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Microfungal Diversity of Botanical garden

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Abstract: *Botanical gardens are hub for the diverse fungi and the present study deals with the micro-fungal investigation in the botanical garden of RMG Arts & Science College, Nagbhid. This study has been conducted for a period of two moths employing petri plate exposure method. Total 537 fungal colonies belong to 13 genera and 24 species were recorded. Deuteromycota dominated with more than two third of total count of colonies, representing a largest contributor followed by Ascomycota. Cladosporium was observed to be the most dominant contributor followed by Fusarium, Alternaria, Curvularia and Aspergillus. The fungal isolates like Nigrospora, Penicillium, Bipolaris, Rhizopus and Torula were recorded at moderate count. Aspergillus dominated with highest species diversity i.e.8 species; Alternaria, Curvularia and Fusarium represented with 2 species each while other genera had single species. Diversity index like Shannon Diversity Index, Simpson Diversity Index and Margalef index showed that the maximum colony count was recorded in the month of February while the maximum diversity of fungal taxa was observed in the month of January.*

Keywords: Aeromycoflora, Fungal spores, *Aspergillus*, Botanical Garden, Diversity Index.

I. INTRODUCTION

Gregory (1952) coined the term "Air spora". His classic book entitled "Microbiology of the atmosphere" is a useful source of information on pollen flora, fungal spores including his extensive microscopic examination of plant parts has been described. This was followed by the work of several others. The spores of phytopathogenic fungi contribute a small but significant portion of airborne fungal spore populations. Among the phytopathogenic fungi whose spores disperse mainly through air are rust, powdery mildews, leaf spotting fungi e.g. *Cercospora, Alternaria, Helminthosporium, Drechslera, Pyricularia* and others. These fungi sporulate abundantly to their successful aerial dispersal (Mallaiah, 1999). With the increasing problems of pollution aerobiological studies have gained new impetus. The large segment of the air borne spore flora is composed of fungal spores and therefore, studies on aeromycology have become important (Bajaj, 1988; Durham, 1946; Verma and George, 1997, Espinosa *et al*., 2024, Ortega-Rosas *et al*., 2025).

Aeromycoflora are considered to act as indicator of the level of atmospheric bio-pollution. The presence of fungal spores, volatile compounds, and mycotoxins in the atmosphere poses a health risk to all demographic groups (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 (Vijayalakshmi and Jeyachandran 2010). 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 airspora are implicated in the deterioration of cellulosic materials (Kayarkar and Bhajbhuje, 2014, Ahmad *et al*., 2025).

The presence of airborne fungi in a botanical garden can vary from one area to another due to variations in climate and plant life (Ghosh *et al*., 2011). The examination of common airborne fungi distribution in a particular region can be

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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. Currently, the prevalence of environmental mycoflora in this area has not been thoroughly examined. Therefore, it appears beneficial to conduct a more detailed and systematic investigation into the occurrence of various microfungal spores during the winter months in the outdoor environment of the botanical garden

II. MATERIAL AND METHODS

Sampling Site

The botanical garden (20º35′36″N, 79º40′34″E) located in front of RMG College, Nagbhid has been selected as sampling site. The samples of different locations were collected for a month (Jan-Feb) on sterile potato dextrose agar (PDA) nutrient medium in petri plates. The fungal taxa were quantified using the culture plate exposure technique (Kayarkar and Bhajbhuje, 2014).

Isolation and Identification

Petri plates containing PDA medium was exposed in triplicate for 5-7 minutes at sampling site in late morning between 10.00 to 11.00 p.m., placed at a meter height. The exposed petri plates were sealed with cellophane-tape, brought them to laboratory and incubated at 25±2°C for 5 to 6. The developed colonies were counted, isolated and sub cultured. The isolated fungi were identified with the help of standard literature like ‘‘Handbook of soil fungi” by Nagamani *et al*., 2006; “Hyphomycetes” by C. V. Subramanian, 1971 “Handbook of Soil Fungi” by Gilman (1945) and “Illustrated Genera of Imperfect Fungi” by Barnett & Hunter, (1972).

Data analysis

The isolated fungal colony count was noted in the form of tabulated data and further analyzed for frequency, Shannon Diversity Index and Simpson Diversity Index by using following formulas.

$$\text{Frequency} = \frac{\text{Number of individual colony}}{\text{Total number of all fugal colonies}} X\ 100$$

$$\text{Shannon index H}' = -\sum_{i=1}^{R} \text{Pi lnPi}$$

$$\text{Simpson's Index(D)} = \frac{\Sigma\, n(n-1)}{N(N-1)}$$

III. RESULT AND DISCUSSION

The existence of viable fungal propagules in the outdoor environment of 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. 2025) 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 537 fungal colonies belongs to 13 genera and 24 species has been recorded for a month (Jan- Feb. 2025) in botanical garden of RMG College. Deuteromycota comprised over two-thirds (60.34%) of the fungal airspora, demonstrating a higher concentration followed by Ascomycota, contributed 23.46%. The depletion

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of concentration was recorded with Zygomycota, contributing 6.70% while sterile mycelia represented 9.5% of the total colonies count (Table 1).

In the month of Feb 2025, 53.63% of fungal flora was isolated on PDA media whereas it was reported 46.37% in January 2025 (Table 1). The distribution of viable fungal propagules in outdoor environment relates to vegetation and environment factors including light intensity temperature and humidity.

In Zygomycota, total 36 fungal colonies were recorded representing two genera. Higher count fungal colonies were observed in the month of January than in month of February i.e. 19 and 17 respectively. Among isolates of Zygomycota, Rhizopus stolonifer encountered with higher concentration (4.28%) than Mucor pusillus (2.42%). Rhizopus stolonifer was isolated with nearly same frequency in both the months whereas Mucor pusillus was observed more (8 colonies) in the month of January than February (Table 1).

A total of 126 fungal colonies of Ascomycota were observed which contributes 23.46% to the total isolates. Diversity of 10 species comprising of only two genera i.e. Aspergillus and Penicillium was recorded during investigation. Aspergillus was recorded with the maximum species diversity (8 species) contributing 18.44% among all the isolates during present study. Aspergillus niger was observed to the most dominated fungi with the colony count of 35 contributing 6.52% among isolated Ascomycetes followed by Aspergillus flavus (3.35%). Penicillum was observed at 5.03% out of which Penicillium oxalicum was dominant with 2.98% of contribution. A. fumigatus, A. japonica and A. sulphureus were observed at moderate frequency while A. ochraceus, A. oryzae and A. versicolor were observed at very low concentration (Table 1, Fig 3).

Deuteromycota observed with 324 fungal colonies representing 7 genera and 10 species of diverse nature. The dominant fungal genera recorded in this group belong to Alternaria (13.04%), Curvularia (13.97%), Fusarium (12.85%) and Cladosporium (9.87%). Others were observed in abundance varies between 5 – 2%. Alternaria, Curvularia and Fusarium were isolated with the two species each. The contribution of white sterile mycelia was comparatively greater over back sterile mycelia (Table 1, Fig 2, Fig 3).

Cladosporium cladosporiodes was observed to be the most dominant contributor representing 9.87% of the total colonies count followed by Curvularia lunata (7.45%), Alternaria alternata (7.08), Fusarium oxysporum, (6.89%), Curvularia brachyspora (6.52%), Aspergillus niger (6.52%), Fusarium monoliformae (5.96%). The isolates, Bipolaris, Nigrospora, Torula, Penicillum, Mucor, Rhizopus and Sterile mycelia were appeared to be moderately significant with 5.0 – 2.4% colony count. Aspergillus dominated with 8 species exhibiting highest count of species diversity; followed by Penicillium, Alternaria, Curvularia and Fusarium represented with 2 species while other genera had single species count (Table 1, Fig 3).

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 in central India is characterized by delightful weather, featuring an average minimum temperature of approximately 10°C and elevated humidity levels. 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 down that the colony count of Zygomycota and Ascomycota was comparatively higher in the month of January begins declining in subsequent period and recorded least as soon the average day temperatures (Fig 2). But the rise in temperature in the month of February favours the growth of Deuteromycetous fungi which isolated at higher concentration than the previous month. These results are in confirmation with earlier findings of Ghosh et al., 2011; Chelak and Sharma 2012; Bhajbhuje 2013, Kayarkar and Bhajbhuje, 2014.

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 was dominated in studied botanical garden with 9.87% of colonies. Same finding was also observed by 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.

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The genus Fusarium, one of the most dominant toxin-producing deuteromycetous fungal organism contributed with 12.85% of the total colonies count (Table 1), 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 demonstrated a range of harmful effects in both laboratory animals and livestock, and there are concerns regarding their potential toxicity in humans as well. 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 8 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, glycosides of leather provide protein rich substrate for many fungal species. Microbial deterioration of cellulose fibers (Menghare and Bhajbhuje 2019) was very well established facts in garden area. Aspergilli and Penicilli were abundantly reported on these nutrient rich substrates, involved in degradation (Sharma etal., 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 favoured 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).

Statistical analysis revels that, altogether 24 fungal taxa were recorded in the month of January 2025 whereas it was only 21 in February but with the higher colony count (288) than previous month. Dominance (D) of fungal taxa for January was found to be 0.05 while that of 0.06 in the month of February which indicate that the occurrence of fungal taxa was more prevalent in the month of February. Simpson_1-D diversity index for both the month was nearly same i.e. 0.9. Shannon_H diversity index was observed 3.061 for the month of January while it was 2.89 in February reflecting that the diversity of fungal taxa was more in the month of January. Species evenness was also calculated and found to be almost same for both the months of study period. Margalef diversity index was found to be more (4.169) in January compared with that of February (3.532) indicating that the fungal species was more prevalently captured on nutrient PDA medium in January (Table 2, Fig 1).

Table 1: Fungal flora of Botanical garden in RMG College, Nagbhid

S.N	Fungal Isolates	Colony Count		Total	Frequency	
		Jan-25	Feb-25	colony	Species	Genera
[A]	**Zygomycota**	19 (3.54)	17 (3.17)	36 (6.70)	6.70	6.70
1	*Mucor pusillus*	8	5	13	2.42	2.42
2	*Rhizopus stolonifer*	11	12	23	4.28	4.28
[B]	**Ascomycota**	65 (12.10)	61 (11.36)	126 (23.46)	23.46	23.46
3	*Aspergillus flavus*	10	8	18	3.35	18.44

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4	*A. fumigatus*	5	3	8	1.49	
5	*A. japonicus*	8	6	14	2.61	
6	*A. niger*	15	20	35	6.52	
7	*A. ochraceus*	3	0	3	0.56	
8	*A. oryzae*	5	0	5	0.93	
9	*A. sulphureus*	6	8	14	2.61	
10	*A. versicolor*	2	0	2	0.37	
11	*Penicillium citrinum*	5	6	11	2.05	5.03
12	*P. oxalicum*	6	10	16	2.98	
[C]	**Deuteromycota**	**143 (26.63)**	**181 (33.71)**	**324 (60.34)**	**60.34**	**60.34**
13	*Alterneria alternata*	15	23	38	7.08	13.04
14	*Alternaria tenuissima*	13	19	32	5.96	
15	*Bipolaris tetramera*	12	16	28	5.21	5.21
16	*Cladosporium cladosporiodes*	21	32	53	9.87	9.87
17	*Curvularia brachyspora*	16	19	35	6.52	13.97
18	*Curvularia lunata*	18	22	40	7.45	
19	*Fusarium monoliformae*	13	19	32	5.96	12.85
20	*Fusarium oxysporum*	17	20	37	6.89	
21	*Nigrospora oryzae*	10	5	15	2.79	2.79
22	*Torula herbarum*	8	6	14	2.61	2.61
[D]	**Other Type**	**22 (4.10)**	**29 (5.40)**	**51 (9.50)**	9.50	9.50
23	Black sterile mycelium	10	13	23	4.28	4.28
24	White sterile mycelium	12	16	28	5.21	5.21
	Total colony count	249	288	537	100	100
	Frequency	46.37	53.63	100		

*Values in parenthesis indicate contribution over total colonies recorded.

Table 2: Diversity Index of sampling site

	Jan-25	Feb-25
Taxa_S	24	21
Individuals	249	288
Dominance_D	0.05095	0.06197
Simpson_1-D	0.949	0.938
Shannon_H	3.061	2.89
Evenness_e^H/S	0.89	0.8571
Margalef	4.169	3.532

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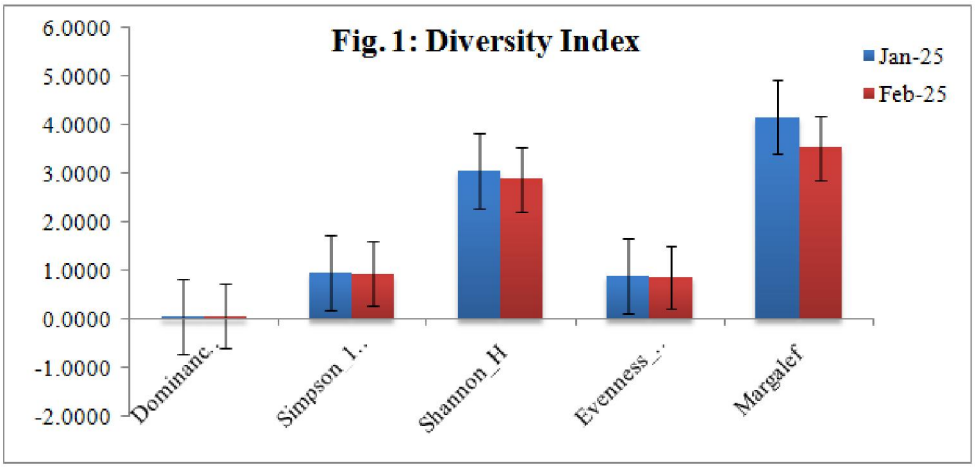

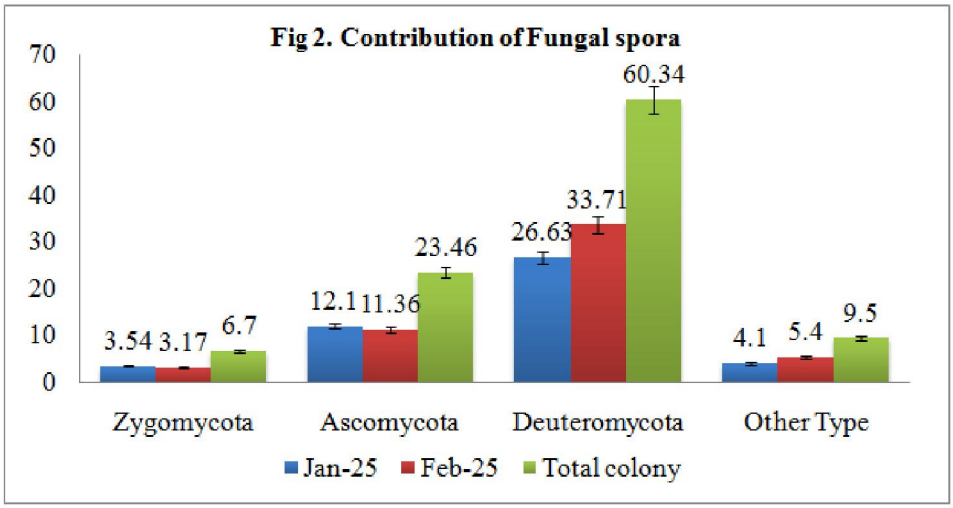

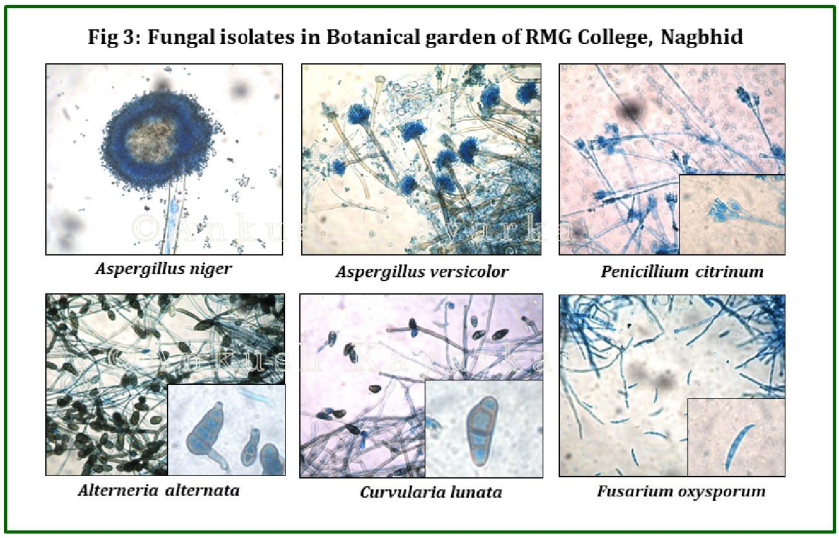

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IV. CONCLUSION

Environmental microfungal agents are responsible causing variety of disease to flora and fauna including allergic, respiratory and other disorders in human beings. Total 537 fungal colonies classified under 13 genera and 24 species were recorded during two month survey of RMG college botanical garden. Deuteromycota represented largest contributors of the total airborne fungal spores followed by Ascomycota. Among genera, Cladosporium, Alternaria, Curvularia, Fusarium and Aspergillus were largest contributor. Higher concentration of fungal propagules remained viable in February. The climate of initial period of winter supports fungal growth. Statistical analysis by employing different diversity index indicate that the species diversity of fungi was found to be more in the month of January compared with that of February. 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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