSION

During the period of present investigation, apart from dust particles, 48 types of biopollutants were trapped of which 43 belonged to the fungal spore type origin, while remaining 5 types belonged to group 'other types' comprising of trichomes (hairs), hyphal fragments, insect scales (parts), pollen grains and unidentified fungal spores. Out of 43 fungal spore types, 2 belonged to Mastigomycotina, 2 to Zygomycotina, 13 to Ascomycotina, 1 to Basidiomycotina, and 25 to Deuteromycotina. Of the various groups during kharif season, fungal spore types belonging to the Deuteromycotina (63.96%) contributed highest catches to the total airborne spores followed by 'other types' (15.13%), Ascomycotina (11.99%), Zygomycotina (6.22%), Mastigomycotina (2.28%) and Basidiomycotina (0.42%).

From the group Mastigomycotina, 2 spore types were trapped in sampler, with their contribution to the total airspora was recorded as *Albugo* (1.27%) and *SCLEROSPORA* (Oospores) (1.01%). The **maximum monthly mean concentration 50/m**³ was recorded in the month of August for *Albugo* and 60/m³ was recorded in the month of September for *Sclerospora* (Oospores). These results conform with the reports of Dransfield (1966) and Sreeramulu (1967).

Cunninghmella spore contribution to the total airspora was recorded as 1.69% with maximum monthly concentration of 60/m³ in month of September. However, in general, the incidence of these spores in the air was recorded in the rainless nights of the rain preceding days as has been recorded by Reddi (1978). Occurrence of *Rhizopus* and *Mucor* spores was continuous. Their contribution to the total airspora was recorded as 4.53% with maximum concentration of 225/m³ during September, and minimum 60/m³ in month of June. Hyde & Williams (1949) reported these spore types with significant concentration. The relevance of these spores in environment and human health hazards is reported by Karne & Pande (2006).

As has been recorded in India and elsewhere, rust spores (uredospores) were common in air in the present investigation. Their contribution to the total airspora was less (0.42%) with occurrence only in July (20/m³) and August (30/m³) (Table 1). Allergenic nature of rust spores has been pointed out by Agarwal & Shivpuri (1974).

MONITORING OF AIRBORNE FUNGAL BIOPOLLUTANTS

Among 13 ascospore types from group Ascomycotina, spores of *Leptosphaeria* contributed 2.54% followed by *Chaetomium* 1.18%, *Pleospora* 1.14%, *Cucurbitaria* 1.13%, *Pringsheimia* 1.10%, *Bitrimonospora* 0.97%, *Hypoxylon* 0.93%, *Monilia* 0.84%, *Massarina* 0.59%, *Didymospheria* 0.59%, *Pleomassaria* 0.42%, *Valsaria* 0.33% and *ASCOTRICHA* 0.05% (Table 1). These spore types were encountered in the air during rainy season. The concentration of ascospores gradually increased according to increase in rainfall (Table 3). The maximum incidence of these spores was recorded in the month of August, when there was 26.04°C temperature, 72.29% relative humidity and 84.6 mm rainfall. Similar results were reported by Tilak & Bhalke (1979) and Karne (2007). Role of ascospores like *Leptosphaeia*, *Chaetomium*, *Pleospora*, etc. is reported in causing allergy and acute asthma by Karne & Pande (2006).

From group Deuteromycotina, *Cladosporium* (19.17%), *Alternaria* (8.97%), *Curvularia* (6.30%), *Epicoccum* (5.58%), *Nigrospora* (4.78%), *Helminthosporium* (2.28%), Aspergilli (2.24%), *Cercospora* (1.73%), *Memnoniella* (1.69%), *Heterosporium* (1.35%), *Bispora* (1.22%), *Hirudinaria* (1.22%), *Spegazzinia* (1.01%), *Fusarium* (1.01%), etc. spore types dominated the airspora during the study period. Spore types like *Alternaria*, *Helminthosporium*, *Cercospora*, *Fusarium*, *Curvularia* and *Drechslera* may cause diseases to brinjal plants when environmental conditions are warm and humid.

The spores of *Cladosporium* were most dominant with highest spore catch (1080/m³) recorded in month of September having 27.68°C temperature, 69.9% relative humidity and 175.2 mm rainfall. High incidence of these spores is reported by De-meena (1955). The lowest contribution was recorded of the spore type *Harknessia* (10/m3) with only 0.08% contribution in September.

The 5 non-fungal biocomponents trapped during the study period belonging to group 'other types' comprised of hyphal fragments (4.19%), trichome (hairs) (2.41%), insect scales (parts) (2.32%), pollen grains (3.72%) and unidentified fungal spores (2.49%). The total number per metre cube of air was 495, 285, 275, 440 and 295 respectively. The maximum monthly concentration of hyphal fragments 120/m³, trichomes (hairs) 120/m³, insect scales (parts) 115/m³, pollen grains 150/m³ and unidentified spores was 170/m³. The incidence of particulate matter was reported by Benninghoff (1965). The counts of 'other types' were necessary to record and maintain the original total spectrum of the airspora over brinjal field.

During the period of present investigation, the aerobiopollutants like *Cladosporium, Alternaria, Nigrospora, Curvularia, Helminthosporium, Epicoccum,* Aspergilli, *Pithomyces, Rhizopus,* rust spores, *Chaetomium, Heterosporium, Drechslera, Leptosphaeria, Pleospora, Fusarium,* hyphal fragments and pollen grains may be responsible for inducing allergenic reactions to sensitive individuals (Agarwal & Shivpuri 1974). They may be significant potential source of allergens and inhalation of these biopollutants may be main causative factor for respiratory allergic diseases in human beings as reported by Feinberg (1935), Shivpuri (1980), Karne & Pande (2006) and Karne (2008).

In Petri plate exposer method, altogether 431 colonies were obtained during 16 exposers conducted over the brinjal field. These were assigned to 16 genera of fungi (Table 2). Out of the total colonies identified, Deuteromycotina (75.54%) dominated the total population followed by mycelia sterila a sterile mycelium group and unidentified type (14.27%), Zygomycotina (88.34%) and Ascomycotina (1.85%).

From the dominant group Deuteromycotina, *Cladosporium* contributed highest colonies (65) with 15.08% followed by *Alternaria* with 13.45% (58 colonies) and Aspergilli 7.88% (34 colonies).

Table 1: Monthwise total airspora (spores/m³ of air) and percent contribution of different spore types during kharif season over Brinjal field.

Sr.	Spore type		Kharif Season P				Percentage
No.		June	July	Aug.	Sept.	Total	contribution
	Mastigomycotina						
Ι.	Albugo	20	40	50	40	150	1.27
2.	Sclerospora (Oospores)	-	15	45	60	120	1.01
	Total	20	55	45	100	270	2.28
	Zygomycotina						
	Cunninghamella	-	10	30	60	200	1.69
	Rhizopus, Mucor	60	95	155	225	535	4.53
	Total	60	105	185	285	735	6.22
	Ascomycotina						
	Ascotricha	-	_	30	30	60	0.05
	Bitrimonospora	10	20	40	45	115	0.95
	Chaetomium	-	35	45	60	140	1.18
	Cucurbitaria	-	-	40	115	155	1.13
	Didymosphaeria	15	25	30	-	70	0.59
	Hypoxylon	25	20	35	30	110	0.93
	Leptosphaeria	25 45	20 60	85	110	300	2.54
•	Massarina	-	15	20	35	300 70	0.59
	Monilia	-	20	20 80	-	100	0.39
0.	Pringsheimia	20	20 35	35	- 40	130	1.10
0. 1.	Pleospora	20 25	35	40	40 35	130	1.10
1. 2.	Pleomassaria	-	20	40 30	-	50	0.42
2. 3.		-	20 15	25	-	30 40	0.42
5.	Valsaria Total	- 140	300		- 500	40 1475	
		140	500	535	300	1475	11.99
	Basidiomycotina		20	20		50	0.40
	Urediospores (Rust spores)	-	20	30	-	50	0.42
	Total	-	20	30	-	50	0.42
	Deuteromycotina	25	60	255	500	10.00	0.07
	Alternaria	25	60	255	720	1060	8.97
	Aspergilli	15	45	80	125	265	2.24
	Biospora	-	40	45	60	145	1.22
	Cercospora	10	45	65	85	205	1.73
•	Chlamydomyces	-	20	-	-	20	0.16
•	Ceratophorum	-	-	20	25	45	0.38
•	Cladosporium	190	380	560	1080	2210	19.17
	Corynespora	-	-	25	35	60	0.50
•	Curvlaria	125	275	130	225	755	6.30
0.	Dictyoarthrinium	-	-	10	15	25	0.21
1.	Diplodia	15	25	30	45	115	0.97
2.	Drechslera	-	10	30	20	60	0.50
3.	Epicoccum	40	180	190	250	660	5.58
4.	Fusarium	-	40	30	50	120	1.01
5.	Harknessia	-	-	-	10	10	0.08
6.	Helminthosporium	10	35	85	140	270	2.28
7.	Heterosporium	15	35	50	60	160	1.35
8.	Hirudinaria	-	40	50	55	145	1.22
9.	Memnoniella	-	40	60	100	200	1.69
20.	Nigrospora	40	100	230	195	565	4.78
1.	Phaeotrichoconis	-	-	5	10	15	0.12
2.	Pithomyces	10	25	15	65	115	0.97

Table cont...

Vol. 8, No. 3, 2009 • Nature Environment and Pollution Technology

Cont	table							
23.	Spegazzinia	-	15	40	65	120	1.01	
24.	Sporidesmium	5	60	40	20	125	1.05	
25.	Tetraploa	-	-	10	20	30	0.25	
	Total	500	1470	2055	3475	7500	63.96	
	Other types							
1.	Trichomes (Hairs)	25	40	100	120	285	2.41	
2.	Hyphal fragments	40	80	140	235	495	4.19	
3.	Insect Scales (Parts)	25	50	85	115	275	2.32	
4.	Pollen grains	40	150	150	100	440	3.72	
5.	Unidentified spores	20	45	60	170	295	2.49	
	Total	150	365	535	740	1790	15.13	
	Grand Total	870	2315	3435	5180	11810	100	

Table 2: Total number of colonies of various species of fungi identified from exposed Petri plates in the entire sampling period and their percent contribution to the total airspora.

Sr. No.	Name of Fungus	Total No. of Colonies	Percentage contribution						
	Zygomycotina								
1.	Mucor sp.	09	2.08						
2.	Rhizopus sp.	15	3.48						
	Rhizopus stolonifer	12	2.78						
	Total	36	8.34						
	Ascomycotina								
1.	Chaetomium sp.	08	1.85						
	Total	08	1.85						
	Deuteromycotina								
1.	Alternaria sp.	58	13.45						
2.	Aspergillus sp.	20	4.64						
	Aspergillus flavus	08	1.85						
	Aspergillus nidulans	06	1.39						
3.	Cladosporium sp.	65	14.8						
4.	Chlamydomyces sp.	15	3.48						
5.	Curvularia sp.	36	8.35						
6.	Drechslera sp.	03	0.69						
7.	Fusarium sp.	26	6.03						
8.	Helminthosporium sp.	05	1.16						
9.	Memnoniella sp.	16	3.71						
10.	Monilia sp.	24	5.56						
11.	Nigrospora sp.	16	3.71						
12.	Penicillium sp.	27	6.26						
13.	Tricothecium sp.	02	0.46						
	Total	327	75.55						
	Sterile Mycelium Group								
1.	White sterile mycelium	22	5.10						
2.	Black sterile mycelium	17	4.30						
3.	Yellow sterile mycelium	09	2.08						
	Total	48	11.48						
	Unidentified	12	2.78						
	Grand Total	431	100						

Nature Environment and Pollution Technology

Vol. 8, No. 3, 2009

Months	Temperature (°C)		Humidity %			Rainfall	Spore	
	Min.	Max.	Mean	Dry	Wet	Relative Humidity	(mm)	percentage
June	26.00	30.00	28.00	26.22	22.10	65.00	13.2	7.36
July	26.63	30.67	28.65	26.33	22.16	65.83	9.9	19.60
August	25.32	26.77	26.04	24.70	21.41	72.29	84.6	28.87
September	26.76	28.60	27.68	25.50	21.80	69.90	175.2	44.17

Table 3: Comparative data of temperature, relative humidity, rainfall and spore percentage during the study period.

Aspergilli included *Aspergillus* sp. 4.64% (20 colonies), *A. flavus* 1.85% (8 colonies) and *A. nidulans* 1.39% (6 colonies), which were dominant and frequent during the study period. The moderately recorded genera were *Curvularia* (8.35%) *Penicillium* (6.26%), *Fusarium* (6.03%), *Monilia* (5.56%), *Nigrospora* (3.71%) and *Memnoniella* (3.71%) with lowest count of *Tricothecium* (0.46%).

The non-sporulating colonies or sterile mycelium group contributed 11.49% (48 colonies) which included white cottony floccose mycelium (22 colonies), black smoky, green smooth colonies (17) and yellow sterile sticky, spherical mycelium with concentric rings (9 colonies). The incidence of these colonies was more. Unidentified colonies contributed 2.78% (12 colonies). Similar findings were reported by Hyde & Williams (1949). Zygomycotina contributed 8.34% (46 colonies) with *Mucor* 2.08% (9 colonies), *Rhizopus* 3.48% (15 colonies) and *R. Stolonifer* 2.78% (12 colonies). Ascomycotina was represented by *Chaetomium* only with 1.85% (8 colonies). It is evident that environmental factors like temperature, relative humidity and rainfall play an important role in the distribution of airspora (Gregory 1971). *Cladosporium, Alternaria*, Aspergilli, *Penicillium, Curvularia*, and *Fusarium* showed higher incidence in the kharif season in association with the higher relative humidity, moderate air temperature, rainfall and availability of the wet plant debris in and around the test field. However, the significant numerical variation shown by the colony counts appears to be correlated with the vegetation around the field and the meteorological parameters. These observations support to the earlier findings of Richards (1956), Hudson (1969), Bajaj (1978) and Raha & Bhattacharya (1992).

ACKNOWLEDGEMENT

Authors are thankful to the Principal Shri Sanjay Patil, Shahajiraje Mahavidyalaya, Khatav for support, motivation and for providing library and laboratory facilities for the experiments. The help rendered by Dr. C. J. Khilare, Dr. Geeta P. Kulkarni, V. K. Adsul, R. K. Sakhare, J. J. Godase and Ayyaj Mulla is duly acknowledged.

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Vol. 8, No. 3, 2009 • Nature Environment and Pollution Technology

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