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Biodiversity Fungal Flora of Outdoor Environment of Botanical Garden

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Abstract:

Mycological survey of outdoor environment of at various location of botanical garden of P.G. Department of Botany, RTM Nagpur University; Nagpur has been conducted for a period of a month at an interval of a week employing petri plate exposure method. The petri plates with sterile nutrient jelly were exposed to air in afternoon in weekly visit and after incubation diverse group of several fungal propagules spores adhered to film of nutrient jelly forms colonies in variable count. A count of altogether 3026 fungal colonies belongs to 15 genera and 21 species were recorded. *Deuteromycota* dominated with more than two-third of total count of colonies, representing a largest contributor followed by *Ascomycota. Cladosporium* contributed one-quarter of total colony count, exhibiting most dominant contributor followed by *Fusarium*, *Alternaria, Curvularia* and *Aspergillus*. The fungal isolates, *Nigrospora, Penicillium, Pseudotorula, Rhizopus* and *Torula* represented 1-4% of sum total colonies while others were recorded frequently with less than one per cent of total count. Greater count of fungal propagules remained viable in 2-week of January, thereafter their viability gradually declined to 20.8% in 1-week of February. *Aspergillus* dominated with highest four species; *Alternaria, Curvularia* and *Fusarium* represented with 2 species while other genera had single species. The prevalence of diverse group of viable fungal propagules in outdoor environment of botanical garden may cause allergic, respiratory and other incurable disorders to visitors including garden employees.

Keywords: *Fungal spores, allergy, environment, frequency, mycelia*

Introduction:

Aeromycoflora are considered to act as indicator of the level of atmospheric bio-pollution. The existence of fungal propagules, volatiles and mycotoxins in the air can cause a health hazard in all segments of the population (Kayarkar and Bhajbhuje, 2014). More than 80 genera of fungi have been associated with respiratory tract allergy (Ghosh et al, 2011). Majority of allergenic fungi are class under Ascomycota and Deuteromycota with a few in other fungal divisions (Bhajbhuje, 2013). Disease expression is affected by the degree of exposure. Repeated exposures to large concentrations of spores may cause severe symptoms of respiratory allergy (Sunita Kumari, 2011). The prevalence of respiratory allergy to fungi is estimated at 20 to 30% among atopic individuals and up to 6% in general population (EFSA, 2011). It is of the most importance that allergens, viable microbes, and other noxious agents that prevail in any particular environment are induced by changes in meteorological conditions. Very little was reported on the impact of airborne fungi in outdoor environments (Ghosh et al., 2011). Fungal air-spora are implicated in the deterioration of cellulosic materials (Kayarkar and Bhajbhuje, 2014).

Airborne fungi occur as single units, spores and occasionally as hyphal fragments, conidiophores, associated with inorganic particles (Ghosh et al., 2011). The count and type of fungi vary with time of day, weather and seasonal fluctuation, condition of the surrounding areas, climatic conditions and with the

presence of a local source of spores (Chelak and Sharma, 2012). The plants are known to produce essential oils and aerosol, which showed sporistatic, fungistatic, and fungicidal activities possibly reduces viability of airspora. Increase of CO_2 concentration stimulates fungal sporulation suggesting that levels of the airspora correlate with air pollution (Sharma et al., 2011). In addition, increase of SO_2 can reduce airborne fungi concentration (Nafis and Sharma, 2012).

The distribution of airborne fungi in botanical garden may differ from location to location because of differences in climate and vegetation (Ghosh et al., 2011). The examination of common airborne fungi distribution in a particular region can be helpful in identifying association between fungal sensitization and clinical diagnosis and clinical prevention of the seasonal allergic diseases (Chelak and Sharma, 2012). A little is known about aeromycoflora of botanical garden. Presently, prevalence of environmental mycoflora has so far not been highlighted from this area, it seemed to be worthwhile to undertake a more comprehensive and systematic study of the prevalence of diverse group of microfungal spores during winter in outdoor environment of botanical garden.

Material and Methods:

The botanical garden located in front of P.G. Department of Botany, RTM Nagpur University, Nagpur has been selected as sampling site. The samples of different locations were collected at one week interval for a month (Jan-Feb) on sterile potato dextrose agar (PDA) nutrient medium in petri plates composed of peeled potato (400gm^{-1}), dextrose (20gm^{-1}) and agar (20gm^{-1}) in a liter of distilled water. Fungal airspora was counted employing culture plate exposure method (Kayarkar and Bhajbhuje, 2014).

Petri plates containing PDA nutrient jelly was exposed in triplicate for 5-7 minutes at sampling site in weekly visit, in afternoon between 11.00 to 11.30 p.m., placed at a meter height. The exposed petri plates were sealed with cellophane-tape, brought them to laboratory and incubated at laboratory climate for 3 to 5 days depending upon growth of colonies. The developed colonies were counted, isolated and identified after sub-culturing on tube slants containing Czapek's nutrient media. Literature, Micro- & macro morphology and reverse surface coloration of colonies on Czapek's medium were used for species identification and finally authenticated by authority.

Result and Discussion:

The existence of viable fungal propagules in the outdoor environment in botanical garden is receiving the greater attention with the framework of potential health hazards to diverse group of biotic elicitors including human beings. The viable microfungal propagules in atmosphere, may remain in the same environment or carried to a long distance far away from existing condition by abiotic elicitors particularly wind, may deposited on healthy flora can caused many plant diseases, hence the knowledge of their periodicity is of great concern in terms of predicting the plant epidemics (Chelak and Sharma, 2012). The present survey aims to record biodiversity of fungal airspora in outdoor environment of botanical

garden employing culture plate exposure method during winter (Jan-Feb. 2015) as majority reports revealed prevalence of higher concentration of fungal airspora during initiation of winter season. Results are presented in table 1.

During present survey a count of total 3026 fungal colonies belongs to 15 genera and 21 species has been recorded for a month (Jan- Feb. 2015) in outdoor environment of botanical garden *Deuteromycota* dominated with more than two-third (86.8%) fungal airspora exhibiting greater concentration followed by *Ascomycota,* contributed 7.1%. The depletion of concentration was recorded with *Zygomycota*, contributing 1.8% while sterile mycelia represented 3.5% of the total colonies count (Table 1). Fungal spores from *Oomycota Mestigo-* & *Basidiomycota* did not seem to survive at sampling site (Table 2).

The distribution of viable fungal propagules in outdoor environment relates to vegetation and environment factors including light intensity temperature and humidity. In 2-week of January, of the total, a count of 34.4% fungal colonies was encountered on nutrient jelly medium, representing higher colony count. Altogether, 22.7% and 21.9% colonies isolated in 3-week and 4-week of January while 1-week of February had least count of colonies (Table 1).

In *Zygomycota*, total 56 fungal colonies were recorded in winter season representing *two* genera. Higher, a count of 27 colonies was encountered on agar jelly in 3-week of January followed by 22 colonies in 1-week of February and least colonies in 4-week of January. Fungal species did not appear in 2-week of January. Among genera, *Rhizopus stolonifer* contributed in maximum (1.4%) concentration while *Mucor pusillus* had least count. Altogether 222 fungal colonies of *Ascomycota* were encountered representing *two* genera and *four* species. Greater count of colonies (169) was reported 2-week of January and least in 3-week. *Aspergilli* contributed in high (5.4%) concentration as compared to *Penicillium* sp which contributed in least concentration (Table 1).

In *Deuteromycota*, 2641 fungal colonies were recorded representing *nine* genera and *twelve* species of diverse nature. Greatest count of colonies was recorded in 2-week of January followed by a count of 640 and 602 colonies in 3-week and 4-week of January *respectively* while least count was confined in 1-st week of February. The dominant fungal species recorded in this group belong to genera *Cladosporium* (25.1%), *Fusarium* (24.4%), *Curvularia* (15.5%), and *Alternaria* (13.7%). Others were appeared in abundance varies between 0.2-.3.2%. Among other types, sterile hyphae with few *chlamydospores* contributed 3.5% of total colonies recorded. Maximum 57 fungal colonies appeared in 1-week of February while a count of moderate count was encountered in month of January. The contribution of white sterile mycelia was comparatively greater over back sterile mycelia (Table 1).

The common isolate, *Cladosporium cladosporiodes* was recorded most dominant contributor representing 25.1% of the total colonies count followed by *Fusarium* (24.4%), *Curvularia* (15.5%) and *Alternaria* (13.7%). The isolates, *Aspergillus, Nigrospora, Torula, Sterile white mycelia* were appeared to be moderately significant with 2.0 – 5.4% colony count. Other members, *Penicillium,*

Rhizopus, Pseudotorula, Helminthosporium, Mucor, Bipolaris and *sterile black mycelia* represented 0.2 – 1.7% of the total colonies *Aspergillus* dominated with four species exhibiting highest count of species; followed by *Alternaria, Curvularia* and *Fusarium* represented with 2 species while other genera had single species (Table 1).

The members of *Deuteromycota, Cladosporium, Penicillium, Curvularia* and *Alternaria* contributed as major components; represented a group of taxa of cosmopolitan fungal organism that can exploit virtually any organic substrate (FESA, 2011). Minor components included *eleven* airborne microfungal genera. Major components included most frequently encountered genus *Cladosporium* and *Fusarium* while minor components included *less frequent* and *sporadic* types. Other stable components recorded were *Mucor pusillus, Rhizopus stolonifer* and sterile mycelia. *Nigrospora, Torula* and *Pseudotorula* were rare in samples, reported only 2-4 times during sampling (Table 1).

The use of culture plate exposure method was proved to be more appropriate in present study due to certain advantages (Sharma 2010; Bhajbhuje, 2013). The winter season lof central India is known for very pleasant weather with average minimum temperature of around 10^{0}C and high humidity. This moderately cold season is seemed to be ideal for rapid multiplication and enhancement the growth rate of biotic community including fungal organism. It was interesting to note that maximum, 34.4% outdoor environmental fungal flora of diverse nature was appeared in 2-week of January, begins declining in subsequent period and recorded least (20.8%) viable in 1-week of February, where average minimum temperature was below 7.0 ^{0}C (Fig. 2). Marginal reduction in number of fungal colonies to 22.7% and 21.3% has been recorded in 3-week and 4-week of January, with slightly cold environment where an average daily mean temperature was seemed decline to 23.0^{0}C. These results are in confirmation with reported earlier (Ghosh et al., 2011; Chelak and Sharma. 2012; Bhajbhuje, 2013, Kayarkar and Bhajbhuje, 2014). The variation in population of fungal flora of industrial environment in winter season may be attributed to fluctuating temperatures and relative humidity, which support fungal growth for some group of organisms and act inhibitory for others. The initial period of winter correlate closely with a period of highest atmosphere Deuteromycetous followed by Ascomycetous microfungal flora. It was interesting to note that fungal spores from *Oomycota* and *Basidiomycotina* did not appear may be possibly attributed to variation in environmental climate (Ghosh et al., 2011).

Diverse group of microfungal species of saprophytic nature grow profusely on organic substrates with different shades as compared to other group of microbes, producing allergens, secondary metabolites and other toxins that are seemed to cause many allergic and respiratory disorders (MBL, 2012). *Cladosporium* and *Fusarium Aspergillus* was dominated in botanical garden with 25.1 and 24.4% colonies respectively (Fig.3). It is in agreement with an earlier finding of Ianovici (2008) who reported higher concentration of spores of *Cladosporium* in outdoor environment. *Cladosporium* has been most correlated with meteorological parameters, may be attributed to appearance of dry conidia in

chains, which can easily carried through air reasonably dispersion of spores was more influenced by meteorological parameters than *Alternaria* spores.

The genus *Fusarium*, a most prevalent toxin-producing deuteromycetous fungal organism contributed with 24.4% of the total colonies count (Fig.3), reported to secrete diverse range of mycotoxins includes *trichothecenes* (*T-2 toxin, HT-2 toxin, deoxy-nivalenol & nivalenol)*, *zearalenone* and *fumonisins* many of them had significant impacts on human health (MBL, 2012). These toxins have been shown to cause a variety of toxic effects in both experimental animals and livestock and also suspected of causing toxicity in human. The *zearalenone* is naturally occurring endocrine disturbing compound induce gastro-intestinal effects and precocious pubertal changes. Many other secondary metabolites (*monoliformin, beauvericin*, and *fusaproliferin*) are known to be secreted by different *Fusaria* and their effects on human health, either alone or in combination with other mycotoxins, are largely unexpected (MBL, 2012). *F. moniliformae* causes *invasive mycoses* in immune-compromised people. It has inhalation and deep skin inoculation health risks to persons with weak immune system. (Shephard, 2012).

The genus *Aspergillus* represented with four species, exhibiting higher count of species among other genera reported (Table 1). Sharma et al (2011) have reported comparatively higher concentration of *Aspergillus niger, A. fumigatus* and *A. flavus* than others in outdoor the aeromycoflora. *Aspegillosis* becomes a common disorder among the human population. The dead remains of plants and some quantity of garbage in the garden contribute to make the environment extremely supportive for fungal attack to the nutrient rich substrate. The cellulosic raw material and its products are rich source of sugar while lipids, glycides of leather provide protein rich substrate for many fungal species. Microbial deterioration of cellulose fibers (Anderson et al 2012) was very well established facts garden area. *Aspergilli* and *Penicilli* were abundantly reported on these nutrient rich substrates, involved in degradation (Sharma et. al., 2011). Ramamurthy et al., (2011) reported 32% *Penicillium* and 28% *Aspergillus* on cellulosic material. These substrates may act as carbon and nitrogen source to microfungal organisms.

The liberation of spores follows dispersion mechanism; both are interrelated and related to wind velocity, weather and other existing environmental conditions. The concentration of fungal flora in outdoor environment has been confined greater for *Deuteromycotina* (Fig. 2) may relate to existing weather in winter. The spores' liberation of *Aspergilli* and *Penicilli* of *Ascomycotina* were favored by high air humidity and while those of *Alternaria, Cladosporium* and *Helminthosporium*, of *Deuteromycotina* were liberated mechanically by the action of wind (Ianovici, 2008). Spore dispersal of Deuteromycota is therefore favored by slight higher temperature with low humidity while high relative humidity and low temperature supports spore dispersal of *Ascomycota*. The prevalence of such variable conditions at different times may help to explain differences in the observed periodicities (Chelak and Sharma, 2012).

Rhizopus stolonifer and *Mucor pusillus* of Mucorales contributed 1.8% of the total colony count have been linked with a common disorder, Zygomycosis and

mucormycosis allergies, and mold sensitivity. Its spore's inhalation caused mucocutaneous & rhinocerebral infections, septic arthritis, renal infections, gastritis and severe pulmonary infection, and difficulty in breathing (Smith, 2013). Its metabolic products induced significant inhibition of human serum vitamin C and Fe, Cu concentrations to a greater extent and the inhibition was proportional with an increase in concentrations of the metabolic products (Al-Jubury et al., 2012).

Deuteromycotina contributed with 87.3% of the total colonies count (Fig. 1). The dominant microfungal genera of this group includes *Cladosporium Alternaria, Curvularia, and Fusarium* are known cellulosic, grows profusely as parasite on plant vegetation as well as involved as saprophyte in biodegradation of debris of plant dispersing numerous conidia in the outdoor environment. *Alternaria* was appeared to be dominated with 13.6% of total colonies count, dispersed heat resistant conidia in afternoon in existing industrial environment. These conidia have implication to asthmatic and allergy patients. During mycelial and reproductive growth, *Alternaria* has been reported secretion of mycotoxins such as *Altersolarol-A, alternaric* acid, *dibenzopyron, tetranic acid, altertoxin-I & II, alternariol, alternariol monomethyl ether, tentoxin, tenuazonic acid, altertoxins, stemphyltoxin III* (Holensein and Stoessi, 2008) can affect respiratory system, skin, and nails in humans (Skjoth, 2012) and also induced reduction in seed germination and seedling emergence with chromosomal abnormalities in plants (Bhajbhuje, 2013). Altertoxins induced micro-mutation in diverse group of experimental animals (ESFA, 2011). The genus *Curvularia lunata* represented 15.5% of total fungal flora of outdoor environment. Its conidial inhalation from environment have been reported to cause infection to lungs, heart, nose, skin and eye, resulting in sneezing, coughing, general weakness, swelling around the eyes, and partial loss of vision. Once conidia gets into the blood stream may lead to endocarditis (MBL, 2012).

The distinction between dry-air spores and wet-weather air spores is well known. Dry-air spores included the conidia of *Cladosporium, Alternaria, Helminthosporium, Trichothecium, Cephalosporium and Curvularia*. Members of dry-airspora were found in greatest abundance in the atmosphere characterized by low humidity, generally during warmer afternoon hours (Ianovici, 2008). With regard to spore species, viability can differ considerably; spores genera of *Deuteromycotina* remained viable after a certain period. High conc. and long lasting presence of allergenic fungal spores in the air may cause and intensify clinical symptoms in human population suffering from sensitivity and extend the period of presence of allergens in the atmosphere (Ianovici, 2008).

The spores of fungal origin of diverse group, excluding Oomycota and Basidiomycota were remained frequent throughout the survey period of month duration (Fig. 1). The peak period of fungal spore concentrations was recorded in the 2-week of January of the winter season as the moderate cold climatic condition during this time (temperature ranges between 32.2^{0}C(max.) to 23.1^{0}C(min) and relative humidity (74%) supports for dissemination of fungal spores in the environment. Marginal reduction in fungal spore concentration in 1-st week of

February may be attributed fluctuation or marginal declining of minimum temperature. These results confirmed with reported earlier (Sharma, 2010). Considerable reduction in fungal airspora at the end of survey may attributed to cold winter.The cold weather of this period may perhaps become barrier for rapid multiplication therefore growth rate slightly declined at 1-week of February for majority of microfungal organisms. It was interesting to note that a period of January of winter season correlate closely with a period of highest atmosphere *deuteromycetous* followed by *ascomycetous* mold of outdoor environment of botanical garden.

Table 1: Genera wise distribution of outdoor environment fungal flora of botanical garden

S.N	Fungal organism	Number of fungal colonies				Total colonies	Per cent Contribution	
		2-wk (Jan)	3-wk (Jan)	4-wk (Jan)	1-wk (Feb)		Species	Genera
A	**Oomycota**	-	-	-	-	-	-	**-**
B.	**Zygomycota**	**-**	**27 (0.9)**	**7 (0.2)**	**22 (0.9)**	**56**	**1.9**	
1	*Mucor pusillus*	-	12 (0.4)	-	-	12	0.4	**0.4**
2	*Rhizopus stolonifer*	-	15 (0.5)	7 (0.5)	22 (0.9)	44	1.5	**1.5**
C.	**Ascomycota**	**169 (5.6)**	**5 (0.2)**	**41 (1.4)**	**7 (0.2)**	**222**	**7.3**	
3	*Aspergillus flavus*	47 (1.6)	-	-	-	47	1.5	**36.7**
4	*A. fumigatus*	83 (2.7)	-	-	-	83	2.7	
5	*A. niger*	-	5 (0.2)	8 (0.3)	7 (0.2)	20	0.6	
6	*A. terreus*	-	-	20 (0.7)	-	20	0.6	
7	*Penicillium sp.*	39 (1.3)	-	13 (0.4)	-	52	1.7	**1.7**
D.	**Basidiomycota**	-	-	-	-	-	-	**-**
E.	**Deuteromycota**	**853 (28.2)**	**640 (21.1)**	**602 (19.9)**	**546 (18.0)**	**2641**	**87.3**	
8	*Alternaria porri*	-	24 (0.8)	120 (4.0)	-	144	4.7	**13.7**
9	*Alternaria sp.*	232 (7.7)	-		42 (1.4)	274	9.0	
10	*Bipolaris tetramera*		-	06 (0.2)	-	6	0.2	
11	*Cladosporium cladosporiodes*	263 (8.7)	206 (6.8)	148 (4.9)	144 (4.8)	761	25.1	**25.1**
12	*Curvularia lunata*	21 (0.7)	-	163 (5.4)	75 (2.5)	259	8.5	**15.5**
13	*Curvularia tetramera*	18 (0.6)	112 (3.7)	-	82 (2.7)	212	7.0	
14	*Fusarium moniliformae*	161 (5.3)	144 (4.8)	-	144 (4.8)	419	13.8	**24.4**
15	*Fusarium oxysporum*	132 (4.4)	98 (3.2)	92 (3.0)	-	322	10.6	**0.8 3.2**
16	*Helminthosporium tetramera*	26 (0.9)	-	-	-	26	0.8	
17	*Nigrospora sp.*	-	21 (0.7)	41 (1.4)	36 (1.2)	98	3.2	
18	*Torula sp.*	-	35 (1.2)	20 (0.7)	31 (1.0)	86	2.8	**2.8 1.1**
19	*Pseudotorula sp.*	-	-	12	22 (0.7)	34	1.1	

				(0.4)				
	Other types	**18 (0.6)**	**17 (0.6)**	**15 (0.5)**	**57 (0.7)**	**107**	**3.5**	
20	*Sterile black mycelia*	-	17 (0.6)	04 (0.1)	25 (0.8)	46	1.5	**1.5 2.0**
21	*Sterile white mycelia*	18 (0.6)	-	11 (0.4)	32 (1.1)	61	2.0	
	Total colonies	**1040**	**689**	**665**	**632**	**3026**	99.8	
	Per cent of total colonies	34.4	22.7	21.9	20.8			
Values in parenthesis calculated in terms of percentage over total colonies recorded								

Table 2 : Division wise distribution of outdoor environment fungal flora of botanical garden

S.N	Fungal group	Number of fungal colonies				Total colonies	Per cent Contribution
		2-wk (Jan)	3-wk (Jan)	4-wk (Jan)	1-wk (Feb)		
1.	Oomycota	-	-	-	-	-	-
2.	Zygomycota	-	27 (0.9)	7 (0.2)	22 (0.9)	56	1.9
3.	Ascomycota	169 (5.6)	5 (0.2)	41 (1.4)	7 (0.2)	222	7.3
4.	Basidiomycota	-	-	-	-	-	-
5.	Deuteromycota	853 (28.2)	640 (21.1)	602 (19.9)	546 (18.0)	2641	87.3
6.	Sterile mycelia	18 (0.6)	17 (0.6)	15 (0.5)	57 (0.7)	107	3.5
	Total colonies	**1040**	**689**	**665**	**632**	**3026**	**100.0%**
	Per cent contribution	34.4	22.7	21.9	20.8		

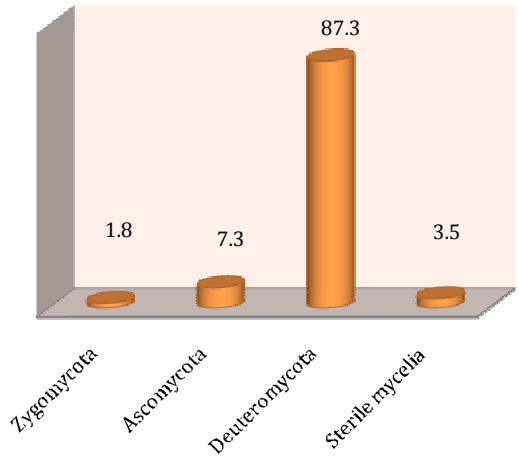

Figure. 1- Division wise per cent distribution of ouutdoor aeromycoflora of Botanical garden

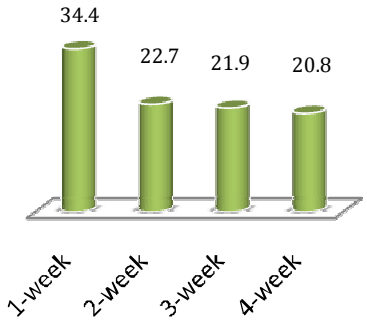

Figure. 2- Weekly distribution of aerommycoflora of outdoor environment of Botanical garden

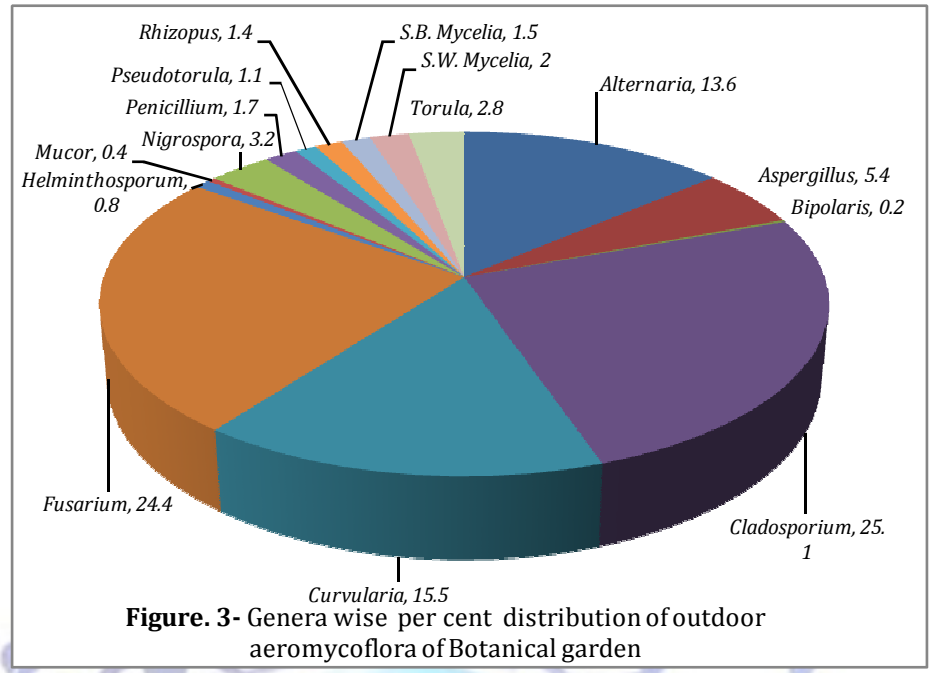

Figure. 3- Genera wise per cent distribution of outdoor aeromycoflora of Botanical garden

Conclusion:

Environmental microfungal agents are responsible causing variety of disease to flora and fauna including allergic, respiratory and other disorders in human beings. Total 3026 fungal colonies classified under 15 genera and 21 species were recorded during four week survey of outdoor environment of botanical garden. *Deuteromycota* represented largest contributors of the total airborne fungal spores followed by *Ascomycota.* Member of *Oomycota* & *Basidiomycotina* did not appear. Among genera, *Cladosporium* and *Fusarium* was largest contributor. Higher concentration of fungal propagules remained viable in 2-week of January. The climate of initial period of winter supports fungal growth. The existence of diverse group of viable fungal propagules in variable frequencies in outdoor environment of botanical garden may increase chances of allergic and respiratory disorders to population of visitors and garden employees. Monitoring of aeromycoflora of sampling site can be helpful in prevention of fungal allergic and respiratory disorders.

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