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Diversity and Distribution of Airborne Fungi: A comparative Study of High-Density Sites in Nagbhid City (MS)

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Abstract: *A comparative study to monitor the incidence of fungal air spores was undertaken during June 2022 to May 2024 in two different heavily crowded traffic areas (City Bus stop and Railway station) of Nagbhid city, Chandrapur district in Maharashtra state. In the present study, fungi were isolated by exposing petri plate containing Potato Dextrose Agar (PDA) in different seasons like Summer, Winter and Rainy. A total of 3520 fungal colonies belonging to 15 genera and 26 species along with 2 sterile types have been isolated from both the sites during study period. Out of which 2118 fungal colonies were reported from City Bus stand while 1402 colony count was noted from Railway station. Members of Deuteromycotina were most prevalently isolated while Ascomycotina and Zygomycotina were least prevalent. Cladosporiumcladosporides was found to be the most dominating contributor followed by Aspergillusniger, A. flavus, Rhizopusstolonifer, Penicilliumoxalicum, Fusariumoxysporum, andAlternariaalternata. Other fungi like Curvularia, Nigrospora, Bipolaris, andHelminthosporium were incident at moderate frequency. The incidence of aeromycoflora was observed to be high during winter and summer compared with rainy season from both localities. The occurrence of aeromycoflora was correlated with meteorological parameters like Humidity, Temperature, rainfall etc. The diversity of fungi was calculated through Shannon Diversity Index, Simpson Diversity Index and Margalef index. An attempt was made to monitor the concentration of aeromycoflora in Nagbhid city.*

Keywords: Aeromycoflora, *Aspergillus*, Bus stop, Railway station, Diversity Index

I. INTRODUCTION

Air quality refers to the degree to which the air is suitable for respiration by humans, flora, and fauna. It encompasses the state of the air surrounding us, which is primarily influenced by elements such as gases, humidity, tiny particles, pollen, microbial contamination, and various pollutants. Among these factors, microbiological quality is a significant aspect, recognized as a source of infection and a contributor to numerous communicable diseases in humans (Jaiswal and Prasad 2021). Often, bacterial and fungal spores can trigger allergic reactions. The microbiological quality of air in educational institutions, workplaces, transportation hubs, hospitals, and other public venues is particularly critical when located near highways, agricultural fields, forests, and waste landfills. In such environments, dust particles, pollen, and aerosols can transport microbes through the air (Enitanet al., 2017).

The atmosphere is populated by a diverse array of microorganisms, predominantly fungal spores, which pose significant health risks to humans, plants, and animals (Kasprzyket al., 2021). The examination of microbes, especially fungal spores in the air, is referred to as aeromycology. Fungal spores are an integral component of the atmosphere, and their abundance and characteristics are influenced by factors such as the time of day, weather conditions, seasons, and the local climatic environment, as well as the availability of nearby spore sources. The identification of airborne fungal spores is essential for various reasons, including the evaluation of air quality, the detection of pathogenic organisms,

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epidemiological considerations, and the potential health hazards they pose to humans (D'Amato and Spieksma 1995; Nagadesi and Jayraj 2016).

The levels of airborne fungi are influenced by various factors, including seasonal and daily fluctuations in meteorological conditions such as temperature, humidity, wind speed, rainfall, solar radiation, vegetation, air pollution, and human activities related to agriculture and industry. Certain fungal spores can trigger allergic reactions. Human beings continuously inhale fungal propagules present in the ambient air, with spores being deposited on sensitive mucosal surfaces. This exposure can lead to allergic conditions in humans, including asthma, rhinitis, and a variety of cardio-respiratory diseases, which are linked to the inhalation of airborne fungal spores and pollen grains. Additionally, airborne fungi are sometimes connected to respiratory illnesses, including chronic bronchitis, asthma, allergies, hypersensitive pneumonitis, and infectious diseases like aspergillosis (Singh, 2017; Jones and Harrison 2004).

Considering theses key characters the present investigation was carried out from two heavily crowded areas of Nagbhid city i.e. railway station and Bus stand to analyze the composition and concentration of airborne allergenic/ pathogenic fungi which enables people to avoid exposure at certain hours and days during the fungal spore season in the concern areas.

II. MATERIALS AND METHODS

Study Area

The aerobiological investigation was carried out in Nagbhid city, located in the Chandrapur district of Maharashtra. To evaluate the air quality in high human density places, two prominent public transit sites were selected for comparative study:

1. Site I: Nagbhid Railway Station (20°57'48"N 79°69'00"E).
2. Site II: Nagbhid City Bus Stop (20°58'55"N 79°67'45"E).

The monitoring period spanned 24 consecutive months, beginning in June 2022 and concluding in May 2024, ensuring a comprehensive analysis of fungal fluctuations across two complete seasonal cycles (Rainy, Winter, and Summer).

Fig 1: Sampling sites under study

Site 1: Railway Station

Site 2: City Bus Stop

Sampling

The Gravity Settling Method (Petri Plate Exposure Method) was utilized for the collection of airborne fungal spores. This technique allows for the assessment of viable aeromycoflora that settle under the influence of gravity. Sterilized Petri plates containing Potato Dextrose Agar (PDA) were primarily used to sample the aeromycoflora. To prevent the growth of saprophytic bacteria, Streptomycin (100 µg/ml) was added to the medium before pouring. At each site, three

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Petri dishes were exposed simultaneously at a height of 1.5 to 2 meters above ground level to simulate the human inhalation zone. Sampling was conducted at fortnightly intervals (twice a month). Each plate was exposed to the atmosphere for a duration of 5 to 10 minutes during peak operational hours (10:00 AM to 12:00 PM) when commuter density was highest.

Laboratory Processing and Incubation

After exposure, the plates were immediately covered, sealed with parafilm, and transported to the laboratory. The plates were kept in a BOD incubator at 26° ±2° C for a period of 5 to 7 days. The plates were monitored for fungal colonies.

Identification and Data Analysis

The identification of the isolated fungi was based on both macroscopic (colony color, diameter, and texture) and microscopic features. Fungal structures were teased out and mounted in Lactophenol Cotton Blue on a glass slide. The morphology of mycelia, conidiophores, and spores was studied under a compound microscope. Standard manuals, including "A Manual of Soil Fungi" (Gilman, 1945) and "DematiaceousHyphomycetes" (Ellis, 1971), were consulted for taxonomic validation. The isolated fungal colony count was noted in the form of tabulated data and was further analyzed for frequency, Shannon Diversity Index and Simpson Diversity Index by using 'PAST' software.

Meteorological Correlation

Throughout the study period (June 2022 – May 2024), data regarding local weather parameters—specifically Temperature (°C), Relative Humidity (%), and Rainfall (mm)—were recorded and Tabulated. These factors were subsequently correlated with the fungal spore concentration to understand the environmental influence on the Nagbhid aeromycoflora.

III. RESULT AND DISCUSSION

A total count of 3520 fungal colonies belonging to 15 genera and 26 species along with 2 sterile type has been isolated from both the sites during study period out of which 2118 fungal colonies were reported from City Bus stand while 1402 colony count was noted from Railway station. Members of Deuteromycotina were most prevalently isolated (42.94 % in Railway station and 46.51% in City Bus stop) followed by Ascomycotina and Zygomycotina. Cladosporiumcladosporides was found to be the most dominating contributor followed by Aspergillusniger, A. flavus, Rhizopusstolonifer, Penicilliumoxalicum, Fusariumoxysporum, andAlternariaalternata. The present report was agreed with the previous workers elsewhere (Burge and Rogers, 2000; Khan and Karuppayil, 2012). Aspergillus, Alternaria, Curvularia, FusariumandPenicillium occurred regularly throughout the study period in the extramural environments, which was correlated with the previous workers done in various working environments. Other fungi like Curvularia, Nigrospora, BipolarisandHelminthosporium were incident at moderate frequency. On a seasonal basis, the incidence of aeromycoflora was observed to be high during winter compared with rainy and summer season from both localities. This may due to the ambient environment which favours the growth of many funguses (Table 1, Fig. 2).

In Oomycotina only single species i.e. Pythiumaphanidermatum was isolated with the count of 14 colonies from Railway station while 12 colonies from City Bus stand. Pythium was observed at high rate in rainy season from both localities but notable thing is that it was not isolated during summer. This may be due to high temperature and low humidity during summer (Table 1 & 2, Fig. 2).

Zygomycotina contribute 8.30% with two species of i.e. Mucorpussilus and Rhizopusstolonifer during study period. A total 130 colonies with frequency of 9.27% was isolated from Railway station while from city bus stop it was only 7.65%. Rhizopus (5.71%) was more frequently isolated than the Mucor (2.59%)(Table 1, Fig 2).

Ascomycotina isolated at the frequency of 40.48% with the count of 3 genera and 10 species. Among all other species in this group Aspergillusniger was isolated with the highest count (8.18%) followed by Aspergillusflavus (6.70%) and Penicilliumoxalicum (5.11%). Genera like Phomopsis were isolated at least count i.e. 0.91% but also found absent in

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Railway station site. Aspergilluswas observed to be the major contributor with the colony count of 609 (28.75%) to the total aeromycoflora followed by Penicillium with count of 221 fungal colonies (10.43%)(Table 1, Fig 2).

Fig 2: Incidance of airborn fungal spores in study area

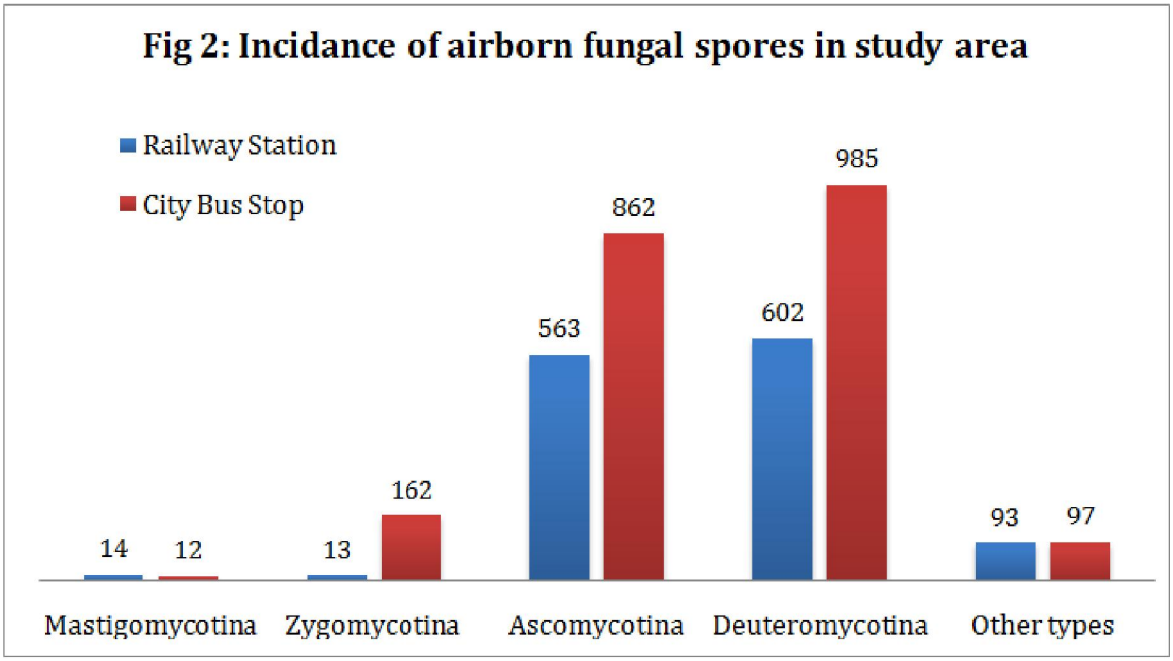
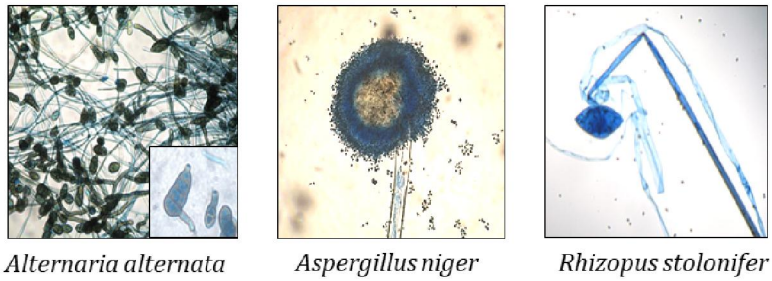
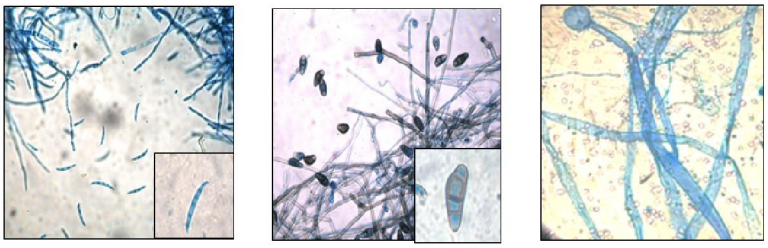


Fig 3: Isolated fungi from study area



Alternaria alternata *Aspergillus niger* *Rhizopus stolonifer*



Fusarium oxysporum *Curvularia lunata* *Mucor pusillus*

Deuteromycotina was the most dominating group with the count of 1587 colonies contributing 45.09% to total aerospora. The species of this group was isolated at more frequency from Bus stand than compared to Railway station i.e. 46.51% and 42.94% respectively. This group was dominated with the count of 7 genera and 11 species.

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Cladosporiumcladosporides was the most dominating fungus isolated from both sites with the frequency of 12.67%. Parasitic or saprophytic species belonging to the genera Alternaria and Cladosporium have been demonstrated to inhabit a diverse array of plant species (Hjelmroos 1993). The two regions examined exhibited elevated spore counts, as both genera are prevalent (Ebneret al., 1989, Hjelmroos 1993, Ricci et al., 1995). Other genera like Alternaria, Bipolaris, Curvularia, Helminthosporium, FusariumandNigrospora were reported at moderate count. Sterile mycelia were reported at the count of 5.40% (Table 1, Fig 2, Fig 3).

Table 1: Isolated Fungi from study area of Nagbhid during 2022 – 2024

SN	Fungal Taxa	Railway Station				City Bus Stop				Total Aero-spora	Freq.
		R	W	S	Total	R	W	S	Total		
	Oomycotina	9 (0.64)	5 (0.36)	0 (0.00)	14 (1.00)	7 (0.33)	5 (0.24)	0 (0.00)	12 (0.57)	26	0.74
1	*Pythiumapha-nidermatum*	9	5	0	14	7	5	0	12	26	0.74
	Zygomycotina	62 (4.42)	47 (3.35)	21 (1.50)	130 (9.27)	78 (3.68)	57 (2.69)	27 (1.27)	162 (7.65)	292	8.30
2	*Mucorpusillus*	19	10	5	34	29	19	9	57	91	2.59
3	*Rhizopusstolonifer*	43	37	16	96	49	38	18	105	201	5.71
	Ascomycotina	142 (10.13)	307 (21.90)	114 (8.13)	563 (40.16)	214 (10.10)	434 (20.49)	214 (10.10)	862 (40.70)	1425	40.48
4	*Aspergillusflavus*	26	56	20	102	28	65	41	134	236	6.70
5	*Aspergillusjaponicus*	14	32	16	62	23	42	33	98	160	4.55
6	*Aspergillusniger*	39	79	14	132	42	89	25	156	288	8.18
7	*Aspergillusochraceaous*	9	16	11	36	16	33	20	69	105	2.98
8	*Aspergillus sulphureus*	8	17	13	38	21	46	31	98	136	3.86
9	*Aspergillusversicolor*	0	0	0	0	14	28	12	54	54	1.53
10	*Penicilliumcitrinum*	14	36	13	63	12	33	14	59	122	3.47
11	*Penicilliumdigitatum*	13	28	5	46	24	30	12	66	112	3.18
12	*Penicilliumoxalicum*	19	43	22	84	32	46	18	96	180	5.11
13	*Phomopsis sp.*	0	0	0	0	2	22	8	32	32	0.91
	Deuteromycotina	101 (7.20)	323 (23.04)	178 (12.70)	602 (42.94)	164 (7.74)	502 (23.70)	319 (15.06)	985 (46.51)	1587	45.09
14	*Alternariaalternata*	8	37	14	59	13	46	35	94	153	4.35
15	*Alternariasolani*	0	0	0	0	12	28	18	58	58	1.65
18	*Bipolaristetramera*	4	26	16	46	10	51	31	92	138	3.92
16	*Cladosporiumcladosporoides*	26	129	48	203	32	146	65	243	446	12.67
17	*Curvularialunata*	14	30	19	63	18	36	30	84	147	4.18
22	*Fusariumculmorum*	0	0	0	0	13	29	20	62	62	1.76
19	*Fusariummoniliformae*	14	26	21	61	17	37	25	79	140	3.98
20	*Fusariumoxysporum*	16	35	23	74	16	41	29	86	160	4.55
21	*Fusariumsemitectum*	0	0	0	0	14	42	25	81	81	2.30
23	*Helminthosporiumtetramera*	11	25	26	62	10	24	27	61	123	3.49
24	*Nigrosporaoryza*	8	15	11	34	9	22	14	45	79	2.24

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	Other types	21 (1.50)	40 (2.85)	32 (2.28)	93 (6.63)		25 (1.18)	44 (2.08)	28 (1.32)	97 (4.58)	190	5.40
25	Sterile black mycelia	15	24	22	61		16	32	23	71	132	3.75
26	Sterile white mycelia	6	16	10	32		9	12	5	26	58	1.65
	Total Colony	335	722	345	1402		488	1042	588	2118	3520	100
	Frequency	23.89	51.50	24.61	100		23.04	49.20	27.76	100		

**Values in the parenthesis indicate per cent (%) contribution*
R-Rainy, W- Winter, S- Summer, Freq- Frequency

Table 2: Nagbhid Weather Data (June 2022 – May 2024)

Month & Year	Avg Temp (°C)	Relative Humidity (%)	Rainfall (mm)
2022			
June	31.5	68	185.0
July	27.8	85	410.2
August	27.2	86	388.5
September	28.1	82	192.4
October	26.5	74	65.2
November	23.4	62	8.5
December	21.8	58	2.1
2023			
January	22.4	55	0.7
February	25.8	48	4.2
March	29.2	42	15.6
April	33.5	38	12.4
May	36.8	35	8.8
June	32.4	62	162.5
July	27.5	88	445.0
August	27.9	84	290.2
September	28.4	81	215.8
October	27.1	70	32.4
November	23.9	64	12.0
December	21.5	60	5.4
2024			
January	23.1	58	0.1
February	26.2	50	18.5
March	30.4	40	10.2
April	34.2	34	5.5
May	37.5	32	14.8

**Source: Regional Meteorological centre Nagpur https://imdnagpur.gov.in/pages/drf.php*

The diversity analysis of fungal isolates across two distinct locations (Railway Station and Bus Stop) over three seasons reveals significant variations in community structure. The Bus stop consistently exhibited higher species richness

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(Taxa_S) compared to the Railway Station, peaking at 26 taxa during both Rainy and Winter seasons. Total fungal abundance (Individuals) was highest during the Winter season at both locations, with the Bus stop reaching a maximum of 1042 isolates (Table 3).

Table 3: Diversity indices for fungal isolates

	Railway Station			Bus stop		
	Rainy	Winter	Summer	Rainy	Winter	Summer
Taxa_S	21	21	20	26	26	25
Individuals	335	722	345	488	1042	588
Simpson_1-D	0.934	0.9242	0.9364	0.9488	0.9445	0.949
Shannon_H	2.876	2.813	2.875	3.102	3.083	3.09
Margalef	3.44	3.039	3.251	4.039	3.598	3.764

The values Shannon Index for the Bus stop (ranging from 3.083 to 3.102) were notably higher than those at the Railway Station (2.813 to 2.876). This indicates a more complex and diverse fungal community at the Bus stop. All values of Simpson Index across both sites were close to 1.0 (ranging from 0.9242 to 0.949), suggesting high evenness and that the communities are not dominated by a single aggressive fungal species. The values of Margalef index showed that the highest richness index was recorded at the Bus stop during the Rainy season (4.039), while the lowest was at the Railway Station during Winter (3.039) (Table 3).

The data indicates that the Bus stop environment supports a more diverse and rich fungal population than the Railway Station. This disparity may be attributed to differences in substrate availability, human-mediated dispersal, or localized microclimatic conditions. The surge in the number of Individuals during the Winter suggests that the cooler, stable temperatures may favor the sporulation or persistence of a wide range of fungal isolates in these urban transit hubs. However, while the abundance was highest in Winter, the Margalef Richness Index peaked during the Rainy season. This suggests that while total numbers grow in winter, the "new" or unique species are more likely to emerge during the monsoon, likely due to increased humidity providing niches for moisture-dependent fungi.

The high Simpson's Index (1-D) values indicate that these fungal communities are relatively stable and well-distributed. Even in the Railway Station, where Taxa count was lower, the evenness remained high (>0.92). The Shannon Index values above 3.0 at the Bus stop are particularly significant. In ecological terms, H > 3 often indicates a healthy, diverse habitat with high "information content," suggesting that the Bus stop may act as a significant reservoir for fungal spores compared to the more anthropogenically stressed Railway Station environment.

IV. CONCLUSION

The data analysis showed that airborne fungi concentrations were very high at city bus stands, and they varied based on weather and available surfaces. The most common allergenic spores were Cladosporium, Aspergillusand Penicillium. This is likely because these fungi can thrive on many kinds of hosts and substrates. The types of fungi present were closely linked to the nearby vegetation necessary for their growth. Changes in work environments and weather influenced the presence of fungi in the outdoor areas of the bus stands and railway stations studied. The presence of microfungi highlights the need for both allergists and mycologists interested in health and environmental pollution to help those suffering from allergic conditions in these areas. Specific actions should be taken to reduce fungal levels in these environments to protect people from allergic reactions.

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