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Studies on Diversity of Aeromycoflora over Vegetables growing field under irrigation in Rubi Season

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Abstract: Fungal propagules including viable spores in the environment are known to be prominent allergen bioparticulates, implicated to cause allergic symptoms and respiratory disorders in animals and various diseases to plants. Aeromycological survey over vegetable growing field under irrigation at different locations for two month period in rubi season revealed existence of significant count of viable fungal spores of diverse group adhere to film of nutrient medium that forms colonies of different colours in variable frequency. Out of the total 2085 fungal colonies fall under 19 genera and 35 species, more than half count of colonies was appeared in the month of January by petri plate exposure method. The peak period of spore concentration was confined second week of January, marked by ambient climate of low temperature and high humidity. Ascomycota contributed greater colony count over others. An individual ascomycetous genus, was reported dominant exhibiting higher count of species and contributed 36 per cent of the total colony count followed by Fusarium (12.7%), Alternaria (7.0%) and Curvularia (6.6%). Cladosporium, Penicillium, Helminthosporium, Rhizopus and sterile black mycelia were reported equally dominant. Least concentration of spores has been recorded for Cunninghamela, Phytophthora, Pythium, and Phomopsis whereas pathogenic fungal genera including *Chaetomium*, Nigrospora, Trichothecium and sterile white mycelia were reported to appear in moderate concentration. It may be concluded that distribution of diverse group of viable fungal spores in variable concentration in response to climate of high humidity and low temperature over vegetable growing field may cause allergic disorders to related farmers.

Keywords: Fungal propagules, allergy, climate, deterioration, mycelia.

I. INTRODUCTION

Fungal spores in ambient particulate matter is one of the major constituents which can adversely affect human health. These spores contain significant amounts of mycotoxins hence due to their inhalation causes allergy, asthma, pneumonitis, hypersensitivity pneumonitis, tremors, chronic fatigue syndrome, kidney failure, and cancer. Airborne fungal spores originally created from plant, animal and soil sources, due to minute size, they remain suspended in the atmosphere for a long time, get airborne during day in the afternoon, carried to a long distance, suddenly deposits on epidermal region of plant parts and may cause diseases to diverse group of healthy plants [1, 2, 3]. They are implicated in damage of food commodities, spoilage of stored grains, fruits, food stuff, in deterioration of organic material and their high concentration of mycotoxins may cause health hazards [4]. An irrigation area of vegetable cultivation field is great potential of ambient atmospheric humidity where the severity of these disorders in the farmers has been lined to airborne level of fungal spores. The distribution of propagules of these microbes in the atmosphere varies from place to place attributed to variation in climate, season, geographical location, vegetation flora combination [5, 6, 7].

The farming practices like irrigation of land for vegetable cultivation for significant productivity help to enhance a level of atmospheric humidity to greater extent together with plant transpiration whereas respiratory activity of biotic components increase CO₂ concentration that stimulate fungal sporulation suggesting that levels of the airspora correlate with humidity and temperature [8]. Inhaled fungal spores by farmers may land on sensitized lining of a nose, the conjunctiva of eye, and mucous membranes of airway or they get inhaled into the depth of lungs and their allergy causes incessant sneezing, itchy eyes and severe seasonal asthma [9]. Usually these spores cause no trouble to most of the human population but they can be harmful by provoking allergic responses or infections and cause disorders, bronchial asthma, allergic rhinitis, migraine, urticaria, eczema, atopic dermatitis etc. in some segments of human population [10]. Several investigations have been made on aeromycoflora on various parts of the globe due to their relationship with plants, animals and human disorders [11, 3, 12, 7]. Literature survey revealed that allergy asthma is a common disorder amongst the farmers in cold winter season, there is a great need for undertaking, aerobiological studies of these fields, it seemed to be worthwhile to report a more comprehensive and systematics of aeromycoflora over vegetable cultivation area under irrigation in Rubi season.



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II. MATERIALS AND METHODS

The field under cultivation of vegetables has been selected as sampling site. At the height of three feet from ground level, the petri plates containing sterile Potato Dextrose Agar nutrient medium composed of peeled potato (200gm⁻¹), dextrose (20gm⁻¹) and agar (20gm⁻¹) in one liter distilled water were exposed in triplicates for 10-15 minutes in afternoon between 11.00 to 11.30 a.m., in different locations at one week interval for two month (Dec-Jan). The exposed petri plates were sealed with cellophane tape and brought to laboratory. Fungal airspora was counted employing culture plate exposure method [13].

After incubation of the exposed petri plates containing viable fungal propagules at 25 to 28^oC in B.O.D. incubator for 3 to 5 days, the developed colonies were counted, isolated and identified after sub-culturing on Czapek's nutrient medium in tube slants. Literature, micro- & macro morphology and reverse surface coloration of colonies on Czapek's medium were used for species identification and finally authenticated by authority.

III. RESULTS AND DISCUSSION

Aeromycoflora is known to cause damage to diverse group of healthy plants resulting economic loss by reducing yield potential and their high concentration of mycotoxins may cause health hazards [1, 2, 7]. The existence of viable fungal propagules over the vegetable cultivated field under irrigation is receiving the great attention with the framework of potential health hazards to both vegetation flora and fauna including human beings. Great concern has been expressed about potential health hazards to a segment of farmers' remained engaged in farming in the field with special focus on allergenic or toxigenic microfungal flora and their association with air quality. The present study aims to record diverse group of viable propagules of fungal origin over vegetable cultivated area under irrigation following culture plate exposure method during Rabi season of winter.

Aeromycological analysis revealed prevalence of a population of altogether 2085 fungal colonies categorize under 19 genera and 35 species in the area understudy. Ascomycota dominated with 44.7% fungal spora exhibiting greater concentration followed by Deuteromycota contributing 41.1%. Moderate concentration was recorded with Zygomycota and sterile white mycelia while Oomycota had least count. Basidiomycetous spores did not persist. Among the isolates, an ascomycetous genus *Aspergillus* dominated the aeromycoflora representing higher count of species followed by *Fusarium* and *Alternaria* with four and three species each respectively. *Cladosporium, Curvularia, Helminthosporium* and *Penicillium* detected with two species each while individual species was detected for remaining genera (Table I).

Members of Oomycota appeared in 31 colonies representing 2 genera and 2 species. *Phytophthora infestans* was recorded dominant over *Pythium aphanidermatum*. Altogether 168 colonies were detected on agar jelly for Zygomycota representing 3 genera and 3 species. Among these, *Rhizopus stolonifer* was recorded dominant over other two isolates, *Mucor pusillus* and *Cunninghamela elegans*. Ascomycota dominated with a count of 932 colonies representing 4 genera and 11 species. *Aspergilli* contributed with higher (36.0%) concentration followed by *Penicilli* (5.9%) while *Chaetomium glabosum* and *Phomopsis* had least colony count. Deuteromycota dominated as second highest group with 857 fungal colonies representing 8 genera and 17 species of diverse nature. The dominant isolates in this group included *Fusarium* (12.7%), *Alternaria* (7.0%) and *Curvularia* (6.6%). Both genera, *Helminthosporium* and *Cladosporium* were detected in equal concentration (4.9%) while remainings were confined in the frequency ranged between 1.1 - 2.4%. Among other types, sterile hyphae with few chlamydospores contributed 4.6% of total colonies. Among these, black sterile mycelia was dominant over white sterile mycelia (Table II).

Among the isolates, *Aspergillus* contributed one-third of the total colony count followed by *Fusarium, Penicillium, Alternaria, Rhizopus, Curvularia, Cladosporium, Helminthosporium* and *sterile black mycelia* with chlamydospores. *Fusarium* was recorded second highest dominant genus against remainings followed by *Alternaria*. The genera, *Cladosporium* and *Helminthosporium* were detected equally significant while *Penicillium, Rhizopus, Curvularia, Helminthosporium* were recorded subdominant. Fungal spora of well-known storage fungal genera, *Aspergillus amestolodami, A. niger, A. flavus, A. candidus, Rhizopus stolonifer, Curvularia lunata were* seemed to be prevalent in significant concentration followed by *Fusarium oxysporum, Penicillium citrinum, P. digitatum, Alternaria alternata, Cladosporium cladosporoides, C. fulvum, Helminthosporium speciferum* and *sterile black mycelia*. Excepting isolates, *Phytophthora infestans, Phomopsis sp., Pythium aphinodermatum, Cunninghamela elegans* other members confined in

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range of 1.1 – 2.7 % (Table I). *Rhizopus* of Zygomycota, *Aspergilli & Penicilli* of Ascomycota; *Alternaria, Cladosporium, Curvularia, Fusarium, Helminthosporium* of Deuteromycota contributed as major components; represented a group of taxa of cosmopolitan fungal microbes that can exploit virtually any organic substrate (Jyoti and Malik, 2013). Minor components included 14 airborne isolates which are less frequent and sporadic type. Other stable components recorded were *Cunninghamela elegans, Diplodia sp., Pythium aphanidermatum,* and sterile mycelia. *Aspergillus sulphureus, Phomopsis sp. Fusarium culmorum, Nigrospora sp., Trichothecium roseum* were rare in samples, prevailed only 4-6 times during sampling.

The culture plate exposure method was confined to be more precise in isolation of aeromycoflora over vegetable cultivated area in rubi season is attributed to certain advantages [3, 7, 11]. Under set of optimum climate of atmosphere, fungal propagules grow and multiply profusely with different shades against other diverse group of microbes [15]. *Aspergilli* dominated over other isolates, exhibiting higher count of species and contributed 36.0% colonies (Fig.1 & 2) hence *Aspegillosis* is supposed to be a common disorder among the farmers who remain engaged in practices of survey area. It is in agreement with an earlier findings [9] who have reported significant concentration level of *Aspergilli* in the ambient air over some crop field. These results are confirmed with earlier findings [16, 12]. The cellulosic products provide sugar rich substrate for many fungal species The dead remains of plants, cow dungs are the common cellulose rich products in the field that form an ideal nutrient rich substrate for development of fungal organisms as bio-deteriogens. Cellulose degrading species of *Aspergilli* and *Penicilli* are abundantly reported on these nutrient rich substrates [17]. Researcher [18] reported 32% *Penicillium* and 28% *Aspergillus* on cellulosic material. These substrates may act as rich source of carbon and nitrogen for microfungal organisms.

Aspergillus niger has potential to produce ochratoxin-A. Aspergillus flavus secretes aflatoxin and other toxic compounds including strigmatocystin, cyclopiazonic acid, kojic acid, β -nitropropionic acid, aspertoxin, aflatrem, gliotoxin and aspergillic acid. Penicillium secretes penicillic acid, causing systemic penicilliosis [19]. Members of Helminthosporium have been reported to produce Helminthisporin; Curvularia lunata produces 2-methyl-(5-hydroxy methyl) furan-2 carboxylate. Fusarium secretes trichothecenes (T-2 toxin, HT-2 toxin, deoxy-nivalenol & nivalenol), zearalenone and fumonisins that have been suspected of causing toxicity in human. Fusarium solani and F. moniliformae were reported to cause keratitis and also associated with wound and infections of the eyes and fingernails [19]. Several species of Alternaria are reported to secrete Altersolarol-A and alternaric acid dibenzopyron, tetranic acid, altertoxin-I & II, alternariol, alternariol monomethyl ether, tentoxin, tenuazonic acid, altertoxins, stemphyltoxin III [20]. Alternaria solani is a major constituent of fungal bio-aerosol [13]. The dispersion of Cladosporium spores is more influenced by meteorological parameters than Alternaria spores [21].

Fungal spores are appeared frequent throughout post-rubi season particularly in month of Dec.-Jan. The peak period of fungal spore concentrations was confined in second week of January which is marked by ambient climate of 20 -25^oC temperature and 85% to 90% RH favors dissemination of fungal spores in the air. The spore concentration was confined 8.7% in first week of December, their concentration was increased to12.1% and again declined to 10.3% in third week. Gradual increase in spore concentration from fourth week of December to second week of January was recorded and again exhibited declining trend. It is in agreement with earlier findings [22] who reported greater concentration of aeromycoflora over rice field in Rubi season in the state of West Bengal, India. These results are confirmed with earlier findings [16, 11, 3]. It seems possible due to fluctuating temperatures and relative humidity which stimulate fungal growth. The fungi failed to sporulate were categorized under "*sterile forms*" and detected regularly throughout a period of survey. It is interesting to note that post-monsoon season correlate closely with a period of highest atmosphere ascomycetous and deuteromycetous mould over vegetable cultivated area in rubi season.



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Table I: Concentration of diverse aeromycoflora over vegetable cultivated field in Rubi season.

S N		Number of fungal colonies								Total	Per cent frequency			
5.IN	Fungal isolates	1-wk	2.wk	December 3-wk	r 4-wk	Total	1-wk	2.wk	January 3-wk	4-wk	Total	coloines	Species	Genera
А	Oomycota	3	$\frac{2-WR}{2}$	2 (0.10)	5 (0.24)	10 12 (0.58)	5 (0.24)	7 (0.34)	4 (0.19)	3 (0.14)	19	31	1.49	1.49
1	Phytophthora infestans de Bary	2 (0.10)	(0.10) 1 (0.05)	1 (0.05)	3 (0.14)	7 (0.34)	3 (0.14)	5 (0.34)	2 (0.19)	2 (0.14)	12 (0.58)	19	0.91	0.91
2	Pythium aphanidermatum (Edson) Fitzp	1 (0.05)	1 (0.05)	1 (0.05)	2 (0.10)	5 (0.24)	2 (0.10)	2 (0.10)	2 (0.10)	1 (0.05)	07 (0.34)	12	0.58	0.58
В.	Zygomycota	18 (0.86)	16 (0.77)	21 (1.01)	17 (0.82)	72 (3.45)	19 (0.91)	22 (1.06)	24 (1.15)	31 (1.49)	96 (4.60)	168	8.06	8.06
3	Cunninghamella elegans Lendner	3 (0.14)	1 (0.05)	1 (0.05)	-	4 (0.19)	3 (0.14)	-	-	1 (0.05)	4 (0.19)	08	0.38	0.38
4	Mucor pusillus Lindt	5 (0.24)	5 (0.24)	6 (0.29)	2 (0.10)	15 (0.72)	7 (0.34)	10 (0.48)	10 (0.48)	12 (0.58)	39 (1.87)	57	2.73	2.73
5	Rhizopus stolonifer Eh. Ex.Rr.)Lind.	10 (0.48)	11 (0.53)	14 (0.67)	15 (0.72)	26 (1.25)	9 (0.43)	12 (0.58)	14 (0.67)	18 (0.86)	53 (2.54)	103	4.94	4.94
C.	Ascomycota	82 (3.93)	103 (4.94)	97 (4.65)	113 (5.42)	395 (18.9)	126 (6.04)	151 (7.24)	139 (6.67)	121 (5.80)	537 (25.8)	932	44.7	44.7
6	Aspergillus amstelodomi (Mang) Thom & Church	5 (0.24)	10 (0.48)	12 (0.58)	8 (0.38)	35 (1.68)	15 (0.72)	16 (0.77)	15 (0.72)	11 (0.53)	57 (2.73)	92	4.41	
7	Aspergillus candidus Link	7 (0.34)	14 (0.67)	16 (0.77)	12 (0.58)	49 (2.35)	16 (0.77)	18 (0.86)	19 (0.91)	16 (0.77)	69 (3.30)	118	5.66	
8	A. flavus Link.	18 (0.86)	22 (1.06)	24 (1.15)	26 (1.25)	90 (4.32)	18 (0.86)	28 (1.34)	25 (1.20)	23 (1.10)	94 (4.51)	184	8.82	
9	A. nidulans (Eidam) Winter	4 (0.19)	6 (0.29)	1 (0.05)	4 (0.19)	15 (0.72)	4 (0.19)	5 (0.24)	7 (0.34)	6 (0.29)	22 (1.06)	37	1.77	26.0
10	A. niger Van Tieghen	28 (1.40)	25 (1.11)	28 (0.99)	32 (1.64)	113 (5.15)	35 (1.52)	36 (1.70)	38 (1.35)	35 (1.23)	144 (5.79)	257	12.3	36.0
11	A. sulphureus (Fres.)Thom & Church	2 (0.10)	4 (0.19)	-	-	6 (0.29)	6 (0.29)	7 (0.34)	2 (0.10)	3 (0.14)	18 (0.86)	24	1.15	
12	Aspergillus versicolor (Vuill.) Tiraboschi	6 (0.29)	2 (0.10)	2 (0.10)	8 (0.38)	18 (0.86)	5 (0.24)	8 (0.38)	6 (0.29)	3 (0.14)	22 (1.06)	40	1.92	
13	Chaetomium glabosum Kunze & Schm	-	6 (0.29)	4 (0.19)	2 (0.10)	12 (0.58)	8 (0.38)	6 (0.29)	5 (0.24)	6 (0.29)	25 (1.20)	37	1.77	1.77
14	<i>Penicillium citrinum</i> (C & S) Pitt.	7 (0.34)	8 (0.38)	6 (0.29)	8 (0.38)	29 (1.39)	11 (0.53)	11 (0.53)	9 (0.43)	5 (0.24)	36 (1.73)	65	3.12	
15	<i>Penicillium digitatum</i> (Pers. Ex. Fr.) Sacc.	5 (0.24)	6 (0.29)	4 (0.19)	8 (0.38)	23 (1.10)	8 (0.38)	12 (0.58)	8 (0.38)	7 (0.34)	35 (1.68)	58	2.78	5.90
16	Phomopsis sp.	-	-	-	5 (0.24)	5 (0.24)	-	4 (0.19)	5 (0.24)	6 (0.29)	15 (0.72)	20	0.96	0.96
D.	Basidiomycota	- 72	- 120	- 83	- 91	366	- 98	- 164	- 122	- 107	491	-		
Е.	Deuteromycota	(3.45)	(5.56)	(3.98)	(4.36)	(17.6)	(4.70)	(7.87)	(5.85)	(5.13)	(23.6)	857	41.1	41.1
17	(Fr.) Keissler	(0.34)	(0.29)	(0.24)	(0.34)	(1.20)	(0.34)	(0.53)	(0.43)	(0.38)	(1.68)	60	2.88	
18	(E & M) Jones & Grout	3 (0.14)	7 (0.34)	6 (0.29)	6 (0.29)	22 (1.06)	8 (0.38)	10 (0.48)	8 (0.38)	5 (0.24)	31 (1.49)	53	2.54	7.0
19	Alternaria porri (Ells) Cif.	3 (0.14)	4 (0.19)	5 (0.24)	-	12 (0.58)	4 (0.19)	9 (0.43)	4 (0.19)	4 (0.12)	21 (0.76)	33	1.58	
20	Cladosporium cladosporoides Link	6 (0.29)	8 (0.38)	8 (0.38)	9 (0.43)	31 (1.49)	6 (0.29)	7 (0.34)	8 (0.38)	9 (0.43)	30 (1.44)	61	2.93	4.85
21	<i>Cladosporium fulvum</i> Cooke.	1 (0.05)	6 (0.29)	5 (0.24)	3 (0.12)	15 (0.64)	5 (0.24)	10 (0.48)	6 (0.29)	4 (0.19)	25 (1.20)	40	1.92	ч.0 <i>3</i>
22	Curvularia lunata (Wakker) Boedijn	7 (0.34)	9 (0.43)	8 (0.38)	8 (0.38)	32 (1.53)	10 (0.48)	16 (0.77)	14 (0.67)	14 (0.67)	54 (2.59)	86	4.12	
23	Curvularia ovoides (H & W) Munt-Cvetk)	4 (0.19)	7 (0.34)	6 (0.29)	6 (0.29)	23 (1.10)	8 (0.38)	11 (0.53)	7 (0.34)	5 (0.24)	31 (1.49)	54	2.59	6.61
24	Diplodia sp	6 (0.29)	9 (0.43)	-	6 (0.29)	21 (1.01)	7 (0.34)	10 (0.48)	6 (0.29)	5 (0.24)	28 (1.34)	49	2.35	2.35

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25	Fusarium moniliformae Sheldom	6 (0.29)	7 (0.34)	6 (0.29)	6 (0.29)	25 (1.20)	8 (0.38)	12 (0.58)	6 (0.29)	6 (0.29)	32 (1.53)	57	2.73	
26	Fusarium oxysporum Schlecht	8 (0.38)	12 (0.58)	10 (0.48)	10 (0.48)	40 (1.92)	9 (0.43)	11 (0.53)	9 (0.43)	11 (0.53)	40 (1.92)	80	3.84	
27	<i>Fusarium solani</i> (Mert.) APP. & Wollenw	9 (0.43)	10 (0.48)	8 (0.38)	8 (0.38)	35 (1.68)	8 (0.38)	12 (0.58)	8 (0.38)	8 (0.38)	36 (1.73)	71	3.41	12.7
28	Fusarium semitectum Berk & Rav.	-	4 (0.19)	4 (0.19)	4 (0.19)	12 (0.58)	4 (0.19)	8 (0.38)	8 (0.38)	2 (0.10)	22 (1.06)	34	1.63	
29	<i>Fusarium culmorum</i> (W.G. Smith) Sacc.	-	4 (0.19)	4 (0.19)	-	8 (0.18)	-	7 (0.34)	6 (0.29)	2 (0.10)	15 (0.72)	23	1.10	
30	Helminthosporium spiciferum (Bain.) Nicol	7 (0.34)	14 (0.67)	6 (0.29)	8 (0.38)	35 (1.68)	-	10 (0.48)	9 (0.43)	8 (0.38)	27 (1.29)	62	2.97	4.04
31	Helminthosporium tetramera G & A	2 (0.10)	5 (0.24)	-	4 (0.19)	11 (0.53)	8 (0.38)	7 (0.34)	8 (0.38)	7 (0.34)	30 (1.44)	41	1.97	4.94
32	Nigrospora sp.	-	4 (0.19)	2 (0.10)	2 (0.10)	8 (0.38)	4 (0.19)	7 (0.34)	-	4 (0.19)	15 (0.72)	23	1.10	1.10
33	<i>Trichothecium roseum</i> Link	3 (0.14)	4 (0.19)	-	4 (0.19)	11 (0.53)	2 (0.10)	6 (0.29)	6 (0.29)	5 (0.24)	19 (0.91)	30	1.44	1.44
	Other types	7 (0.34)	10 (0.48)	11 (0.58)	8 (0.38)	36 (1.73)	15 (0.72)	12 (0.58)	17 (0.82)	17 (0.82)	61 (2.92)	97	4.65	4.65
34	Sterile black mycelia	5 (0.24)	8 (0.38)	9 (0.43)	8 (0.38)	30 (1.44)	9 (0.43)	9 (0.43)	11 (0.53)	13 (0.62)	42 (2.01)	72	3.45	3.45
35	Sterile white mycelia	2 (0.10)	2 (0.10)	2 (0.120	-	6 (0.29)	6 (0.29)	3 (0.14)	6 (0.29)	4 (0.19)	19 (0.91)	25	1.20	1.20
	Total colonies	182	251	214	234	881	263	356	306	279	1204	2085	100	
	Per cent frequency	8.7	12.1	10.3	11.2	42.3	12.6	17.1	14.6	13.4	57.7			

Table II : Division wise distribution of aeromycoflora on brinjal cultivated field in rubi season.										
			Period of							
S.N	Fungal group	Dece	ember	Jai	nuary	Total	Per cent contribution			
		Colony	Percent	Colony	Percent	colonies				
		count	contribution	count	contribution					
1.	Oomycota	12	0.6	19	0.9	31	1.5			
2.	Zygomycota	72	3.5	96	4.6	168	8.1			
3.	Ascomycota	395	18.9	537	25.8	932	44.7			
4.	Basidiomycota	-	-	-	-	-	-			
5.	Deuteromycota	366	17.6	491	23.5	857	41.1			
6.	Sterile mycelia	36	1.7	61	2.9	97	4.6			
Total colonies		881	42.3	1204	57.7	2085	100			





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IV. CONCLUSIONS

Environmental fungal organisms are responsible for spread of diseases in response to transport of their viable propagules even to a small distance in favourable season. The results of present survey revealed variation in content of fungal spores relates to ambient climate of high humidity and low temperature over vegetable cultivation area in *rubi* season. Some viable fungal propagules in air may be responsible for a plant diseases and variety of respiratory diseases in humans and animals. Prevalence of higher concentration of viable fungal spores over vegetable cultivation may cause allergic disorders to a segment of farmers involved in farming. Monitoring of environmental mycoflora can be helpful in prevention of *Aspegillosis* and other fungal allergic disorders.

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