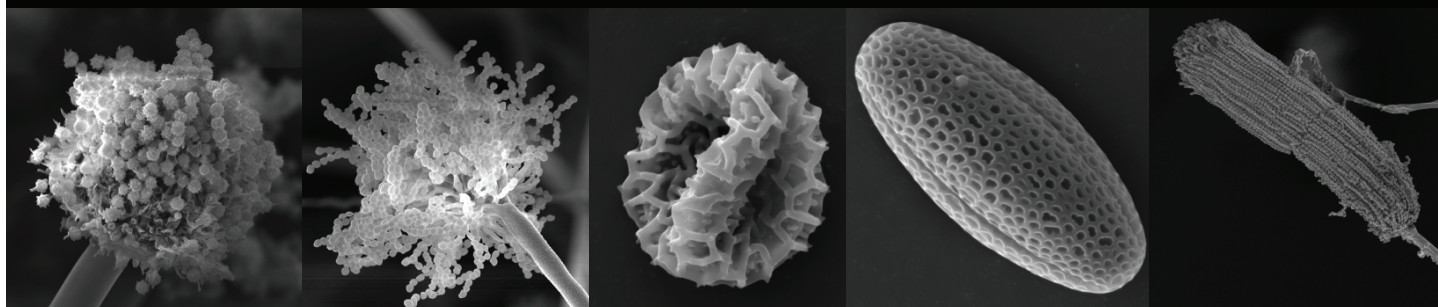
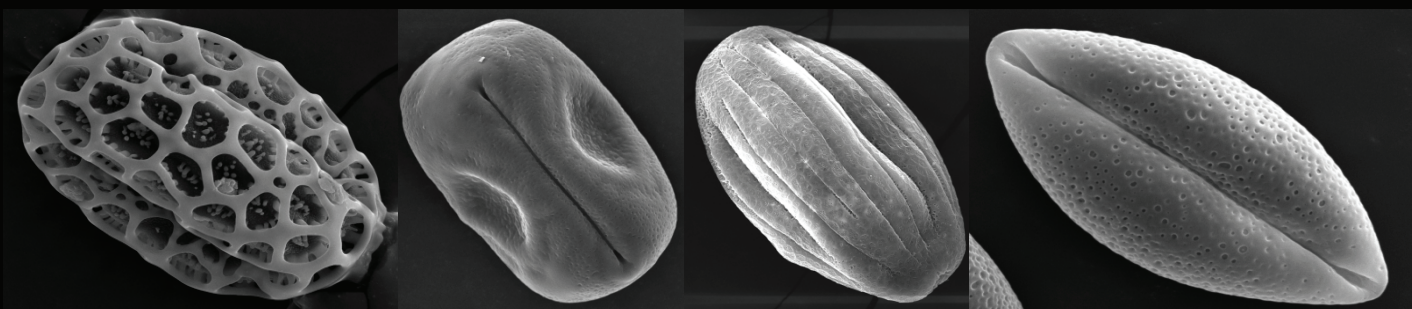


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Research Article

IMPACT OF AIRBORNE AND STORAGE FUNGI ON LOSS OF SEED QUALITY OF VIGNA MUNGO (L.) HEPPER AT TWO DISTINCT BIOZONES OF PURULIA, WEST BENGAL

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An aeromycological study was conducted over the course of a year to examine the presence and impact of airborne fungi in two different ecological zones—rural plain lands and the Ajodhya hilly region. The investigation focused on measuring fungal load, identifying sources and dispersal mechanisms, and assessing their effects on plants and animals. In rural houses of the plain lands, the study recorded a total fungal load of 118,980.30 CFU/m³, comprising 18 fungal species from 14 genera. In contrast, rural houses in the Ajodhya hills showed a higher fungal load of 149,531.82 CFU/m³, involving 23 fungal species from 17 genera. Across both regions, *Aspergillus niger* emerged as the most dominant airborne fungus. Fungal concentrations were significantly higher during the monsoon season, likely due to increased relative humidity, which was found to strongly influence fungal proliferation in the air. In parallel, the study also examined the seed mycoflora of stored *Vigna mungo* (black gram) in households from both biozones. Seeds stored in the hilly villages exhibited a higher rate of fungal infection, along with a greater loss of carbohydrates, proteins, and free fatty acids, and a notable decline in germination capacity. The most common fungi associated with the stored seeds were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. Notably, aflatoxin contamination was detected and quantified using ELISA. After one year of storage, seeds from hilly villages contained 15.34 ppb of total aflatoxins, while seeds from the plain lands had 8.639 ppb, indicating a significantly higher risk of toxin accumulation in the hill region. This study highlights the environmental and health implications of airborne and seed-borne fungi, particularly in relation to storage conditions and regional climatic variations.

Key Words: Aeromycology, Aflatoxin, Seed deterioration, Storage fungi, *Vigna mungo*

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INTRODUCTION

The ability of fungi to decompose organic matter makes them one of the most vital parts of the ecosystem. The fungi typically proliferate asexually and sexually by generating spores and conidia, which make up airspora and can be detrimental to humans, animals, and plants¹. Nearly all fungi carry out the biodegradation and bio-deterioration of biological components to meet their primary needs for carbon, nitrogen, and other nutrients². The detection of fungal load, sources, release method, dispersal, deposition, and their effects on plant and animal systems are all covered through aeromycological research³. Seasonal variations and atmospheric factors including temperature, humidity, precipitation, and wind speed affect the fungal abundance of an area⁴. Seeds are the most crucial propagules for the world's food crops. Generally, small-scale farmers keep their seeds in their homes for the next year cropping and their own consumption, but these seeds are gradually tainted with

infections and their quality declines at the same time⁵. Seeds contaminated with pathogenic fungi during storage lose vigour and viability and undergo detrimental chemical changes that cause degradation⁶. Storage fungi are very sneaky and can cause significant damage to seeds while being stored before their existence is detected⁷. These seed-borne fungi of contaminated seeds deteriorate the quality of seeds during storage and produce mycotoxins, making them useless or even unsuitable for consumption as human and animal feed⁸. Generally, the ideal storage conditions are rarely present all year round in the villages. In addition to several seed-borne diseases, the seeds become infected with a range of field fungi during the crop's maturation, harvesting, threshing, processing, and storage phases⁹.

In India, *Vigna mungo* (L.) Hepper (black gram) also referred to as Urd bean, is an essential pulse crop. It is nutritionally balanced and high in lysine and sulfur containing amino acids. India produces more than 70%

of the world's black gram, making it the leading producer in the world¹⁰. Seed ageing results in reduced germination rates, delayed germination, and occasionally total loss of seed viability. Higher temperatures and moisture content cause seed quality to deteriorate more quickly, which could impact crop performance and yield¹¹. Black grams are rich in nutrients, including 40–47% starch, 20–25% proteins, and other vital vitamins and carbohydrates¹².

This study focuses on the comparative aeromycological studies of storage places in indoor houses of two different biozones, as well as to determine the changes in the quality of the seeds due to associated mycoflora of *Vigna mungo* seeds stored in these biozones.

MATERIAL AND METHODS

Selection of Study sites

The study was conducted in two plain land villages—Malthore (240 m) and Golkunda (248 m) in Purulia II CD Block—and two hilly villages—Jamghutu (578 m) and Telia Bhasa (548 m) in the Ajodhya Hills, Baghmundi CD Block, Purulia district, West Bengal. The hilly villages are surrounded by forest patches, while the plain land villages are bordered by agricultural fields.

Aeromycological survey

A year-long study of the aeromycology was conducted between June 2023 and May 2024. The fungi were isolated from the air of indoor houses employing the petriplate exposure method. Chloramphenicol-supplemented Potato Dextrose Agar (PDA) petriplates were exposed for 15 minutes at different rural storage places to isolate viable airborne fungi. The sampling was done at weekly intervals throughout the year between 9 a.m. to 11 a.m. The exposed plates were kept in an incubator at 28°C. The fungal colonies were examined morphologically, stained with lactophenol cotton blue solution, observed under bright field microscope (Leica DM 3000 LED) and identified by standard literatures and experts to construct the fungal spore calendar. The concentration of spores in the air was calculated as CFU/m³. To evaluate the seasonal variation of fungal spores the whole study period was divided into four seasons – monsoon (June – August), post-monsoon (September – November), winter (December – February) and summer (March – May).

The meteorological data of the sampling sites were recorded by Digital Hygrometer and Thermometer (Metravi HTC-1).

Collection of Seed samples

Seed samples of *V. mungo* were collected from the rural houses of same villages of plain and the hilly regions of Purulia where aeromycological monitoring were carried out.

Measurement of Seed moisture content

Seed moisture was determined following Roberts and Roberts¹³. Seed samples of 10g in triplicate were dried for two hours at 130°C and then allowed to cool at room temperature in a desiccator containing fused calcium chloride for 40–45 minutes and finally weighed. The seed moisture content (SMC) of test seeds was calculated by the following formula:

$$\text{SMC} = \frac{(\text{wet weight} - \text{dry weight}) \times 100}{\text{wet weight}}$$

Determination of Germinability and Seed viability

Germinability was determined by randomly placing surface sterilized 100 seeds in three replicates on sterilized petridishes containing three layered moist filter papers. Surface disinfection was done by 2% sodium hypochlorite solution for 1 minute followed by repeated washing by distilled water. Petridishes were incubated at 30°C for seven days with alternate twelve hours of light and twelve hours of darkness¹⁴. Seedlings with normal roots of 5mm were counted as germinated.

Study of Seed mycoflora

Types of fungi associated with stored seeds and the extent of fungal infection were determined by the agar plate method. The isolation of seed borne fungi was determined by placing ten surface sterilized seeds on each petriplates with salt malt agar (10% sodium chloride in 2% malt agar) medium at pH 6.4. The triplicate sets of petridishes were incubated at 30°C in darkness for seven days. Fungi developed on seeds were counted, identified using authentic manuals^{15,16} and maintained as pure cultures.

Study of Major nutrient changes in Seeds during Storage

The major nutrients like carbohydrate, protein, and free fatty acid content of stored Urd bean were measured at

monthly intervals taking samples from storage places of sampling sites.

Total Carbohydrate content

The extraction of total carbohydrates was done using the method of Chow and Landhäusser¹⁷. For extraction of total soluble sugar and total soluble starch, 100 mg of the powdered black gram seed samples was added with 2 ml of 80% ethanol and placed in the water bath for 15 minutes at 90–95°C followed by centrifugation at 3000 rpm for 5 minutes. The supernatant was collected in a separate test tube for total soluble sugar analysis and the extraction procedure was repeated two more times. The residue was oven-dried at 80°C for 2 hours and then mixed with 1.1 % HCl for total starch (insoluble sugar) estimation. After that, the quantitative estimation of the total carbohydrates (soluble and insoluble sugar) was done using the anthrone method. Absorbance was taken at 630 nm using Shimadzu UV-1800 spectrophotometer.

Protein content

For protein extraction, 100 mg of ground *Vigna mungo* seed samples were suspended with 5 ml of 1(M) NaOH and incubated at 80°C for 1 hour followed by centrifugation at 4000 rpm for 10 minutes. Protein content was estimated quantitatively following Lowry *et al.*¹⁸ Absorbance was taken at 660nm using Shimadzu UV-1800 spectrophotometer.

Free Fatty acids content

The extraction of oil from *Vigna mungo* seeds was done

by using hexane in a soxhlet apparatus for 6 to 7 hours. After extraction the solvent was separated by a rotary evaporator (IKA RV 10 with chiller) and free fatty acid was determined following the methods of Mehra¹⁹.

Extraction and Estimation of Total Aflatoxin through ELISA

Total aflatoxin was extracted from the black gram seeds following the protocol supplied along with the Toxin-Fast Total Aflatoxins (AFT) ELISA Kit (Perkin Elmer). In brief, 5 g of seeds were used for the total aflatoxin extraction in 25 ml of 60% methanol followed by centrifugation at 4000 r/min. The upper liquid layer was used for the analysis of total aflatoxin. The aflatoxin was quantified following the instructions mentioned in the protocol by taking absorbance at 450 nm of wavelength in microplate reader (Erba LisaScan EM).

RESULTS

AEROMYCOLOGICAL STUDY

Indoor houses of Lower altitudes

The aeromycological survey was conducted for 1 year in indoor houses at lower altitudes near Purulia town to isolate and identify the aeromycoflora. During this aeromycological investigation, 18 fungal species from 14 genera contributed a total of 118980.30 CFU/m³ (Fig 1.1). The highest fungal concentration was noticed in monsoon (29.87%) followed by post-monsoon (25.42%), winter (24.58%), and summer (20.13%) with a peak during July (10.88%) followed by August

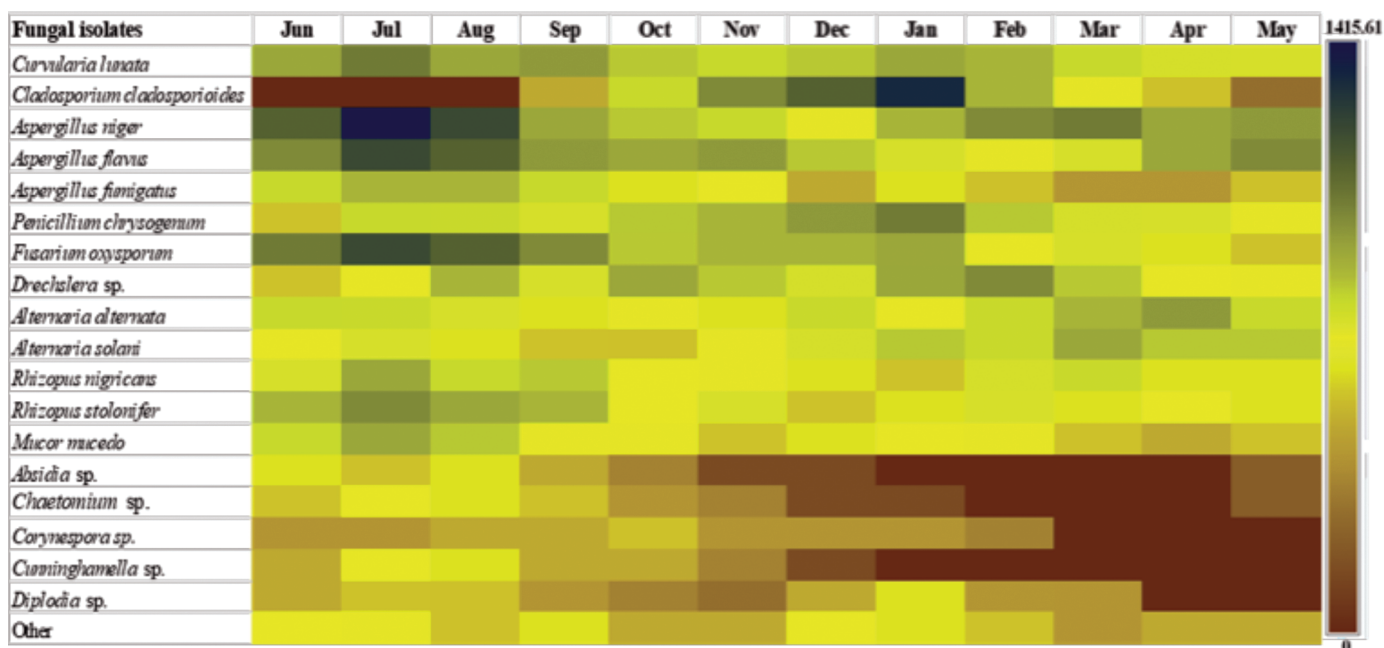


Fig. 1.1: Monthly fungal load (CFU/m³) in indoor houses of lower altitudes

(10.00%) and June (8.99%). *Aspergillus niger* (8.77%), *Aspergillus flavus* (8.06%) and *Fusarium oxysporum* (7.53%) were most prevalent in the air. Three recorded species of the genus *Aspergillus* (*A. niger*, *A. flavus*, and *A. fumigatus*) altogether constitute about 22% of the total fungal load. Two species of *Alternaria* and *Rhizopus* were found during this aeromycological survey and the rest genera were represented by a single species only. Among the total fungal isolates, 13 fungal species were from Ascomycota and the rest 5 were from Mucoromycota.

Indoor houses of Higher altitudes

A number of 23 fungal species from 17 fungal taxa were isolated during this aeromycological investigation from the rural houses of Ajodhya hills (Fig 1.2). The total concentration of fungal spores in the air was detected as 149531.82 CFU/m³ among which *Aspergillus niger* contributed a maximum (6.80%) followed by *Aspergillus flavus* (6.45%) and *Fusarium oxysporum* (6.31%). The highest recorded fungal load was in July (10.37%), followed by January (9.64%) and August (9.36%). The maximum of three fungal species observed from the genus *Aspergillus* and the genera *Penicillium*, *Fusarium*, *Alternaria*, and *Rhizopus* were found to contribute two species each. Among the isolated fungal species, 20 belonged to Ascomycota and 3 were members of Mucoromycota. The maximum fungal load was detected in monsoon (27.85%) followed by winter (27.12%), post-monsoon (25.86%), and summer (19.17%).

SEASONAL VARIATION OF FUNGAL SPORES

Indoor houses of Lower altitudes

Seasonal variation of fungal spores in the air of indoor houses of lower elevation is given in Table 1.1. *Aspergillus niger* (10.18%) was found to be most prevalent in the air during monsoon followed by *Fusarium oxysporum* (8.85%) and *Aspergillus flavus* (8.70%). *Aspergillus flavus* (8.15%) was dominant in post-monsoon subsequently *Fusarium oxysporum* (7.62%) and *Curvularia lunata* (7.10%). *Cladosporium cladosporioides* (10.58%) was the highest in occurrence and *Penicillium chrysogenum* (8.42%) and *Drechslera* sp. (7.71%) were the second and third most dominant airspora during winter. In summer the concentration of airborne *Aspergillus niger* conidia reached to peak (10.72%) like monsoon followed by *Aspergillus flavus* (9.41%) and *Alternaria alternata* (9.19%).

Indoor houses of higher altitudes

Seasonal variation of fungal spores in the air of indoor houses in Ajodhya hills is presented in Table 1.2. *Fusarium oxysporum* (8.18%) was most dominant in monsoon followed by *Aspergillus niger* (8.05%) and *Aspergillus flavus* (7.80%). In post-monsoon, *Aspergillus flavus* (6.37%) was the most frequent airspora whereas *Fusarium oxysporum* (6.10%) and *Fusarium* sp. (5.83%) were the second and third most dominant fungi in the air. During winter *Cladosporium cladosporioides* (8.27%) was the most common in the air followed by *Penicillium chrysogenum* (6.33%), *Curvularia lunata* (5.81%) and

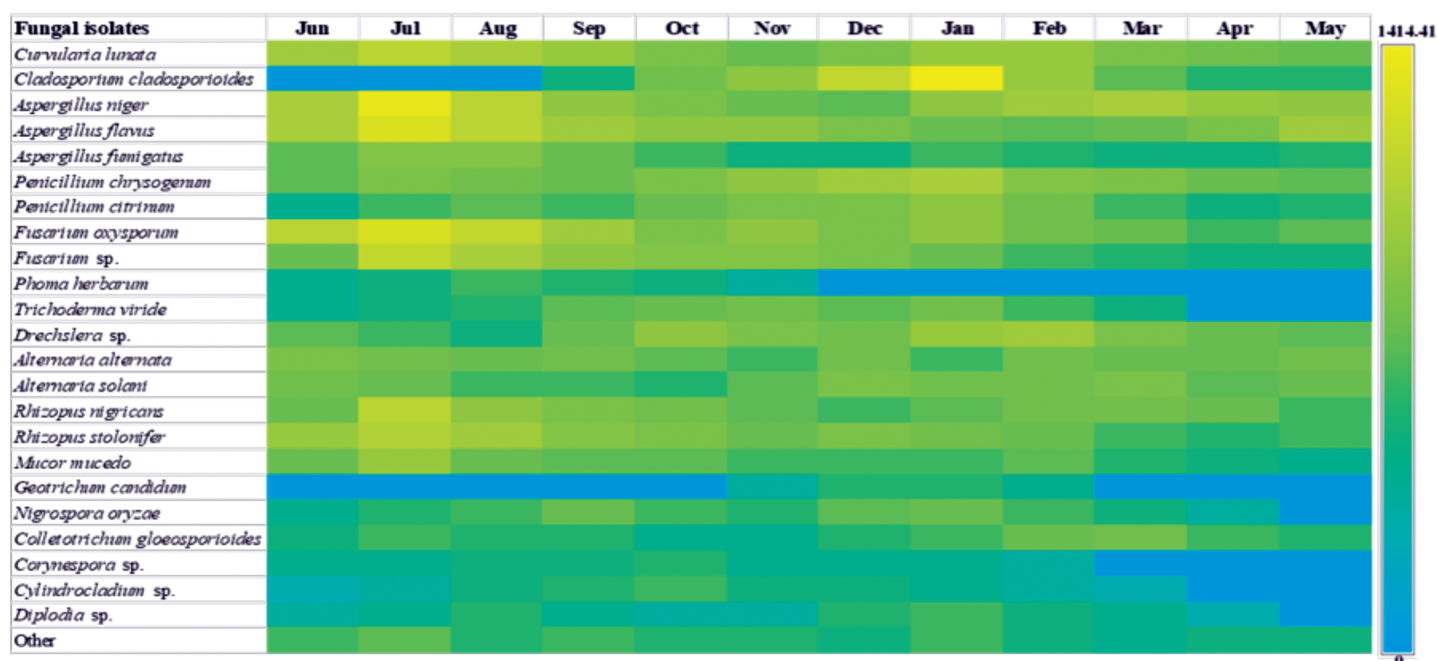


Fig. 1.2: Monthly fungal load (CFU/m³) in indoor houses of higher altitudes

Table 1.1: Seasonal variation of fungal spores in the air of indoor houses of lower altitudes

Fungal isolates	Monsoon (%)	Post-monsoon (%)	Winter (%)	Summer (%)
<i>Curvularia lunata</i>	7.08	7.10	7.53	7.44
<i>Cladosporium cladosporioides</i>	0.00	6.24	10.58	4.81
<i>Aspergillus niger</i>	10.18	6.93	7.35	10.72
<i>Aspergillus flavus</i>	8.70	8.15	6.09	9.41
<i>Aspergillus fumigatus</i>	5.90	5.37	4.30	4.38
<i>Penicillium chrysogenum</i>	4.72	6.59	8.42	7.00
<i>Fusarium oxysporum</i>	8.85	7.62	6.99	6.13
<i>Drechslera</i> sp.	4.72	6.76	7.71	7.22
<i>Alternaria alternata</i>	5.16	4.85	6.09	9.19
<i>Alternaria solani</i>	4.42	4.51	6.45	8.97
<i>Rhizopus nigricans</i>	5.60	5.72	5.02	6.56
<i>Rhizopus stolonifer</i>	6.79	6.07	5.02	6.13
<i>Mucor mucedo</i>	5.90	4.85	5.20	5.03
<i>Absidia</i> sp.	3.83	2.43	0.36	0.66
<i>Chaetomium</i> sp.	3.98	3.29	0.72	0.66
<i>Corynespora</i> sp.	2.80	3.64	3.05	0.00
<i>Cunninghamella</i> sp.	3.84	3.29	0.36	0.00
<i>Diplodia</i> sp.	3.39	2.60	3.94	1.31
Other	4.13	3.99	4.84	4.38

Table 1.2: Seasonal variation of fungal spores in the air of indoor houses of higher altitudes

Fungal isolates	Monsoon (%)	Post-monsoon (%)	Winter (%)	Summer (%)
<i>Curvularia lunata</i>	6.92	5.42	5.81	6.58
<i>Cladosporium cladosporioides</i>	0.00	4.61	8.27	4.75
<i>Aspergillus niger</i>	8.05	5.28	5.43	8.96
<i>Aspergillus flavus</i>	7.80	6.37	4.39	7.49
<i>Aspergillus fumigatus</i>	4.78	3.66	3.10	4.02
<i>Penicillium chrysogenum</i>	3.14	4.47	5.17	4.39
<i>Fusarium oxysporum</i>	8.18	6.10	5.17	5.48
<i>Fusarium</i> sp.	6.29	5.83	4.26	4.02
<i>Phoma herbarum</i>	2.77	2.71	0.00	0.00
<i>Trichoderma viride</i>	2.64	4.47	4.01	1.28
<i>Drechslera</i> sp.	3.27	5.28	5.81	6.22
<i>Alternaria alternata</i>	4.53	4.20	4.26	6.22
				Contd.

Contd. Table 1.2

Fungal isolates	Monsoon (%)	Post-monsoon (%)	Winter (%)	Summer (%)
<i>Alternaria solani</i>	4.03	3.66	4.78	6.22
<i>Rhizopus nigricans</i>	5.79	4.74	4.01	5.85
<i>Rhizopus stolonifer</i>	6.54	5.15	4.65	4.75
<i>Mucor mucedo</i>	4.78	3.93	3.62	3.84
<i>Geotrichum candidum</i>	0.00	0.68	2.84	0.00
<i>Nigrospora oryzae</i>	2.89	3.79	3.88	2.19
<i>Colletotrichum gloeosporioides</i>	3.02	2.71	3.62	5.30
<i>Corynespora</i> sp.	2.39	2.85	2.20	0.00
<i>Cylindrocladium</i> sp.	2.01	3.25	2.33	0.73
<i>Diplodia</i> sp.	2.39	2.17	3.10	1.83
Other	3.40	3.39	2.97	3.66

Drechslera sp. (5.81%). The summer air was dominated by *Aspergillus niger* (8.96%) subsequently *Aspergillus flavus* (7.49%) and *Curvularia lunata* (6.58%).

Monitoring of Environmental parameters

The environmental parameters like average temperature and average relative humidity were recorded from June 2023 to May 2024 (Table 1.3). The temperature was

found to be a maximum of 33°C in indoor rural houses of lower altitudes whereas the maximum of 31°C was recorded in indoor rural houses of higher altitudes. The lowest temperature was observed at 17°C and 14°C in houses of plain land and higher elevation respectively. The relative humidity was found higher in houses at higher elevations in comparison to lower altitudes. The relative humidity reached 84% in the indoor environ-

Table 1.3: Record of environmental parameters from June 2023 to May 2024

Months	Indoor houses of lower elevation		Indoor houses of higher elevation	
	Average temperature (%)	Average relative humidity (%)	Average temperature (%)	Average relative humidity (%)
June	33	61	31	65
July	30	80	29	85
August	29	81	27	84
September	29	84	27	87
October	28	73	26	78
November	24	71	24	75
December	19	74	16	75
January	17	72	14	73
February	23	62	20	68
March	26	59	25	65
April	33	42	31	50
May	32	59	31	61

ment of plain land however, the humidity reached 87% in indoor houses of hilly regions in the monsoon. The minimum relative humidity was noticed at 42% in houses of lower elevations whereas 50% in houses of higher elevations.

Correlation between monthly fungal concentration and indoor atmosphere

The correlation study between monthly fungal concentration and the indoor atmosphere was assessed to determine the influence of meteorological parameters on fungal load in the air (Table 1.4). A significant positive correlation was observed between the fungal concentration and relative humidity. An insignificant positive correlation was detected between temperature and spore load in the rural houses of lower elevation whereas an insignificant negative correlation was noticed in the houses of higher elevation.

Determination of seed-borne fungi

A total of 18 fungal genera were isolated from *Vigna mungo* seed samples stored in two different biozones of Purulia district. In addition to three fungal species did not produce any reproductive structures and 15 genera were identified as *Rhizopus* sp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp., *Penicillium* sp., *Alternaria alternata*, *Curvularia lunata*, *Drechslera* sp., *Trichoderma* sp., *Absidia* sp., *Diplodia* sp., *Mucor mucedo*, *Macrophomina* sp., *Cladosporium cladosporioides*, *Chaetomium* sp., *Cunninghamella* sp., and *Fusarium oxysporum*.

Fresh seeds of lower altitude and higher altitude rural houses showed a high rate of infection of *Fusarium oxysporum*, *Curvularia lunata*, *Cladosporium cladosporioides*, *Alternaria alternata*, *Trichoderma* sp., and a low infection rate of *Rhizopus* sp., *Aspergillus niger*,

Table 1.4: Correlation between monthly fungal concentration and indoor atmosphere

Statistical parameters	Indoor houses of lower altitudes		Indoor houses of higher altitudes	
	Average temperature (%)	Average humidity (%)	Average temperature (%)	Average humidity (%)
P	.060	.760	-.427	.800
r	.854	.004*	.166	.002*

* Significance at the level of 0.05

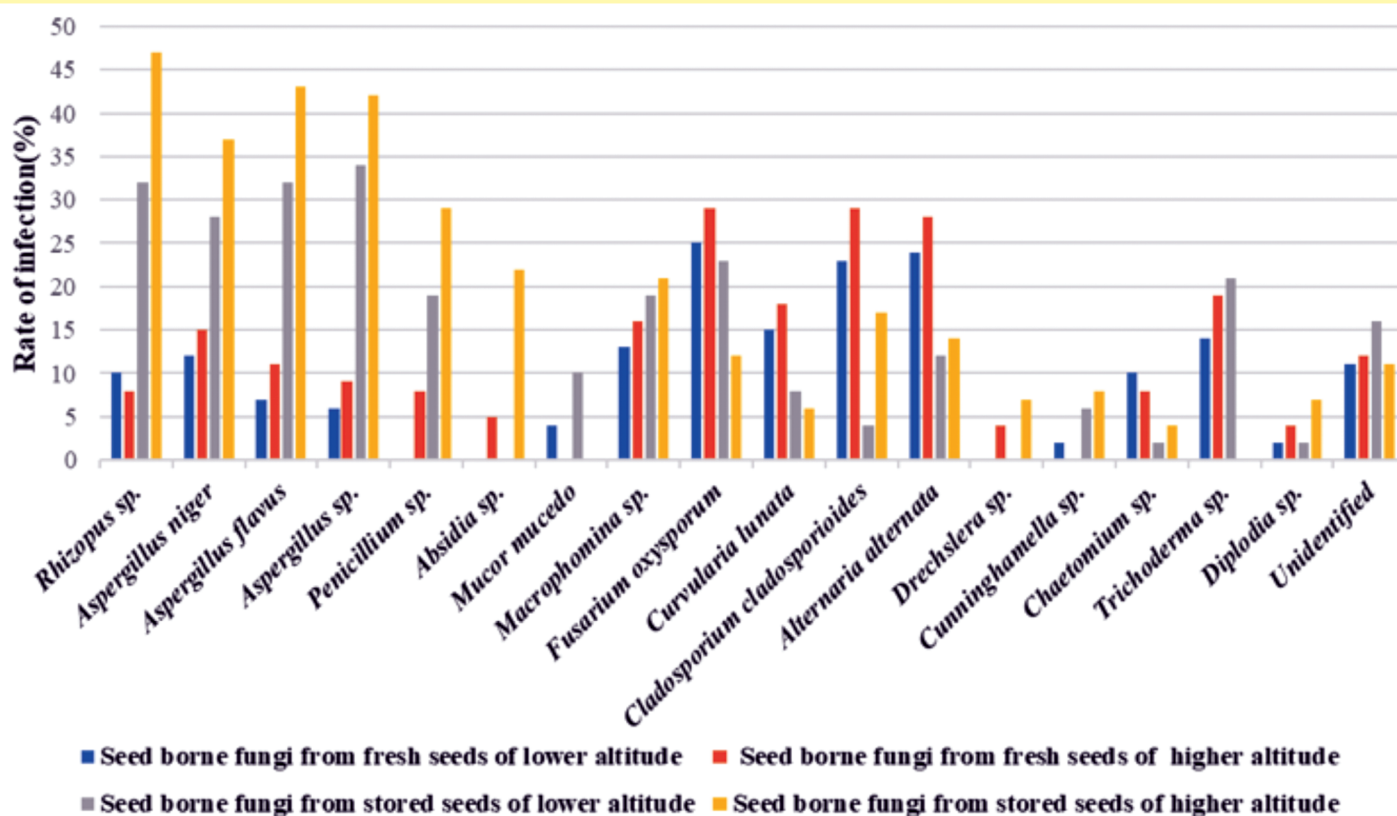


Fig. 1.3: Diversity of seed mycoflora in fresh and one-year stored black gram seeds in two different biozones

Aspergillus flavus, *Aspergillus* sp., *Chaetomium* sp., *Macrophomina* sp., *Diplodia* sp. Three fungal genera viz. *Absidia* sp., *Penicillium* sp. and *Drechslera* sp. were only recorded from the fresh seeds of hilly region whereas two fungal genera viz. *Mucor mucedo* and *Cunninghamella* sp. were only found in lower altitudes (Fig. 1.3). The total infection rate of the seeds stored in higher altitudes exhibited higher than the lower altitude.

After one year of storage, stored seeds of both the biozones had shown a greater percentage of infection by *Rhizopus* sp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus* sp., *Penicillium* sp., *Macrophomina* sp., and other fungi had shown a lower infection. Two genera viz. *Absidia* sp. and *Drechslera* sp. were only found in hilly regions whereas *Mucor mucedo* and *Trichoderma* sp. were only present in lower altitude rural houses after one year of storage of seeds (Fig. 1.3).

Changes in germination rate and moisture content of the seeds during storage in two different storage biozones

Vigna mungo seeds stored in lower-altitude rural houses had shown a decrease in germination rate from 81.66% to 34.33%. In the high-altitude region, the germination rate decreased from 82.66% to 21.66% after one year of storage (Fig. 1.4).

Seeds kept in both the biozones had shown a little increase in their moisture content with the advancement of storage period. The initial moisture content of *Vigna mungo* fresh seeds was 10% which was increased upto 11.90% after one year of storage in the seeds stored in the rural houses of lower altitude and moisture content of the stored seeds of higher altitude increased from 10.50% to 13.90% (Fig. 1.4).

Biochemical changes in the seeds during storage

Protein, carbohydrate and free fatty acids content was found to fluctuate as the storage duration progressed in the seeds stored in the villages of lower and higher altitudes.

Carbohydrate content

The total carbohydrate content of seeds stored in the hilly Ajodhya region decreased from 56.45% to 15.19%, whereas seeds kept in the villages of plain land decreased from 56.36% to 18.19% (Fig. 1.4).

Protein content

Protein content gradually decreased from 23.95% to 7.55% after one year of storage in the seeds stored in the hilly villages of Ajodhya region, in contrast, seeds stored in the lower-altitude rural houses the protein content decreased gradually from 24.24% to 13.74% (Fig. 1.4).

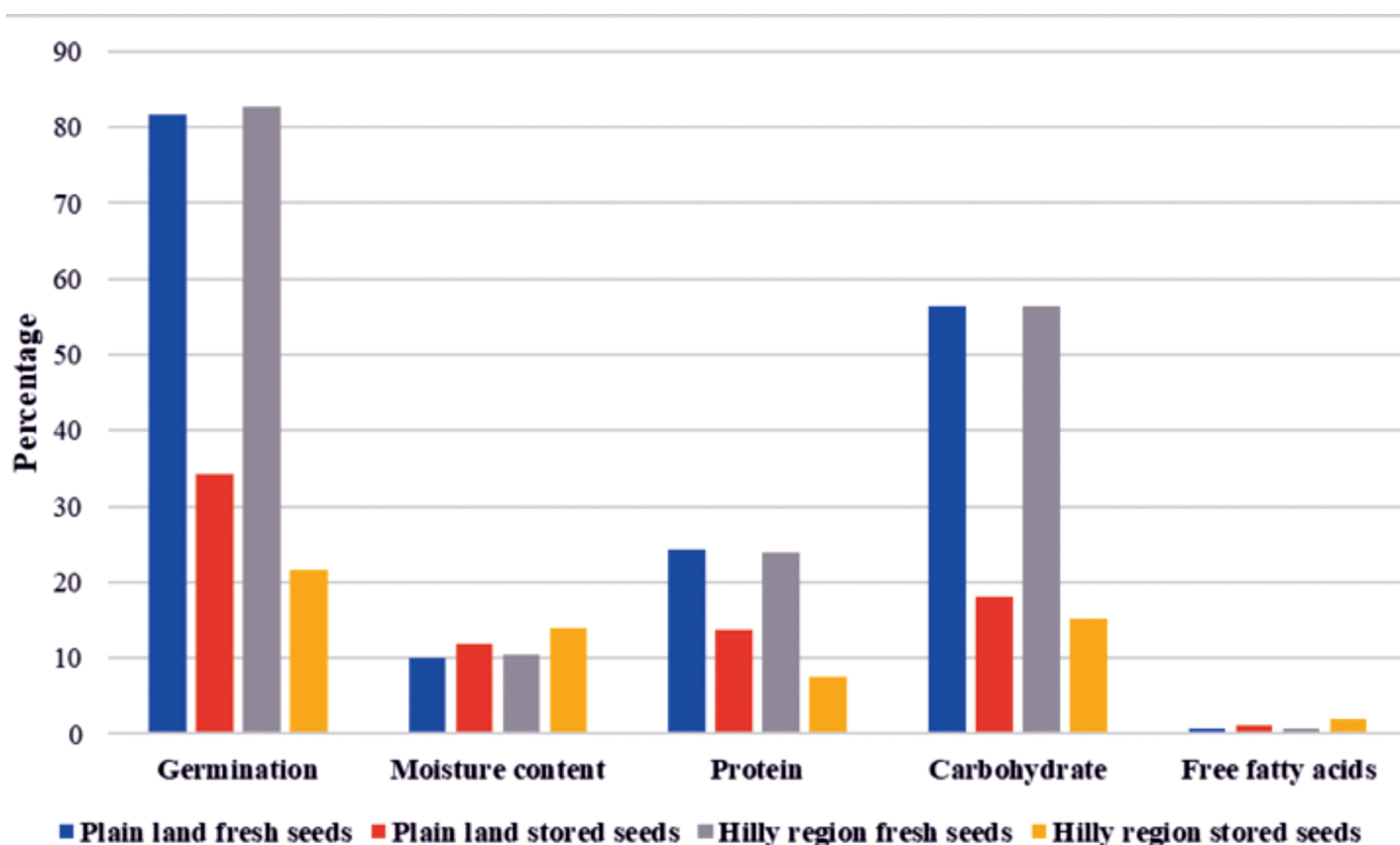


Fig. 1.4: Changes in germination rate, moisture content and nutritional values in seeds

Free Fatty acids content

The free fatty acid content increased from 0.62% to 1.9% in the seeds stored in the villages of Ajodhya hilly region and seeds stored in the villages of the local plain land exhibited an increase in the amount of free fatty acids from 0.62% to 1.1% (Fig. 1.4).

Determination of total aflatoxin

The total aflatoxin was quantified using ELISA kit and it was observed that the stored seeds in plain land were contaminated with 8.693 ppb and stored seeds in hilly villages contained 15.34 ppb of aflatoxin after one year of storage.

DISCUSSION

The aeromycological investigation continued for 1 year to study the fluctuation of fungal load in the air of indoor houses in plain lands and hilly regions. The total fungal load was detected as 118980.30 CFU/m³ from 18 fungal species belonging to 14 genera in plain land houses whereas in houses of hilly regions, the fungal concentration was detected as 149531.82 CFU/m³ from 23 fungal species belonging to 17 fungal taxa. The fungal load and species variation were found to be higher in villages of hilly regions in comparison to lower elevation may be due to the higher relative humidity in the houses, and availability of substrates like forest litter and vegetational as well as crop diversity surrounding the houses. Among the isolated fungal species, *Aspergillus niger* was the most dominant in both the study places. A similar observation was also recorded by Gorai *et al.*²⁰ from the storage places where *Aspergillus niger* was most frequent in the air. The concentrations of viable fungal spores in the air were reported to change with the season^{21, 22} similar to the current investigation. In this piece of work, the maximum fungal load was observed in monsoon and winter and the lowest fungal concentration was noticed in summer supporting the observation made by Adhikari *et al.*²³ and Kasprzyk²⁴, who recorded the lowest spore densities of airborne viable fungi during summer in tropical and subtropical locations. It has also been reported that environmental factors such as humidity and temperature affect the spore load of fungi in the air^{25, 26} which corroborates the current findings where relative humidity was found to be positively correlated with the fungal load in both the sites of study but insignificant correlation with

temperature has been noticed.

Seeds are a major source of air mycoflora under storage circumstances, with *Aspergillus* being the main component^{20, 27, 28}. Most of the early invading fungi increase moisture within the seed bulk which favours different storage fungi^{28, 29}. In the present study, the isolated fungi that unswervingly occurred and proliferated in the *Vigna mungo* seeds were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Alternaria alternata*, *Curvularia lunata*, *Drechslera* sp., *Trichoderma* sp., *Absidia* sp., *Diplodia* sp., *Mucor mucedo*, *Macrophomina* sp., *Cladosporium cladosporioides*, *Chaetomium* sp., *Cunninghamella* sp., and *Fusarium oxysporum*. The findings are similar to the observations of Debbarma *et al.*³⁰ and Netam *et al.*³¹.

Seeds of *Vigna mungo* stored in the villages of the hilly Ajodhya region had shown a higher number of seed-borne fungi and a greater infection rate than local villages of plain land. In both the study localities, fresh seeds had shown a low infection rate and notably infection was increased after one year of storage. The higher rate of seed infection in the hilly Ajodhya region may be due to fluctuation of environmental parameters and higher humidity. The amount of field fungi was decreased with the progression of storage time due to the appearance of storage fungi which all together reduced the protein and carbohydrate content as well as germinability of seeds. The increase in moisture and free fatty acid content along with the decrease in protein and carbohydrate content after one year of storage is due to deterioration by storage fungi is similar to the findings of Raha and Bhattacharya²⁷. Germination of seeds was significantly decreased with the increase in storage time due to fungal infection and moreover, high moisture as well as temperature increase the risk of *Aspergillus* infection in storage conditions³². Additionally, it was reported that *Aspergillus flavus*-infected seeds deteriorate more than those infected by *Aspergillus niger*, *Penicillium* sp., and *Fusarium* sp. because *Aspergillus flavus* can produce aflatoxins, enzymes like alpha-amylase and protease, and induce the ageing process³³. Further analysis of mycotoxin in stored seeds indicated that seeds of hilly regions possessed higher levels of total aflatoxins in comparison to plain land due to the severe infection which was promoted by the higher humidity. The regulatory limit of aflatoxin in India is 15 µg/kg³⁴ and the aflatoxin present in the stored seeds

of hilly regions just crossed the safety limit. Due to increased level of total aflatoxin and lower nutritional values, the deteriorated seeds become useless as food and becomes unfit for sowing purpose also due to decrease in germination rate.

This aeromycological investigation indicated that, compared to houses at lower elevations, the airborne fungal spores load and the species variation were higher in higher-altitude dwellings. The seeds stored at a higher elevation had a greater infection rate and faster degree of nutrient loss than those stored at lower elevations may be due to the relative humidity which was higher in hilly regions. The seeds stored in the hilly regions were found to possess a higher amount of aflatoxin and reached to the permissible limit after one year of storage. As a result, inhabitants of villages at higher elevations are more likely to be exposed to fungal spores and consume seeds that contain higher levels of aflatoxin, which can be detrimental to their health.

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Research Article

REPRODUCTIVE BIOLOGY OF TREES OF WESTERN GHATS, INDIA:
AN OVERVIEWPOCHAMONI BHARATH SIMHA YADAV^{1*}, SUBBIAH KARUPPUSAMY¹ AND PAULRAJ SELVA SINGH RICHARD²¹DEPARTMENT OF BOTANY, BOTANICAL RESEARCH CENTRE, THE MADURA COLLEGE (AUTONOMOUS), MADURAI – 625011, INDIA (AFFILIATED WITH MADURAI KAMARAJ UNIVERSITY)²DEPARTMENT OF BOTANY, MADRAS CHRISTIAN COLLEGE (AUTONOMOUS), TAMBARAM, CHENNAI – 600059, INDIA*CORRESPONDING AUTHOR: bharathpochamoni@gmail.com

Plant reproductive biology is the study of process and mechanism of sexual and asexual reproduction in plants. It may encircle with a study of pollination mechanism, breeding systems, gene flow, and genetic variation between and within population. Understanding of the pollination biology and breeding systems are very essential to know the life cycle of a tree species. In India, very limited number of tree species has been studied with respect to reproductive biology and pollination systems. These studies are mostly confined to Western Ghats and Himalayan regions, specifically on endemic and rare trees or woody species. The present review is highlighting the reproductive biology and pollination systems of trees or woody taxa of Western Ghats, India.

Key Words: Breeding system, phenology, pollination, endemic trees, conservation, Western Ghats.

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INTRODUCTION

The Western Ghats is one of the richest biodiversity hotspots in India located between 08°19'18" – 21°16'24" N and 72°56'24" – 78°19'40" E. It has a total area of 1,64,280 km², covering six states of India such as Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. Anamudi is the height peak in Western Ghats with 2695 m MSL and the average elevation ranges between 900 and 1500 m MSL. Western Ghats is a home for more than 5800 flowering plants among these, 2100 species are endemic to this region, of which 352 are tree species¹. Richard and Muthukumar² recorded 247 arborescent taxa from southern Western Ghats of which 98 are endemic including 27 trees that are confined only to Agasthyamalai region. The southern end of Western Ghats *i.e.* south of Palghat Gap holds 1286 endemic taxa indicating that southern Western Ghats is high in endemism when compared to northern Western Ghats³. On the other hand, the presence of more than 56 endemic and 49 monotypic genera makes Western Ghats unique⁴. Among the endemic taxa over 60% are represented by trees. Sexual and breeding systems of tropical plants are very significant for understanding speciation processes in various tropical

forests; the selective pressure behind the development of sexual and mating systems as well as conserving tropical biodiversity^{5,6}.

Historical perspective

Plant reproductive biology and pollination have long intrigued naturalists. Early work by Kolreuter⁷ and especially Sprengel⁸ provided detailed insights into floral adaptations, highlighting their role in attracting insect pollinators. Sprengel's observations led to the idea that flowers evolved to enhance insect-mediated pollination. Building on this, Delpino⁹ introduced the concept of pollination syndromes—traits reflecting adaptations to specific pollinators—later refined by Faegri and van der Pijl¹⁰. Though once central, pollination syndromes are now seen as starting points for investigating floral biology, as it's unclear if current pollinators match those that influenced floral evolution¹¹⁻¹³. Since pollination interactions rarely fossilize, comparative studies across species are used to infer ancestral pollinators^{14,15}.

Pollination is crucial for plant reproduction, especially for obligate out-crossers and many self-pollinators.

Pollinator decline threatens plant reproductive success and biodiversity. Plants and pollinators have co-evolved, and ecological disruptions can desynchronize their life cycles. In both natural and agricultural systems, pollinators are vital.

The reproductive biology of angiosperms, particularly flowers—structures composed of modified fertile and sterile leaves—reveals key aspects of a species' life history. Studying reproductive biology aids understanding of pollination, gene flow, genetic variation, and seed dispersal, and informs species classification and evolutionary relationships¹⁶. In the Western Ghats, research on tree reproductive biology emphasizes the essential role of pollination in maintaining plant diversity.

Vegetative and Reproductive Phenology

Phenology refers to the study of seasonal life-cycle

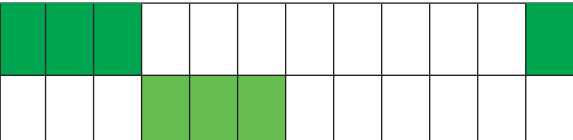
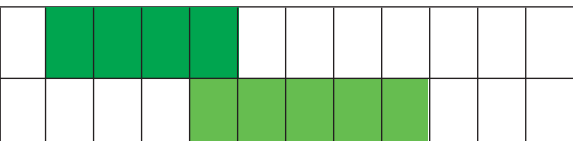
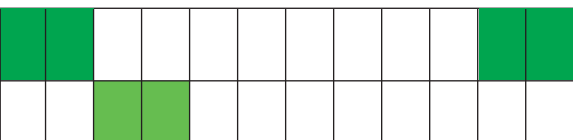
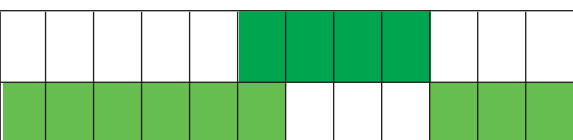
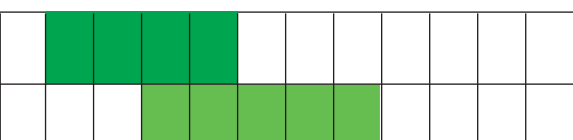
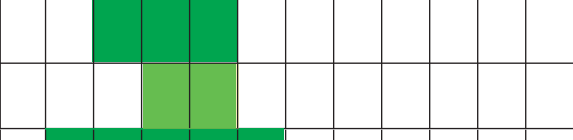
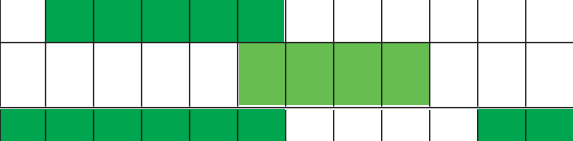
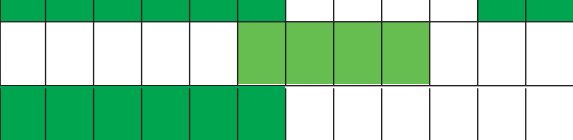
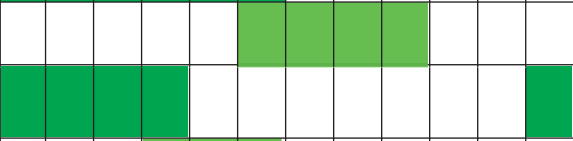
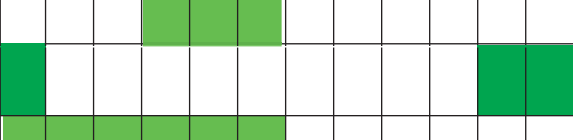
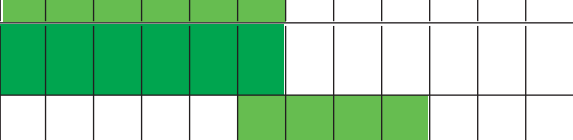
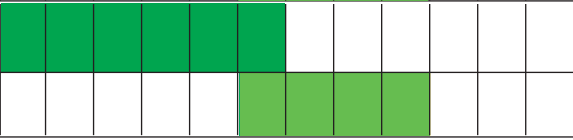

events in organisms, particularly plants, in response to climatic changes such as daylight, temperature, and precipitation. In plants, key phenological stages include bud formation, leafing, flowering, pollination, fruiting, and seed dispersal. These events are crucial for survival, reproduction, and species conservation, especially in ecologically sensitive regions like the Western Ghats.

In the southern Western Ghats, tree species exhibit diverse flowering patterns—classified as continual, sub-annual, annual, and supra-annual—to reduce competition for pollinators. For example: Continual flowering: *Monoon tirunelveliense* flowers year-round with protogynous blooms (Table 2.1). Sub-annual flowering: *Madhuca neriifolia* flowers twice—from October to November, and again from November to January. Multiple peaks: Most trees flower during the dry season (March–May), with a secondary peak at the onset of the rainy season.

Table 2.1: Phenology calendar of Western Ghats, India

Sl. No.	Plant Name and Family	Forest type	Flowering and Fruiting Phenology												Floral traits Floral color	P/O ratio
			J	F	M	A	M	J	J	A	S	O	N	D		
ANNONACEAE																
1.	<i>Manoon tirunelveliense</i>	TWEF													Greenish yellow	660:1
ASTERACEAE																
2.	<i>Vernonia travancorica</i>	TWEF														
CALOPHYLLACEAE																
3.	<i>Calophyllum apetalum</i>	TWEF														
CELASTRACEAE																
4.	<i>Salacia gambleana</i>	TWEF													Light green	187:1
5.	<i>Salacia oblonga</i>	TWEF													Greenish yellow	1573:1
CLUSIACEAE																
6.	<i>Garcinia imberti</i>	TWEF													Green	

Contd.

20.	<i>Cinnamomum sulphuratum</i>	TWEF		Greenish yellow	1292:1
MALVACEAE					
21.	<i>Cullenia exarillata</i>	TWEF			
MELASTOMATAACEAE					
22.	<i>Memecylon flavescens</i>	TWEF?		Bluish white	
MELIACEAE					
23.	<i>Aglaiia simplicifolia</i>	TWEF			
MYRTACEAE					
24.	<i>Eugenia discifera</i>	MWTF		White	
25.	<i>Syzygium caryophyllatum</i>	TWEF		Creamy white	
26.	<i>Syzygium heyneanum</i>	TWEF		White	
27.	<i>Syzygium laetum</i>	TWEF		Pink or Yellow	
28.	<i>Syzygium mundagam</i>	TWEF		White	
29.	<i>Syzygium occidentale</i>	TWEF		White	
30.	<i>Syzygium palghatense</i>			Creamy white	
31.	<i>Syzygium parameswaranii</i>	TWEF		Creamy white	
32.	<i>Syzygium travancoricum</i>	TWEF		White	

PITTOSPORACEAE

33.	<i>Pittosporum dasycaulon</i>	TWEF		Creamy white	2600:1
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POACEAE

34.	<i>Ochlandra scriptoria</i>	ORB			
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PROTEACEAE

35.	<i>Helicia nilagirica</i>	TWEF			
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PUTRANJIVACEAE

36.	<i>Drypetes malabarica</i>	TSEF		Creamy white	6096:1
37.	<i>Hydnocarpus macrocarpa</i>	TSEF		Green	87282:1

RUBIACEAE

38.	<i>Ixora brachiata</i>	TWEF			
39.	<i>Octotropis travancorica</i>				

SAPOTACEAE

40.	<i>Madhuca indica</i>				
41.	<i>Madhuca neriifolia</i>	TWEF			74044:1

Species-specific observations: *Humboldtia* spp. like *H. decurrens*, *H. sanjappae*, and *H. vahliana* share overlapping flowering periods, causing competition for pollinators. *Pittosporum dasycaulon* flowers from February to April (peak in March). In dioecious *Garcinia imberti*, male and female trees flower in February and March, respectively. *Elaeocarpus* species flower during the wet monsoon; *E. blascoi* shows leaf flushing and fruit development in February, with fruit fall in June. *Calophyllum apetalum* flowers from late October to mid-November; fruiting starts by late November. *Dipterocarpus bourdi-*

llonii and *D. indicus* flower between November and January. *Cullenia exarillata* flowers February–May; fruits from May–September. Species such as *Eugenia discifera*, *Syzygium* spp., and *Vateria macrocarpa* flower March–May, with fruiting up to July. Members of Lauraceae, Moraceae, *Myristica*, and *Polyalthia* fruit in April–May, aligning with the breeding season of the Great-pied hornbill, thus supporting bird diets. Phenological patterns in Western Ghats trees are closely tied to the region's dual monsoon system, with flowering and fruiting timed to optimize pollination success

and seed dispersal while minimizing competition. Understanding these patterns is essential for biodiversity conservation and ecological research.

Floral biology and Morphology

The various stages of floral ontogeny will provide valuable anatomical and morphological features of floral biology. This includes the measurements of floral length, width, length of petals and sepals, width of petals and sepals and length of pistil and stamen from bud initiation to until flower senescence. Perusal of literature revealed that the floral biology and morphology has been observed in some tree species by different authors, *Calophyllum apetalum* Willd. Two stages of flower opening were observed in *Cinnamomum sulphuratum*⁴⁵. Flowers are hermaphrodite, zygomorphic and complete in *Eugenia discifera*. Flowers are actinomorphic, complete and scented in *Dipterocarpus bourdillonii*. Flowers small, hermaphrodite, complete, zygomorphic in *Pittosporum dasycaulon*. Flowers are actinomorphic, white, large and sweet scented in *Syzygium occidentale*¹⁶. *Memecylon flavescens* flowers are hermaphrodite, actinomorphic, pin type, protogynous, blue in color, in axillary clusters.

Pollen production, Germination, Viability, and Pollen-ovule ratio

Pollen morphology is important for plant taxonomic classification and gene flow, especially in out crossing plants. Pollen production will be determined from the matured anthers of flowers. Climate change, seasonal variation, anther length, pollen grain size, and the mode of anther dehiscence are all factors that influence pollen production in plants. Number of ovules per ovary used to calculate by taking cross section of ovary. Pollen viability is 82.60%, 80.69% and 87.3% observed by author in *Cinnamomum sulphuratum* during the 2011, 2012 and 2013. In *Dipterocarpus indicus* the average pollen viability is recorded 62.94% and 57.68%. In *Drypetes malabarica* female flower had four ovules, the pollen-ovule (P:O) ratio is 6096:1 and *Hydnocarpus macrocarpa* female flower had 54 ovules and hence the P:O ratio was worked out as 87,282:1 for the species. Pollen-ovule ratio is 1062:1 in *Humboldtia decurrens*. Pollen ovule ratio is 1166:1 in *Humboldtia sanjappae*. The pollen germinations of 73.44% in MTT assay, and 73.10 % in 20% sucrose solution were recorded in *Manoon tirunelveliense*. The pollen ovule ratio is 1349:1 in *Syzygium occidentale*, it is indicating that the

plant permits both anemophily and entomophily¹⁶.

Breeding systems

Breeding systems in plants range from complete self-compatibility to strict self-incompatibility, with many species exhibiting intermediate forms. Self-incompatible species are more negatively affected by habitat loss and show greater pollen limitation than self-compatible ones. Breeding systems reflect the extent of self- and cross-pollination, influencing gene transmission across generations⁵.

Small-flowered plants often self-pollinate without pollinators, while larger-flowered species rely more on cross-pollination or are self-incompatible. Evolutionary shifts from outcrossing to inbreeding are common across plant genera. Breeding experiments show variation among species: *Calophyllum apetalum* and *Dipterocarpus indicus* had highest fruit set with hand cross-pollination and lowest with open pollination. *Cinnamomum sulphuratum* lacks apomixis but shows allogamy, autogamy, and insect-mediated pollination. Four *Syzygium* species showed low apomixis; *S. travancoricum* and *S. heyneanum* are fully self-compatible, while *S. laetum* and *S. mundagam* are partially self-compatible, with better fruit set from cross-pollination. *Eugenia discifera* is fully self-compatible and fertile through outcrossing. *Garcinia imberti* is self-incompatible, with higher fruit set from manual cross-pollination. *Humboldtia* species prefer cross-pollination. *Madhuca neriifolia* is self-compatible with high fruit set in both self- and cross-pollination. *Elaeocarpus blascoi* allows self-pollination but is incompatible with cross-pollination; flower structure favors selfing. *Pittosporum dasycaulon* is self-incompatible and an obligate outcrosser, with no fruit from self-pollination. *Vateria macrocarpa* is self-compatible with moderate fruit set under natural, geitonogamous, and xenogamous pollination. Its flower structure (protogyny and pin-type) favors cross-pollination (Table 2.2)

Pollination and Floral visitor's dynamics and behavior

Pollination—the transfer of pollen from anthers to stigma—occurs via two main types: autogamy (self-pollination) and allogamy (cross-pollination). The mode of pollination influences gene flow, genetic purity, adaptability, and plant breeding outcomes. To assess pollinator diversity and behavior, observations should be made at different times of the day, considering approach mode, visitor type, foraging behavior, contact

Table 2.2: Breeding systems in the plants of Western Ghats, India: Aut - Autogamy, All - Allogamy, Gei-Geitonogamy, Xen - Xenogamy, ASP - Artificial self-pollination, ACP - Artificial cross pollination, OP - Open pollination, Apo - Apomixis

Sl. No.	Plant Name and Family	Breeding systems and fruit set percentage								Fruits type	Modes of fruit dispersal	Seed germination in %
		Aut	All	Geit	Xen	ASP	ACP	OP	Apo			
ANNONACEAE												
1.	<i>Manoon tirunelveliense</i>									Drupe		
CALOPHYLLACEAE												
2.	<i>Calophyllum apetalum</i>						98.8	73.1		Drupe		
CLUSIACEAE												
3.	<i>Garcinia imberti</i>						53.3	36.6	30	Drupe		
DIPTEROCARPACEAE												
4.	<i>Dipterocarpus indicus</i>					59.7	69.5	56.9		Nutlike		
5.	<i>Vateria macrocarpa</i>	18		26	24			22		Drupe		
ELAEOCARPACEAE												
6.	<i>Elaeocarpus blascoi</i>	72						78		Drupe		48
7.	<i>Elaeocarpus gaussonii</i>									Drupe	Zoochory	
8.	<i>Elaeocarpus recurvatus</i>									Drupe	Zoochory	
FABACEAE												
9.	<i>Humboldtia bourdillonii</i>						39.6	16.5		Pod	Dehiscent	
10.	<i>Humboldtia decurrens</i>									Pod		
11.	<i>Humboldtia sanjappae</i>									Pod		
12.	<i>Humboldtia vahliana</i>									Pod		
13.	<i>Saraca asoca</i>			0.46				2.28		Pod		
LAURACEAE												
14.	<i>Cinnamomum sulphuratum</i>	70	75					63		Berry		
MELASTOMATACEAE												
15.	<i>Memecylon flavescens</i>									Pod		negligible
MYRTACEAE												
16.	<i>Eugenia discifera</i>	34		48	42			38		Drupe	Zoochory	
17.	<i>Syzygium caryophyllatum</i>				42			18		Drupe		
18.	<i>Syzygium heyneanum</i>	1.0		37.9	36.9			31.3	7.6	Drupe		
19.	<i>Syzygium laetum</i>	9.1		42.9	62			35.2		Drupe		
20.	<i>Syzygium mundagam</i>	11		36.1	51.7			56.7	12.5	Drupe		
21.	<i>Syzygium travancoricum</i>	5.9		52.1	50			41.2	4.9	Drupe		
PITTOSPORACEAE												
22.	<i>Pittosporum dasycaulon</i>		57							Capsule		

Contd.

PUTRANJIVACEAE

23.	<i>Drypetes malabarica</i>									Drupe	Zoochory	
24.	<i>Hydnocarpus macrocarpa</i>									Drupe	Zoochory	85

SAPOTACEAE

25.	<i>Madhuca neriifolia</i>					97.9	97.9	82.9		Berry		
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with reproductive organs, floral reward preference, visit frequency, and duration¹⁰ (Fig. 2.1).

In tropical rainforests like the Western Ghats, insects—especially bees—are primary pollinators, particularly for canopy trees. Medium to large bees (e.g., *Halictidae*, *Apidae*, *Anthophoridae*, *Megachilidae*) and smaller bees (*Apidae*, *Halictidae*, *Megachilidae*) dominate. Honeybees (*Apis dorsata*, *A. cerana*, *A. florea*, *Tetragonula iridipennis*) and carpenter bees (*Xylocopa pubescens*, *X. violacea*) are key pollinators of endemic tree species such as *Calophyllum apetalum*, *Dipterocarpus bourdillonii*, and *Syzygium caryophyllatum*^{4,5} (Table 2.3).

Butterflies also play a vital role, especially for nectar-producing tubular flowers like *Psychotria nilgiriensis*, *Vernonia ramosamii*, and *V. travancorica*, attracting species like *Appias wardii* and *Ypthima ypthimoides*. Moths and flies contribute to pollination in species like *Syzygium occidentale* and *Vateria macrocarpa*. Ants may rob nectar but also participate in pollination, as seen in *Cinnamomum sulphuratum* alongside wasps and butterflies.

Certain trees, like those in the Annonaceae family, rely on beetles due to large, odoriferous flowers. *Elaeocarpus* species exhibit floral adaptations to attract specific pollinators: sapromyophilous flies in *E. serratus*, moths and beetles in *E. tuberculatus* and *E. venustus*. *Madhuca neriifolia* shows multiple pollination modes (birds, bats, wind, and insects), while *Salacia fruticosa* experiences limited pollination due to low pollen and visitor counts.

Birds and mammals also play a significant role. *Syzygium heyneanum* attracts up to 23 pollinators, including sunbirds and barbets. *Cullenia exarillata* follows a predator-pollination system, attracting fruit bats, macaques, squirrels, civets, and numerous bird species. Notably, the endemic bat *Latidens salimalii* is a key pollinator for *Syzygium mundagam*⁸.

Flower, Fruit and Seed predation

Compare to folivory (herbivory on leaves) or seed predation, florivory (herbivory on flower parts) is relatively

less studied and its impact are unclear. Florivory is an important ecological interaction that can have a major impact on plant reproduction. Grizzled Giant Squirrel is foraging on flowers of *Albizia lebbbeck*, *Cayratia trifolia* *Mangifera indica* and *Tamarindus indica*. Lion Tailed Macaque is predating on flowers or flower buds of *Erythrina indica* and *Moringa oleifera*. At the individual, population, and community levels, seed predation is a major ecological and evolutionary force affecting plant community diversity, demography, and phenology. Seed predation and the quantity of frugivores in Indian forests are poorly understood. Plant spatiotemporal distribution, plant density, seed crop sizes, seed chemistry, seed size, season, fruit ripening, soil humidity, temperature, pollination rates, predator density, and availability of alternative food for generalist predators are all factors that influence seed predation. *Drypetes malabarica* and *Hydnocarpus macrocarpa* fruits largely predated by Malabar Grey Horn Bill (*Tockus griseus*), Flying squirrel (*Petaurista philippensis*), Malabar giant squirrel (*Ratufa indica*) and some mammals' species. Bonnet Macaque, Flying Fox, Indian Crested Porcupine, Lion-tailed Macaque, Malabar giant squirrel, Nilgiri langur, green barbet, Indian white-eye and Red-whiskered bulbul are major fruit and seed predators of *Elaeocarpus gaussonii* and *E. recurvatus*; causing considerable quantity of seed and fruit damage. In *Elaeocarpus blascoi* seeds are with hard endocarp, some of the seeds fully lost their viability within year after detaching from the mother tree and only 48% seeds are viable. *Altica cyanea* (Flea beetle) and *Anomala* sp. (Shining leaf chafers), were known to feed the petals of *Eugenia discifera*, thus affecting the fruit-set; the fruits were majorly predated by *Curculio c-album* (seed weevil) and other some insects. Two group of insects Dipteran and Lepidopteran are mostly predating on fruit and flower of *Dipterocarpus bourdillonii*, weevils are major predators of *Humboldtia burdillonii* flower buds. Malabar giant squirrel, Lion-tailed macaque and birds are major predators of *Cullenia exrillata* fruits and



Fig. 2.1: Pollination mechanism in plants of Western Ghats: (a) Slate flash butterfly foraging nectar from *Alstonia scholaris* flowers, (b) Purple sunbird nectaring on *Alstonia scholaris* flowers, (c) Calliphorid and Tipulid flies consuming nectar from *Schefflera agasthiyamalayana*, (d) A The phritid fruit fly visiting the blossoms of *Elaeocarpus gaussoni*, (e) Oriental white-eye bird foraging nectar from the flowers of *Rhododendron arboreum* subsp. *nilagiricum*, (f) Black bulbul visiting the blossoms of *Acrocarpus fraxinifolius*, (g) A hesperiid butterfly foraging nectar from *Fagraea ceilanica* flower, (h) A calliphorid fly foraging nectar from *Nothapodytes nimmoniana* flower, (i) Beetle eating the petals of *Eugenia mabaeoides*, (j) *Apis cerana* (Asian honey bee) visiting *Baccaurea courtallensis* flowers, (k) *Crematogaster* ant consuming pollen of *Symplocos anamallayana*, (l) Southern birdwing nectaring on *Clerodendrum viscosum* blossoms.

Table 2.3: Pollinators and their functional groups of trees of Western Ghats

Sl. No.	Scientific name	Functional group	Probable pollinator
ANNONACEAE			
1.	<i>Munoon tirunelveliense</i>	Bee	
		Beetle	
		Weevil	
		Fly	
		Thrips	
ARALIACEAE			
2.	<i>Schefflera wallichiana</i>	Bee	<i>Apis cerana</i>
		Bird	<i>Zesterops palpebrosus</i> (Indian White Eye)
ASTERACEAE			
3.	<i>Vernonia travancorica</i>	Bee	<i>Apis dorsata</i> , <i>A. cerana</i>
BIGNONIACEAE			
4.	<i>Spathodea campanulata</i>	Bird	<i>Pycnonotus jocosus</i> (Red-whiskered Bulbul)
CALOPHYLLACEAE			
5.	<i>Calophyllum apetalum</i>	Bee	<i>Apis mellifera</i> , <i>A. dorsata</i> , <i>Xylocopa violacea</i> , <i>X. pubescens</i>
CELASTRACEAE			
6.	<i>Salacia gambleana</i>	Ant	
7.	<i>Salacia oblonga</i>	Ant	
CLUSIACEAE			
8.	<i>Garcinia imberti</i>	Ant	
		Cockroach	
		Snail	
DIPTEROCARPACEAE			
9.	<i>Vateria macrocarpa</i>	Bee	
		Butterfly	
EBENACEAE			
10.	<i>Diospyros foliolosa</i>	Bee	<i>Apis cerana</i> , <i>Amegilla</i> sp.
		Beetle	Chrysomelidae
		Butterfly	<i>Hasora chromus</i> (Banded awl)
		Ant	Formicidae
ELAEOCARPACEAE			
11.	<i>Elaeocarpus blacoi</i>	Bee	<i>Apis cerana</i> , <i>Apis indica</i> , <i>A. dorsata</i>
		Fly	Carrion fly, fruit fly, unidentified fly
		Mites	
12.	<i>Elaeocarpus serratus</i>	Fly	Calliphoridae

Contd.

Contd. Table 2.3

Sl. No.	Scientific name	Functional group	Probable pollinator
13.	<i>Elaeocarpus veustus</i>	Beetle	Phalacridae
ERYTHROXYLON			
14.	<i>Erythroxyton obtusifolium</i>	Bee	<i>Apis cerana</i> , <i>Apis dorsata</i>
EUPHORBIACEAE			
15.	<i>Euphorbia santapaui</i>	Ant	Formicidae
		Fly	Syrphidae
FABACEAE			
16.	<i>Humboldtia bourdillonii</i>	Bee	
		Butterfly	
		Bird	Leptocoma minima (Sun bird)
17.	<i>Humboldtia decurrens</i>	Bee	<i>Apis cerana</i> , <i>Trigona iridipennis</i>
		Butterfly	<i>Euploea core</i> , <i>Pachliopta hector</i> , <i>P. aritolochia</i> , <i>Papilio demoleus</i> , <i>Oecophylla smaragdina</i>
18.	<i>Humboldtia sanjappae</i>	Bee	<i>Apis cerana</i> , <i>A. dorsata</i> , <i>Trigona iridipennis</i>
		Butterfly	<i>Euploea klugii</i> , <i>Jamides alecto</i>
19.	<i>Humboldtia vauhliana</i>	Bee	<i>Apis cerana</i> , <i>A. dorsata</i>
		Burrefly	<i>Euploea core</i> , <i>Pachliopta hector</i> , <i>P. aritolochia</i>
FLACOURTIACEAE			
20.	<i>Homalium travncorium</i>	Bee	<i>Trigona</i> sp.
		Fly	Calliphoridae, Syrphidae
LAURACEAE			
21.	<i>Cinnamomum sulphuratum</i>	Bee	<i>Apis dorsata</i> , <i>A. indica</i> , <i>A. florea</i> , <i>Trigona iridipennis</i>
		Butterfly	<i>Cupha erymanthis</i> (Rustic butterfly)
		Wasp	<i>Vespa</i> sp.
22.	<i>Litsea</i> sp.	Fly	<i>Sciaria</i> sp.
		Beetle	Phalacridae
MALVACEAE			
23.	<i>Cullenia exarillata</i>	Bee	<i>Apis indica</i>
		Butterfly	
		Bird	<i>Leptocoma minima</i> (Sun bird)
		Mammal	<i>Macaca silenus</i> (Lion-tailed macaque), <i>Ratufa indica</i> , <i>Funambulus sublineatus</i> , <i>Petaurista petaurista</i> , <i>Plantacanthomys lasiurus</i> , <i>Paradoxurus jerdoni</i> , <i>Cynopteres sphynx</i> and <i>Latidens salimalii</i>
MYRTACEAE			
24.	<i>Eugenia discifera</i>	Bee	<i>Apis</i> spp, <i>Anomala</i> sp., <i>Altica cyanea</i>

Contd.

Sl. No.	Scientific name	Functional group	Probable pollinator
25.	<i>Syzygium caryophyllatum</i>	Bee	<i>Apis cerana</i> , <i>A. dorsata</i> , <i>Xylocopa</i> sp.
		Butterfly	<i>Euploea core</i> (Common crow), <i>Junonia lemonias</i> (Lemon pansy), <i>Junonia iphita</i> (Chocolate pansy), <i>Tirumala limniace</i> (Blue tiger)
		Wasp	<i>Scolia</i> sp., <i>Sphex</i> sp., <i>Cryptocheilus</i> sp.
26.	<i>Syzygium heyneanum</i>	Bee	<i>Apis dorsata</i> , <i>A. cerana</i> , halictid bee
27.	<i>Syzygium laetum</i>	Fly	Syrphid fly
		Bird	<i>Leptocoma minima</i> (Sunbird)
		Wind	
28.	<i>Syzygium mundagam</i>	Bee	
		Butterfly	
		Bird	<i>Leptocoma minima</i> (Small sunbird), <i>Psilopogon viridis</i> (Small green barbet)
		Beetle	Unidentified
		Moth	Unidentified
29.	<i>Syzygium occidentale</i>	Bee	<i>Apis cerana</i> , <i>A. dorsata</i> , <i>Trigona</i> sp.
		Butterfly	
		Moth	
30.	<i>Syzygium laetum</i>	Bee	
		Butterfly	
		Fly	<i>Calliphora</i> sp. (Blue bottle)
		Wasp	
31.	<i>Syzygium parameshwarnii</i>	Bee	
		Wasp	
		Butterfly	
		Moth	
		Fly	
		Beetle	
32.	<i>Syzygium travancoricum</i>	Bee	<i>Apis cerana</i> , halictid bee
		Fly	Syrphid fly
	<i>Syzygium tamilnadensis</i>	Beetle	Chrysomelidae
		Fly	<i>Sciaria</i> sp.
OLEACEAE			
33.	<i>Olea paniculata</i>	Fly	Tipulidae
PITTOSPORACEAE			
34.	<i>Pittosporum dasycaulon</i>	Bee	
		Butterfly	

Contd. Table 2.3

Sl. No.	Scientific name	Functional group	Probable pollinator
POACEAE			
35.	<i>Ochlandra scriptoria</i>	Bee	<i>Apis cerana</i>
36.	<i>Ochlandra travancorica</i>	Bee	<i>Apis dorsata</i>
		Moth	
PUTRANJIVACEAE			
37.	<i>Drypetes malabarica</i>	Bee	
		Butterfly	
		Moth	
38.	<i>Hydnocarpus macrocarpa</i>	Bee	<i>Apis florea</i>
		Butterfly	<i>Cirrochroa thais</i> (Tamil yeoman), <i>Euploea core</i> (Common crow), <i>Pachliopta aristolochiae</i> (Common rose),
		Bird	<i>Passer doemsticus</i> (Sparrow)
		Moth	Unidentified
RUBIACEAE			
39.	<i>Canthium travancorium</i>	Bee	<i>Apis cerana</i> , <i>Xylocopa</i> sp.
		Beetle	Cerambycidae
		Butterfly	Papilionidae
40.	<i>Psychotria nilgherense</i> var. <i>congesta</i>	Bee	<i>Apis cerana</i> , <i>Amegilla</i> sp., <i>Xylocopa</i> sp. and <i>Lasioglossum</i> sp.
		Butterfly	<i>Graphium doson</i> (common jay), <i>Papilio helenus</i> (large swallow-tail butterfly), <i>Appias wardi</i> (Indian albatross), <i>Cepora Nerissa</i> (common gull), <i>Ypthima</i> sp., <i>Akasinula akasa</i> (gossamer-winged butterfly), <i>Jamides celeno</i> (common cerulean), <i>Nacadubba</i> sp., <i>Abisara echerius</i> (Plum judy)
		Fly	<i>Calliphora</i> sp., <i>Melangyna</i> sp., <i>Episyrphus</i> sp.
		Wasp	Vespidae
RUTACEAE			
41.	<i>Acronychia pedunculata</i>	Ant	Formicidae (<i>Crematogaster</i> sp.)
42.	<i>Vepris bilocularis</i>	Beetle	Chrysomelidae
SAPOTACEAE			
43.	<i>Madhuca nerifolia</i>	Bee	<i>Apis dorsata</i> , <i>A. milifera</i> , <i>Xylocopa pubescens</i>
		Bird	<i>Psittacula krameri</i> (Rose-ringed parakeet), <i>Pycnonotus jocosus</i> (Red-whiskered bulbul), <i>P. cafer</i> (Red-vented bulbul), <i>Iole indica</i> (Yellow-browed bulbul)
		Bat	<i>Cynopterus sphinx</i> (Greater short-nosed fruit bat)
SYMPLOCACEAE			
44.	<i>Symplocos cochinchinensis</i>	Fly	<i>Sciaria</i> sp.
45.	<i>Viburnum punctatum</i>	Ant	Formicidae
		Fly	<i>Sciaria</i> sp.

flowers. Fruit and seed predation are the major factor influencing the low germination ability of *Syzygium caryophyllatum*, inflorescences of this tree species are mainly infested by aphids and ants, ripen fruits are predated by frugivorous birds. *Syzygium palghatense* flowers and fruits are falling due to fungal attack, and seeds attacked by insect species *Euderus*. *Anselmella kerrichi* is one of the major predators of *Syzygium parameswaranii* flowers and fruits. Flowers of *Vateria macrocarpa* infected by Beetle larvae. The seeds of 34 tree species including large fruits and seeds of tree such as *Durio exarillatus*, *Elaeocarpus* sp. and *Palaquium ellipticum* were identified from the feeding roost of *Latidens salimalii*, an endemic and endangered fruit bat of southern Western Ghats. This is very evident that Salim Ali's fruit bat is the only known flying mammal in high-elevation wet evergreen forest that involved in dispersing large-sized seeds¹³.

CONCLUSION

This review has summarized the reproductive biology of tree species in Western Ghats, India highlighting various aspects including the vegetative and reproductive phenology, floral biology, pollen production, germination, viability, pollen-ovule ratio, various breeding systems, pollination mechanisms, floral visitor's dynamics and behavior, flower, fruit and seed predation, threats and challenges. This enables us to examine the gaps in the propagation, management and conservation of endemic trees of Western Ghats. It is also very essential to understand the evolution and survival of endemic tree species to develop an effective conservation management and strategies for potential tree species. Floral biology, breeding mechanisms and reproductive rate of trees could indicate the vulnerability of the tree species in the natural environment; if this is accounted, it is possible to take an immediate action to conserve a potential tree species. Though there are many trees in western Ghats were studied on the reproductive aspects; yet there are much more endemic trees need to be studied in this region, only few forest areas covered by the previous authors in Western Ghats, still many areas not covered especially northern Western Ghats. It is obligatory that these endemic tree taxa have to be studied as their population are declining rapidly due to various factors like habitat loss, inadequate pollinators and seed dispersers, climate change etc. Such further studies could aid in conserving the endemic tree taxa that forms an integral part of a unique ecosystem of the Western Ghats. This knowledge will be significant for researchers, policymakers, and local communities to understand and work towards the protection and conservation of Western Ghats tree species for ensuring long-term sustainability of these ecosystems and to safeguard the invaluable natural heritage for posterity.

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Research Article

EXPLORATION OF AIRBORNE BACTERIAL AND FUNGAL DIVERSITY OF VIDYASAGAR UNIVERSITY AND MIDNAPORE MEDICAL COLLEGE AND HOSPITAL

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A study was conducted during summer (April-May 2023) to assess airborne microbial (bacteria and fungi) populations at two sites in Midnapore town, West Bengal, India—Vidyasagar University campus (Site 1) and Midnapore Medical College and Hospital (Site 2). Air sampling was performed using an Anderson 2-stage volumetric sampler and culture media at regular intervals. Microbial colonies were identified using conventional microbiological techniques. Bacterial concentration was higher at Site 2 (371 CFU/m³) compared to Site 1 (67 CFU/m³). Gram-negative bacilli constituted 62.5% of bacterial isolates at Site 1 and 50% at Site 2. Eight fungal species were identified across both sites, including *Aspergillus* sp., Aspergilli-Penicilli group, *Rhizopus* sp., *Cladosporium* sp., *Trichoderma* sp., *Cryptococcus neoformans*, *Alternaria alternata*, and *Fusarium* sp. *Aspergillus* sp. was the most prevalent fungus, accounting for 58% of isolates at Site 1 and 64% at Site 2.

Pearson's correlation analysis revealed a significant relationship ($P < 0.05$) between temperature/pollutant concentration and fungal CFU levels. The findings highlight the diversity and distribution of airborne microbes and their biochemical profiles in a university and hospital environment of an unexplored biozone of West Bengal.

Key Words: Airborne bacteria, airborne fungi, university campus, hospital area, biochemical test, air quality data, correlation.

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INTRODUCTION

In the dynamic interplay between humans and their environment, the invisible world of airborne microorganisms plays a critical role in shaping our health and well-being. The study of airborne bacterial and fungal communities has gained significant importance, particularly in distinguishing microbial diversity between normal settings and highly sensitive environments, such as hospitals¹. As these microorganisms constantly circulate in the air we breathe, understanding their composition, abundance, and potential implications becomes imperative, especially in the context of infection control and public health. Air contains pollutants, dust, microorganisms and small organic particles such as volatile microbial organic compounds, cell-free DNA, phytochemicals etc. When these microbes, micro-

bial components or freely floating plant wastes enter our body through breathing causes health issues². Airborne fungi and bacteria are sometimes toxic. Some of the species can cause severe infection or extensive allergic reaction when enters our body³.

No prior study has been conducted on the airborne microbial community in the Midnapore district of West Bengal. In this study, Vidyasagar University and the Medical College and Hospital have been selected as Site 1 and Site 2, respectively—both being key locations in Midnapore city. The diversity and concentration of airborne microbes, as well as their physicochemical properties, are influenced by factors such as meteorological conditions, air quality, and nutrient availability. Airborne microbial density was measured using an air sampler, expressed in colony-forming units per

cubic meter of air (CFU/m³). Common airborne bacteria in hospital environments include *Staphylococcus aureus*, *Acinetobacter*, *Bacteroides fragilis*, *Clostridium difficile*, *Enterobacteriaceae*, *Klebsiella*, and *Escherichia coli*, while typical hospital fungi include *Aspergillus* spp., *Candida albicans*, and *Cryptococcus neoformans*.

This study provides important data on the density and diversity of airborne bacterial and fungal communities in a university and hospital setting in Midnapore. It also examines how environmental factors—such as temperature, humidity, rainfall, and air quality—influence these microbial communities.

MATERIALS AND METHODS

Selection of Sampling area

For the study on the diversity of airborne fungi and bacteria, two different sites were selected: Vidyasagar University campus as study site 1 (S.S.1) and Midnapore Medical College and Hospital campus as study site 2 (S.S.2), in Midnapore town of West Bengal, India for April-May, 2023.

Collection of Sample and Analysis

Four samples were collected from each of the Study site. The samples were collected thrice in a week, in the afternoon between 3 p.m. to 5 p.m. The timing of sampling was after the visiting hour in hospital. The temperature of the study site ranges between 37.54°C-32.04°C.

Monitoring of Airborne Bacteria and Fungi

The monitoring was carried out in both the sites simultaneously. The air sampling was conducted at the height of human respiratory level. Andersen Sampler was used, which have the flow rate of 28.3 lit/min. The plates with culture media were kept for 10 minutes inside the suction device for the sedimentation of the air on plate. Total number of bacteria and fungi in the samples were counted as the colony forming unit per cubic meter air (CFU/m³).

Analytical media

For analytical purpose Nutrient Agar (NA) was used as the bacterial sampling media. On the other hand, Potato Dextrose Agar (PDA) was used for the sampling of the Fungus. At the time of sampling Amoxicillin was used

as antibacterial drug and mixed in PDA media, and Itraconazole was used as antifungal drug and mixed in NA media. Mueller Hinton agar media was used to perform the antibiotic sensitivity test of bacterial colonies. The plates of bacterial samples were incubated at 37°C for 24-48 hours and the fungal plates were incubated at 25°C for 5-6 days. Sterilization of various equipment during the study were done in autoclave at 15 psi, at 121°C for 15 mins.

Staining of Bacteria and Fungi

The bacterial colonies were identified at the macroscopic level based on their colony morphology, and the microscopic identification were done by the means of their Gram's character. The fungal colonies were identified at the genus and species level from the macroscopic and microscopic view. Fungal samples were stained using lactophenol and cotton blue for the identification.

Biochemical test of Bacterial sample

The bacterial samples of Study site 1 and Study site 2 were analyzed by their chemical means for the enumeration of different chemical characters and the comparison between the bacterial samples. Single colonies were selected as the representative of colonies having morphologically similar characteristics for all the biochemical tests. We performed catalase test, sugar fermentation test and IMVIC test for coliform bacteria. For the sugar fermentation test glucose, lactose and sucrose was selected.

Antibiotic Sensitivity test

As the samples were collected from the hospital campus, we performed the antibiotic sensitivity test with the bacterial samples from both the study site 1 and 2. We tried to know whether there is/are any antibiotic-resistant bacterial species in the study sites or not. The antibiotic sensitivity test is very crucial to study the presence of susceptible microorganism in the air of study sites. We tested for the sensitivity of Amikacin, Chloramphenicol, Vancomycin, Bacitracin and Streptomycin. There were some bacterial colonies from study site 1 having the resistant property against only bacitracin. On the other hand, a colony was isolated from study site 2 having the resistant property against both bacitracin and chloramphenicol.

Collection of meteorological parameters and air quality data

In this study of diversity of airborne microbial community, meteorological parameters and air quality played an important role. The occurrence of different types of bacterial species in the air is influenced by the current weather condition, such as – temperature of at the time of sampling, humidity of atmosphere and the amount of rainfall. Pollutants like – NO_2 , SO_2 , and air particulate matters (PM_{10} , $\text{PM}_{2.5}$) are also responsible for the diversity of bacterial and fungal species at the site of sampling. We considered the official websites of ‘Vidyasagar University Automatic Weather Station’ (<http://ccnet.vidyasagar.ac.in>) and (<http://emis.wbpcb.gov.in>) ‘West Bengal Pollution Control Board’ for collecting the current data of meteorological parameters and air quality index (AQI) respectively of the study sites. As the two study sites are present in the same location with proximity, the meteorological parameters and AQI were same for both.

Data Analysis

For the descriptive study, different statistical parameters like – maximum, minimum, mean, mode, standard deviation were used to analyse the collected data. Pearson’s correlation coefficient test was performed to determine the correlation between the CFU of bacterial/fungal species and meteorological parameters and AQI at the significance level of $p < 0.05$.

RESULTS

I. Sampling of Culturable bacteria and Fungal spore in the atmosphere of study sites:

The total average load of airborne bacteria at the study site 1 and study site 2 are 213.75 ± 45.15 CFU/ m^3 and 237.75 ± 79.64 CFU/ m^3 (Table 3.1 and 3.2) respectively, the total average load of airborne fungus at the study site 1 and study site 2 are 43.75 ± 14.72 CFU/ m^3 and 34.75 ± 5.53 CFU/ m^3 . The highest number of airborne bacteria was present in study site 2 (371 CFU/ m^3) and the airborne fungal load in higher in study site 1 (67 CFU/ m^3).

II. Meteorological parameters and air quality study:

The meteorological parameters may affect the overall variability in species level and their biochemical characteristics. The highest temperature (37.54°C) was observed on Day-3 and the lowest temperature (32.04°C) is observed on Day-1. The observation of average relative humidity was highest (71.53%) on Day-3 and lowest (63.04%) on Day-2 (Table 3.3). Among the total rainfall of all the sampling days, only rainfall was observed on Day-3 that is 7.11 mm.

Observation of Air quality parameters: This type of data provides us about the level of concentration of pollutants present in air. After observing the air quality of study site 1 and study site 2. The concentration of

Table 3.1: Mean concentrations of airborne bacteria at Study site 1 and Study site 2

Study site 1	CFU/ m^3	Mean \pm SD (CFU/ m^3 of air)	Study site 2	CFU/ m^3	Mean \pm SD (CFU/ m^3 of air)
Day 1	222		Day 1	170	
Day 2	187	213.75 ± 45.15	Day 2	226	237.75 ± 79.64
Day 3	163		Day 3	184	
Day 4	283		Day 4	371	

Table 3.2: Mean concentrations of airborne fungi at Study site 1 and Study site 2

Study site 1	CFU/ m^3	Mean \pm SD (CFU/ m^3 of air)	Study site 2	CFU/ m^3	Mean \pm SD (CFU/ m^3 of air)
Day 1	67		Day 1	42	
Day 2	45	43.75 ± 14.72	Day 2	31	34.75 ± 5.53
Day 3	28		Day 3	38	
Day 4	35		Day 4	28	

Table 3.3: Meteorological parameter								
Characteristics	Day 1 (25.04.2023)		Day 2 (02.05.2023)		Day 3 (10.05.2023)		Day 4 (17.05.2023)	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Temperature (°C)	32.04		34.01		37.54		36.32	
Average relative humidity (%)	69.17		63.04		71.53		68.94	
Total rainfall (mm)	0		7.11		0		0	

Table 3.4: Air Quality Index									
Characteristics		Day 1 (25.04.2023)		Day 2 (02.05.2023)		Day 3 (10.05.2023)		Day 4 (17.05.2023)	
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Concentration of pollutant ($\mu\text{g}/\text{m}^3$)	NO ₂	25		19		16		21	
	SO ₂	5		2		2		2	
	PM ₁₀	81		71		68		70	
	PM _{2.5}	48		36		36		41	
Overall quality	Score	81		71		68		70	
	Remark	Satisfactory		Satisfactory		Satisfactory		Satisfactory	

NO₂ (25 $\mu\text{g}/\text{m}^3$) was high on Day-1 and low on (19 $\mu\text{g}/\text{m}^3$) on Day-3 (Table 3.3), the SO₂ (5 $\mu\text{g}/\text{m}^3$) concentration in air was highest on Day-3 and other days had similar concentration (Table 3.3). These data also provide the concentration of particulate matter PM_{2.5}, PM₁₀ present in the air. The PM_{2.5} on Day-1 was likely higher than other days that was 48 $\mu\text{g}/\text{m}^3$ and lower on Day-2 and Day-3 that was 36 $\mu\text{g}/\text{m}^3$. The PM₁₀ was the most observed on Day-1 (81 $\mu\text{g}/\text{m}^3$) and least observed on Day-3 (68 $\mu\text{g}/\text{m}^3$) (Table 3.4).

After observing the air quality index, we can say that the air quality at the sampling sites was Satisfactory. We

observed the concentration and the amounts of pollutants and found that, Day-1 has the highest air quality index and Day-3 has the lowest air quality index.

III. Bacterial load and Diversity in the atmosphere of the study sites:

Count of colony forming unit (CFU): CFU count represents that the average number of airborne bacteria present in study site-1 and study site 2. Airborne bacteria that were taken from study site 1 showed CFU at a level ranged from 163-283 CFU/ m^3 and airborne bacteria that were taken from study site 2 expressed CFU at a level

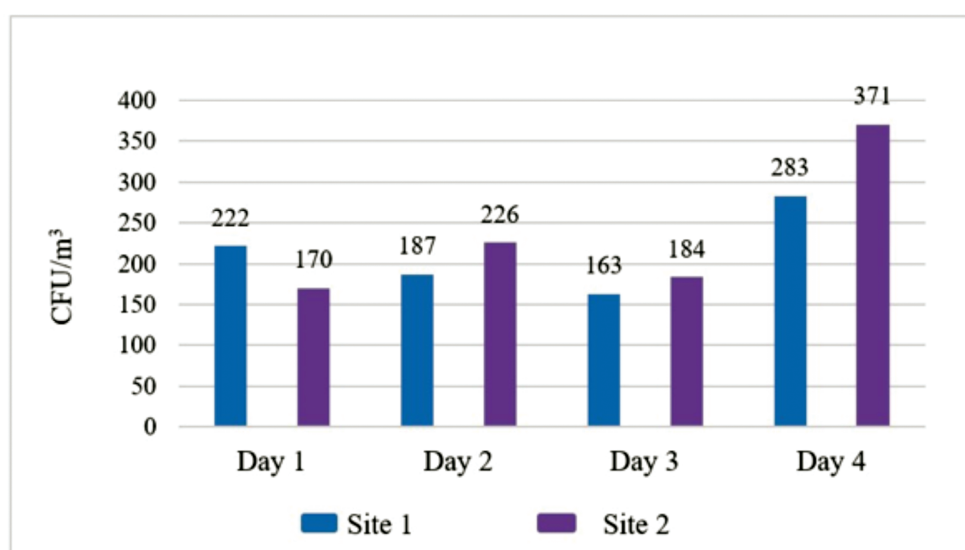


Fig. 3.1: Enumeration of airborne bacteria colony CFU/ m^3 in study sites

ranged from 170-371 CFU/m³ (Fig. 3.1). So, it was understood that the airborne bacterial load of the study site 2 was much higher than the bacterial load of the study site 1.

Among the four days of sampling in study site 1, the highest CFU was observed on Day-4 (283 CFU/m³) and lowest CFU was observed on Day-3 (163 CFU/m³). Study site 2 showed the highest CFU on Day-4 (371 CFU/m³) and showed lowest CFU on Day-1 (170 CFU/m³). This means that bacterial load was highest on Day-4 in study site 1 and Day-4 in study site 2 (Fig. 3.1).

Determination of Gram's Character: The airborne bacteria of sampling were determined after gram staining that Gram positive bacteria were observed only in the Day-2 of study site 1. For study site 2, Gram positive bacteria were observed on all days except Day-1 (Fig. 3.2). In terms of Gram-negative bacteria, both of the study sites showed the highest number of bacteria on Day-1 (Fig. 3.3).

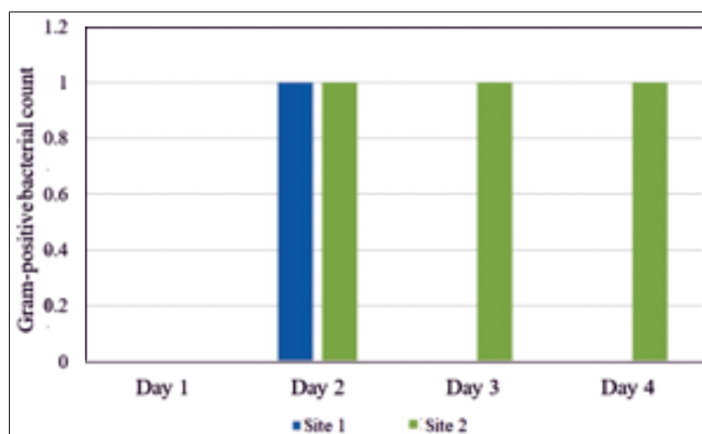


Fig. 3.2: Gram positive characters of airborne bacteria in the study sites

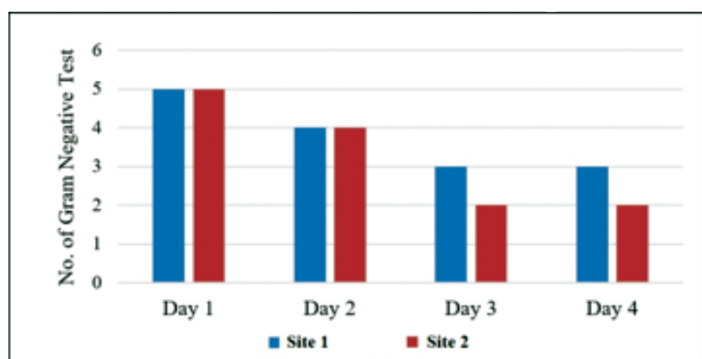


Fig. 3.3: Gram negative characters of airborne bacteria in the study sites

Gram negative coccus and Gram-negative bacillus were seen on Day-1 for both the study sites. Gram negative coccus was found to be the most common in study site 1 and study site 2 on Day-1. Gram negative coccus, Gram negative Bacillus, Gram positive Bacillus, Gram

positive Coccus were seen on Day-1 for both the study sites. Gram negative Bacillus are the most common in study sites on Day-2. Gram negative Bacillus were also most common in study sites on Day-3 and Day-4.

IV. Observation of biochemical character of airborne bacteria from study sites

Catalase Test. In the catalase test, study site 1 sample provides 3 among the 5 are catalase positive and study site 2 sample showed 4 among the 5 are catalase positive tests that means the Day-1 samples provides more positive results than the other days (Fig. 3.4). For both study site catalase test will give negative result. Most of the tests were positive on Day1 and 3 (Fig. 3.5).

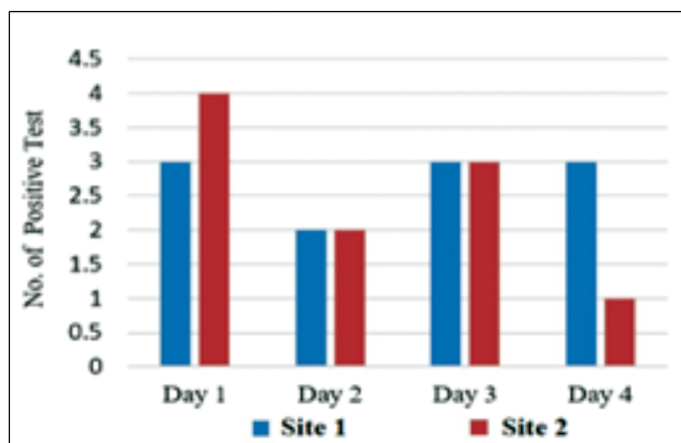


Fig. 3.4: Catalase test positive result for airborne bacteria in the study sites.

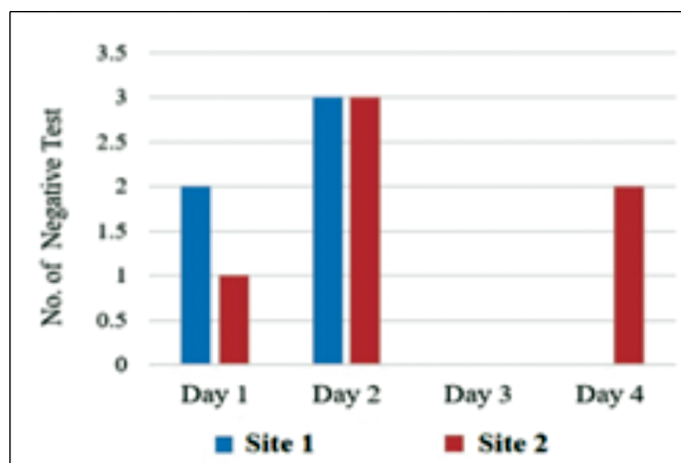


Fig. 3.5: Catalase test negative result for airborne bacteria in the study sites.

Study for sugar fermentation potential for airborne bacteria: In sugar fermentation test for positive result, 4 among the 5 sample are glucose fermenters were present in study site 1 and 4 among the 5 sample are glucose fermenters were present in study site 2, lactose fermenters were relatively higher in study site 2 than study site 1 (Fig. 3.6). Sucrose fermenters were relatively higher in study site 1 than study site 2.

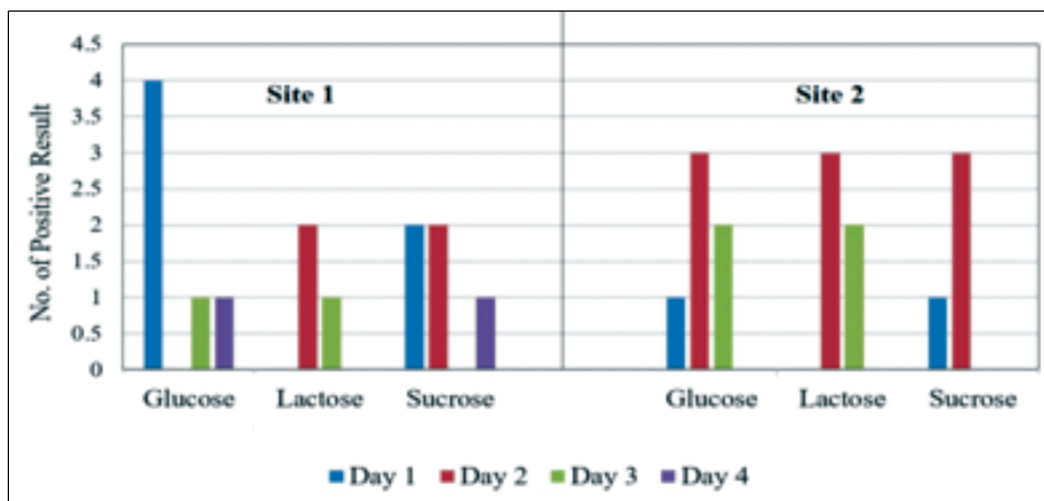


Fig. 3.6: Sugar Fermentation positive result

Indole, methyl red, Voges Proskauer and citrate (IMVIC) test to identify coliform bacteria: In IMVIC test, bacterial samples in study site 2 provide more positive indole test results than study site 1 (Fig. 3.7a), same number of methyl red positive test results were seen in site 1 and site 2 (Fig. 3.7b), for Voges- Proskauer test study site 1 provide more positive result than study site 2 (Fig. 3.7c), for citrate test study site 1 provide more positive result than study site 2 (Fig. 3.7d).

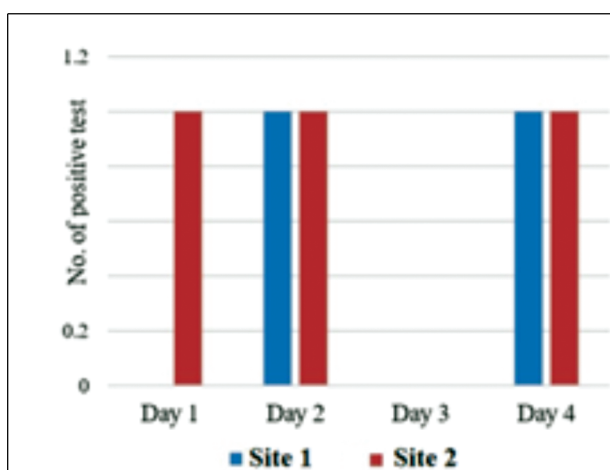


Fig. 3.7a: Indole positive test

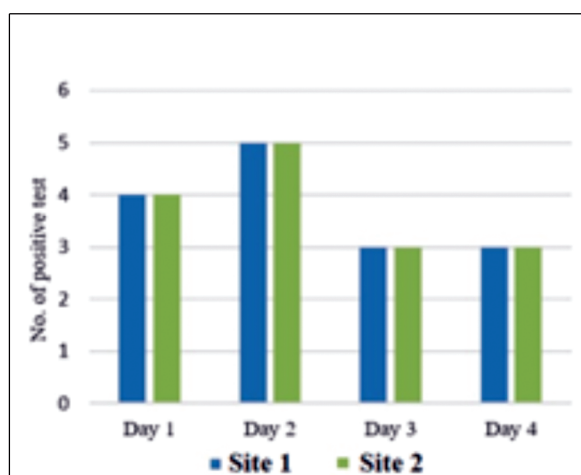


Fig. 3.7b: Methyl red positive test

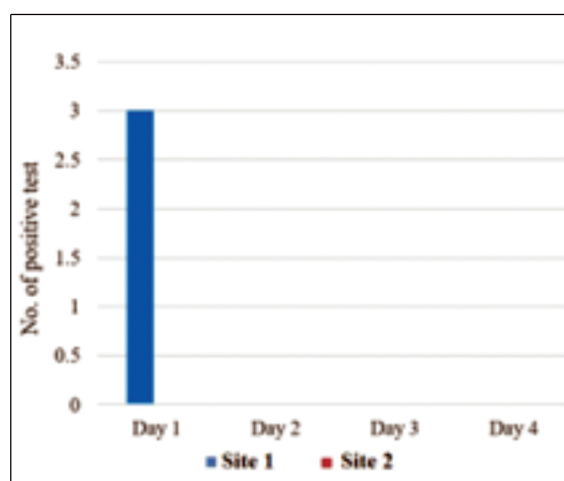


Fig. 3.7c: Voges-Proskauer test

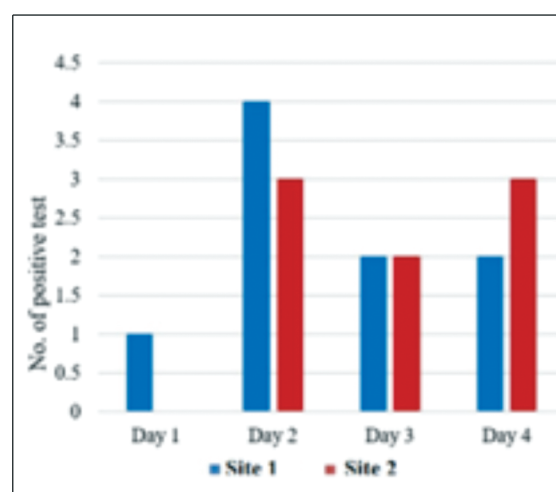


Fig. 3.7d: Citrate positive test

Antibiotic sensitivity test

In the antibiotic sensitivity test, most of the colonies from study site 1 and 2 showed antibiotic sensitive property against Amikacin, Chloramphenicol, Vancomycin, Bacitracin, Streptomycin on Day-1, only one colony C2 (Fig. 3.8) from study site 2 on Day 1 showed antibiotic resistance property to chloramphenicol and bacitracin

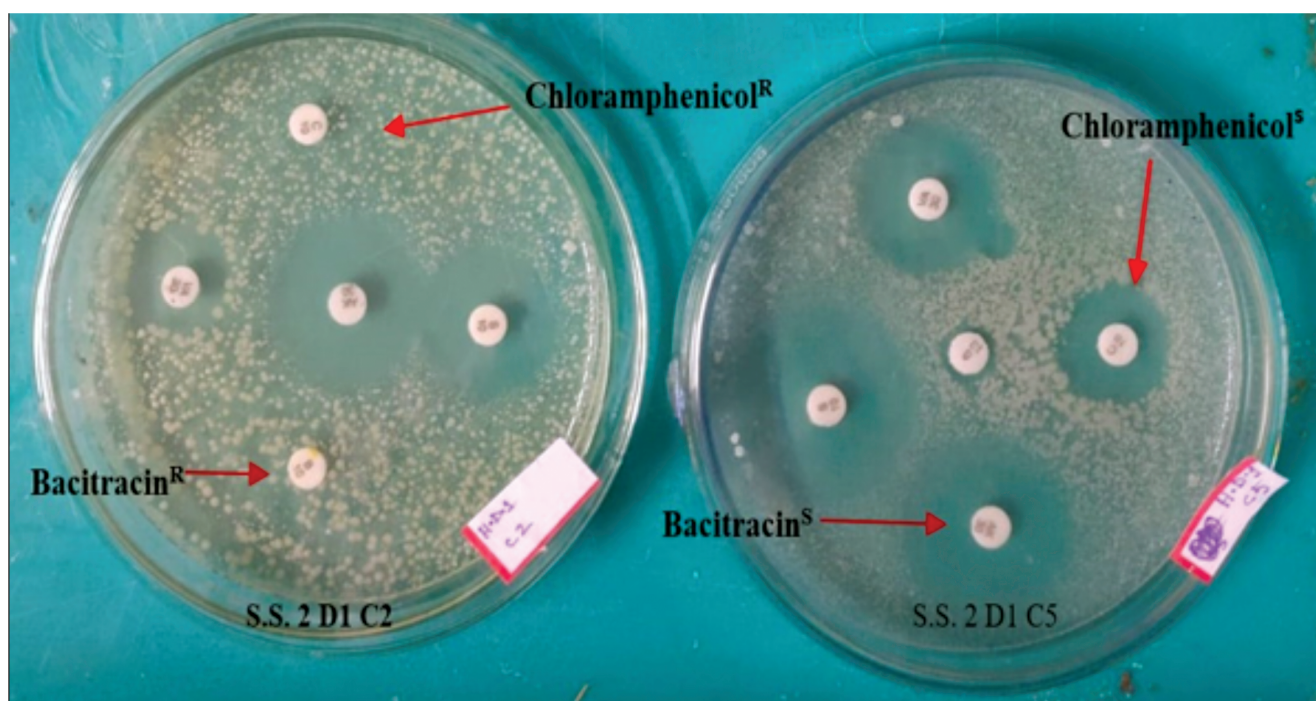


Fig. 3.8: S.S. 2 D1 C2 showed antibiotic resistance property against Chloramphenicol and Bacitracin, comparing with S.S. 2 D1 C5 showing antibiotic sensitive property against Chloramphenicol and Bacitracin.

Table 3.5: Antibiotic sensitivity test for bacteria trapped from air in Day 1

Colony	Amikacin	Chloramphenicol	Vancomycin	Bacitracin	Streptomycin
S.S. 1 – D 1 – C 1	+	+	+	+	+
S.S. 1 – D 1 – C 2	+	+	+	+	+
S.S. 1 – D 1 – C 3	+	+	+	+	+
S.S. 1 – D 1 – C 4	+	+	+	+	+
S.S. 1 – D 1 – C 5	+	+	+	+	+
S.S. 2 – D 1 – C 1	+	+	+	+	+
S.S. 2 – D 1 – C 2	+	–	+	–	+
S.S. 2 – D 1 – C 3	+	+	+	+	+
S.S. 2 – D 1 – C 4	+	+	+	+	+
S.S. 2 – D 1 – C 5	+	+	+	+	+

Table 3.6: Antibiotic sensitivity test for bacteria trapped from air in Day 2

Colony	Amikacin	Chloramphenicol	Vancomycin	Bacitracin	Streptomycin
S.S. 1 – D 2 – C 1	+	+	+	+	+
S.S. 1 – D 2 – C 2	+	+	+	+	+
S.S. 1 – D 2 – C 3	+	+	+	+	+
S.S. 2 – D 2 – C 1	+	+	+	+	+
S.S. 2 – D 2 – C 2	+	+	+	+	+
S.S. 2 – D 2 – C 3	+	+	+	+	+

****N.B.** Antibiotic sensitive property is denoted by plus (+) sign and antibiotic resistant property is denoted by minus (–) sign.

Table 3.7: Antibiotic sensitivity test for bacteria trapped from air in Day 3

Colony	Amikacin	Chloramphenicol	Vancomycin	Bacitracin	Streptomycin
S.S. 1 – D 3 – C 1	+	+	+	+	+
S.S. 1 – D 3 – C 2	+	+	+	+	+
S.S. 1 – D 3 – C 3	+	+	+	+	+
S.S. 2 – D 3 – C 1	+	+	+	+	+
S.S. 2 – D 3 – C 2	+	+	+	+	+
S.S. 2 – D 3 – C 3	+	+	+	+	+

Table 3.8: Antibiotic sensitivity test for bacteria trapped from air in Day 4

Colony	Amikacin	Chloramphenicol	Vancomycin	Bacitracin	Streptomycin
S.S. 1 – D 4 – C 1	+	+	+	+	+
S.S. 1 – D 4 – C 2	+	+	+	+	+
S.S. 1 – D 4 – C 3	+	+	+	+	+
S.S. 2 – D 4 – C 1	+	+	+	+	+
S.S. 2 – D 4 – C 2	+	+	+	+	+
S.S. 2 – D 4 – C 3	+	+	+	+	+

(Table 3.5). On Day-2, all colonies in study site 1 and 2 showed antibiotic sensitive property against all experimental antibiotics (Table 3.6). In the case of Day-3 all the colonies showed antibiotic sensitive property against all experimental antibiotics in both study site (Table 3.7). On Day-4, all colonies in study site 1 and 2 showed antibiotic sensitive property against all experimental antibiotics (Table 3.8).

V. Characterization of bacterial colonies with similar color, elevation and form

Bacterial colonies with morphologically similar characters were identified and used for further characterization like Gram's character, catalase test and total number of colonies. It was observed that total eight different types of colonies were present in the samples. Among those, colonies with morphologically off white, Raised, Round

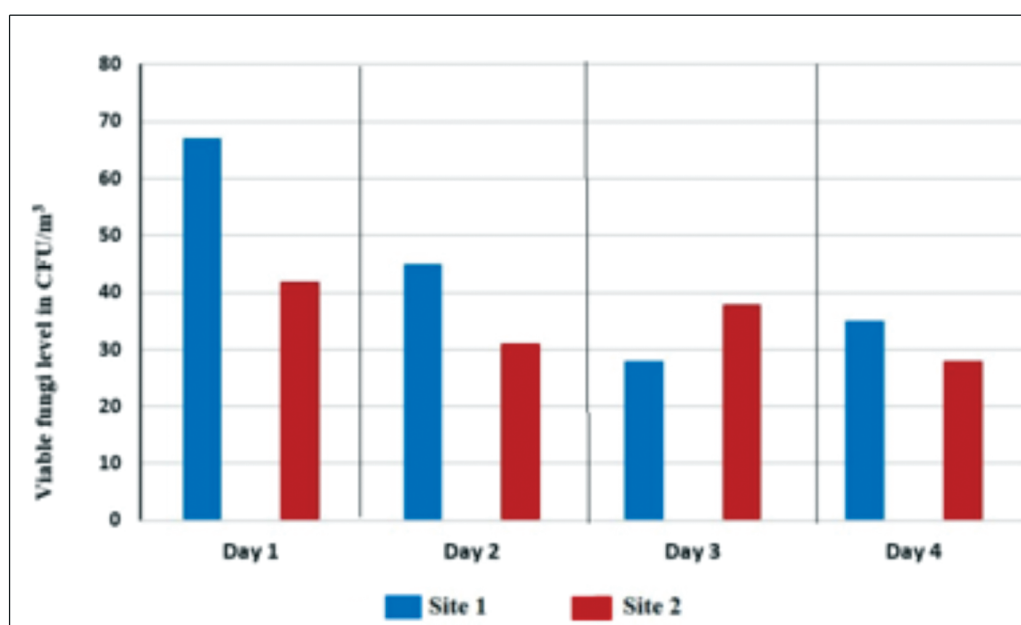
**Fig. 3.9:** Level of airborne fungi (CFU/m³) in study sites

Table 3.9: Comparative study table of bacterial samples

Characteristics	Day 1 (25.04.2023)		Day 2 (02.05.2023)		Day 3 (10.05.2023)		Day 4 (17.05.2023)	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Pink, Raised, Round	00	05	02	04	02	01	00	00
	--	Negative cocci	Negative bacilli	Negative bacilli	Negative bacilli	Negative bacilli	--	--
		Catalase positive	Catalase positive	Catalase positive	Catalase positive	Catalase positive		
Off white, Flat Irregular	18	07	09	15	00	16	32	22
	Negative cocci	Negative cocci	Negative bacilli	Negative bacilli	--	Negative bacilli	Negative cocci	Negative cocci
	Catalase negative	Catalase positive	Catalase negative	Catalase negative		Catalase positive	Catalase positive	Catalase negative
Off white, Raised, Round	30	16	04	06	00	11	26	9
	Negative bacilli	Negative bacilli	Negative bacilli	Negative bacilli		Negative bacilli	Negative bacilli	Negative bacilli
	Catalase negative	Catalase positive	Catalase negative	Catalase negative		Catalase positive	Catalase positive	Catalase positive
Light yellow, Flat, Round	04	09	06	08	05	03	06	14
	Negative cocci	Negative bacilli	Negative bacilli	Negative bacilli	Negative bacilli	Positive bacilli	Negative bacilli	Positive bacilli
	Catalase positive	Catalase positive	Catalase negative	Catalase positive	Catalase positive	Catalase positive	Catalase positive	Catalase positive
Dark yellow, Flat, Round	02	03	12	16	10	09	09	10
	Negative cocci	Negative bacilli	Negative bacilli	Negative bacilli	Negative bacilli	Negative bacilli	Negative cocci	Positive bacilli
	Catalase positive	Catalase positive	Catalase negative	Catalase negative	Catalase positive	Catalase positive	Catalase positive	Catalase negative
White, Flat, Round	05	07	15	09	11	07	05	23
	Negative cocci	Negative cocci	Negative cocci	Negative cocci	Negative bacilli	Negative bacilli	Negative bacilli	Negative bacilli
	Catalase negative	Catalase positive	Catalase positive	Catalase negative	Catalase positive	Catalase positive	Catalase positive	Catalase negative
White, Flat, Irregular	02	01	05	06	18	03	00	12
	Negative bacilli	Negative bacilli	Negative bacilli	Positive cocci	Negative bacilli	Negative bacilli	--	Negative bacilli
	Catalase negative	Catalase positive	Catalase positive	Catalase negative	Catalase positive	Catalase positive		Catalase positive
Red, Flat, Round	00	00	00	00	00	00	02	15
	--	--	--	--	--	--	Negative bacilli	Negative bacilli
							Catalase positive	Catalase negative

character, had the highest number of occurrences, and they were Gram negative bacilli. Only on Day-4, red, flat, round colonies were present in both the study site 1 and 2, and they were Gram negative bacillus and gave positive result for catalase test (Table 3.9).

VI. Airborne fungal load and diversity study

Colony forming unit (CFU Count): CFU count describes that the average number of airborne fungi present in study site 1 and study site 2. Airborne fungus that was taken from study site 1 showed CFU at a level ranged from 28-67 CFU/m³ and airborne fungus that were

taken from study site 2 expressed CFU at a level ranged from 28-42 CFU/m³ (Fig. 3.9). So, that means the airborne fungal load of the study site 1 was greater than the fungal load of the study site 2.

Among the four days of sampling in study site 1, the highest CFU was observed on Day-1 (67 CFU/m³) and lowest CFU was observed on Day-3 (28 CFU/m³). Study site 2 showed the highest CFU on Day-1 (42 CFU/m³) and showed lowest CFU on Day-4 (28 CFU/m³). This means that the fungal load was highest on Day-4 in both study site1 and study site 2.

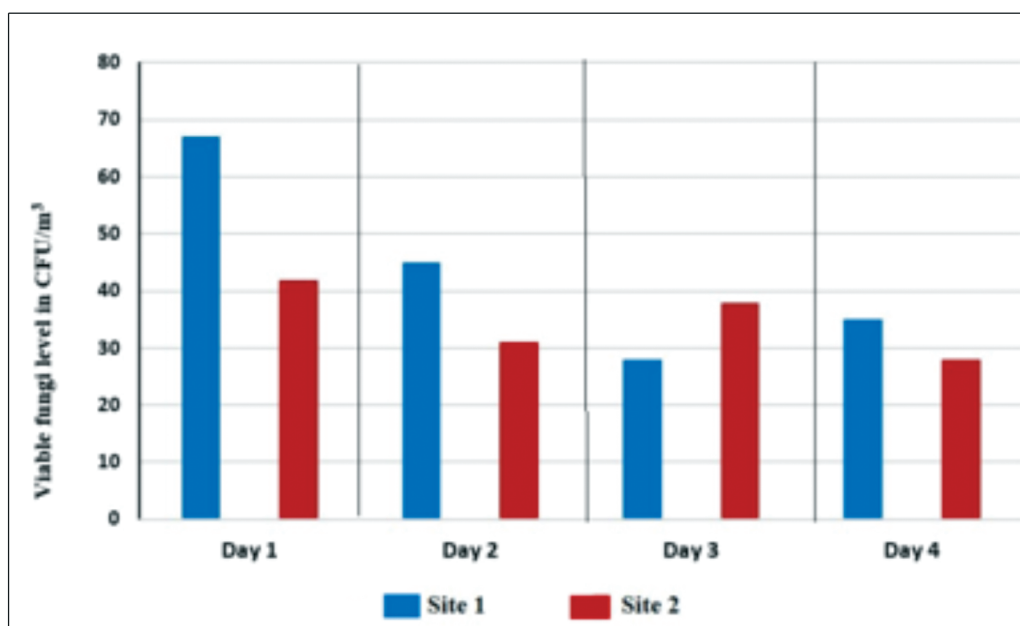


Fig. 3.9: Level of airborne fungi (CFU/m³) in study sites

Table 3.10: Identification of fungal colonies under microscope

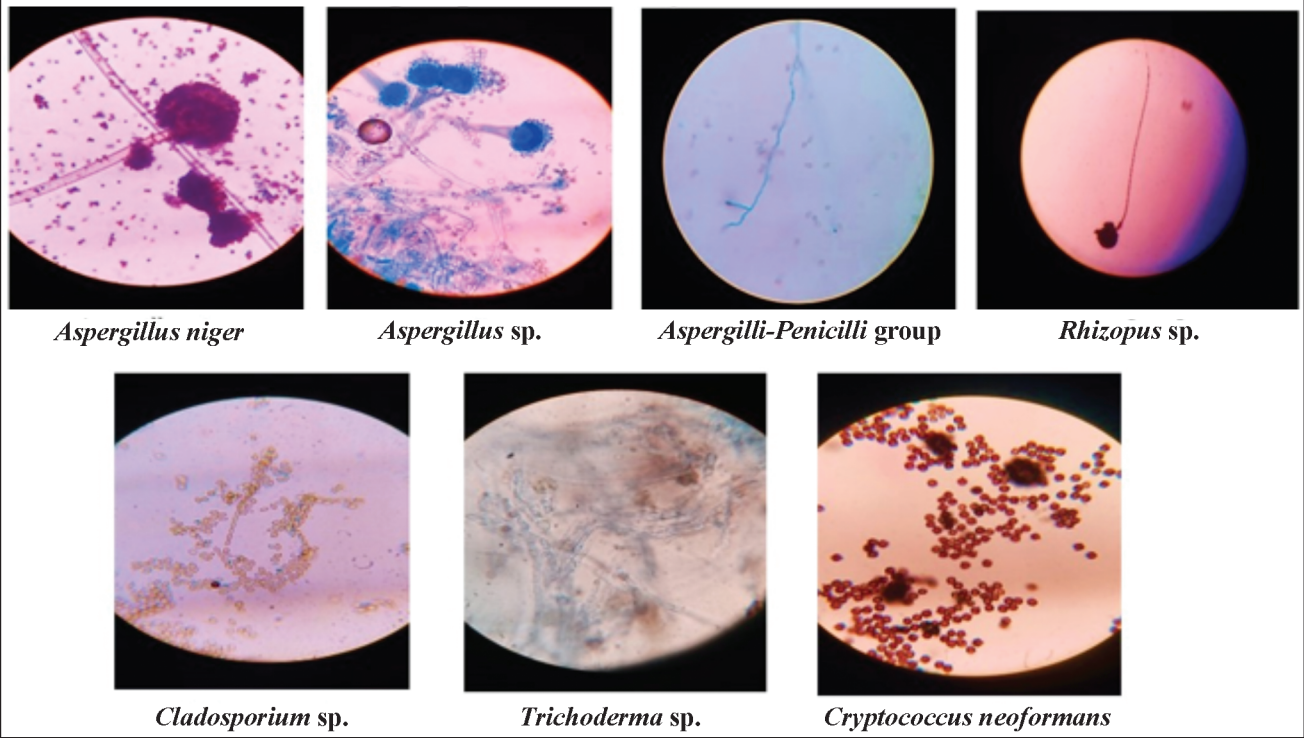
Study side	Colony	Day 1	Day 2	Day 3	Day 4
Study side 1	C1	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	<i>Aspergillus</i> sp.
	C2	<i>Aspergillus</i> sp.	<i>Aspergilli-Penicilli</i> group	<i>Aspergillus</i> sp.	<i>Cryptococcus neoformans</i>
	C3	<i>Aspergilli-Penicilli</i> group	<i>Aspergillus niger</i>	<i>Rhizopus</i> sp.	<i>Aspergillus niger</i>
	C4	<i>Rhizopus</i> sp.	<i>Trichoderma</i> sp.	<i>Aspergillus niger</i>	—
	C5	<i>Cladosporium</i> sp.	<i>Aspergillus</i> sp.	—	—
Study side 2	C1	<i>Aspergillus niger</i>	<i>Aspergillus</i> sp.	<i>Aspergillus</i> sp.	<i>Rhizopus</i> sp.
	C2	<i>Aspergillus</i> sp.	<i>Aspergillus</i> sp.	<i>Alternaria alternata</i>	<i>Aspergillus niger</i>
	C3	<i>Aspergillus niger</i>	<i>Aspergillus</i> sp.	<i>Alternaria alternata</i>	<i>Fusarium</i> sp.
	C4	<i>Aspergillus</i> sp.	<i>Aspergillus niger</i>	<i>Alternaria alternata</i>	—
	C5	<i>Aspergillus niger</i>	<i>Aspergilli-Penicilli</i> group	—	—

Staining and Microscopic Study: The fungal staining is mainly done by two reagents, one is the lactophenol and another is the cotton blue. After observing the microscopic view, we find that *Aspergillus niger* is mostly present on study site 1 samples on Day-1, also *Aspergillus* sp. is mostly present on Day-2. *Alternaria alternata* is mostly present on Day-3 and Day-4 contains a

greater number of *Aspergillus* sp. (Fig. 3.10, Table 3.10).

Statistical Analysis: Pearson’s correlation coefficient test was determined to find out the relationship between microbial CFU and meteorological parameters/air quality data. There was a significant correlation between fungal CFU of study site 1 and temperature, NO₂, SO₂, PM₁₀, PM_{2.5} (Table 3.11). There was also a significant

Fungal species in Study site 2



Fungal species in Study site 2

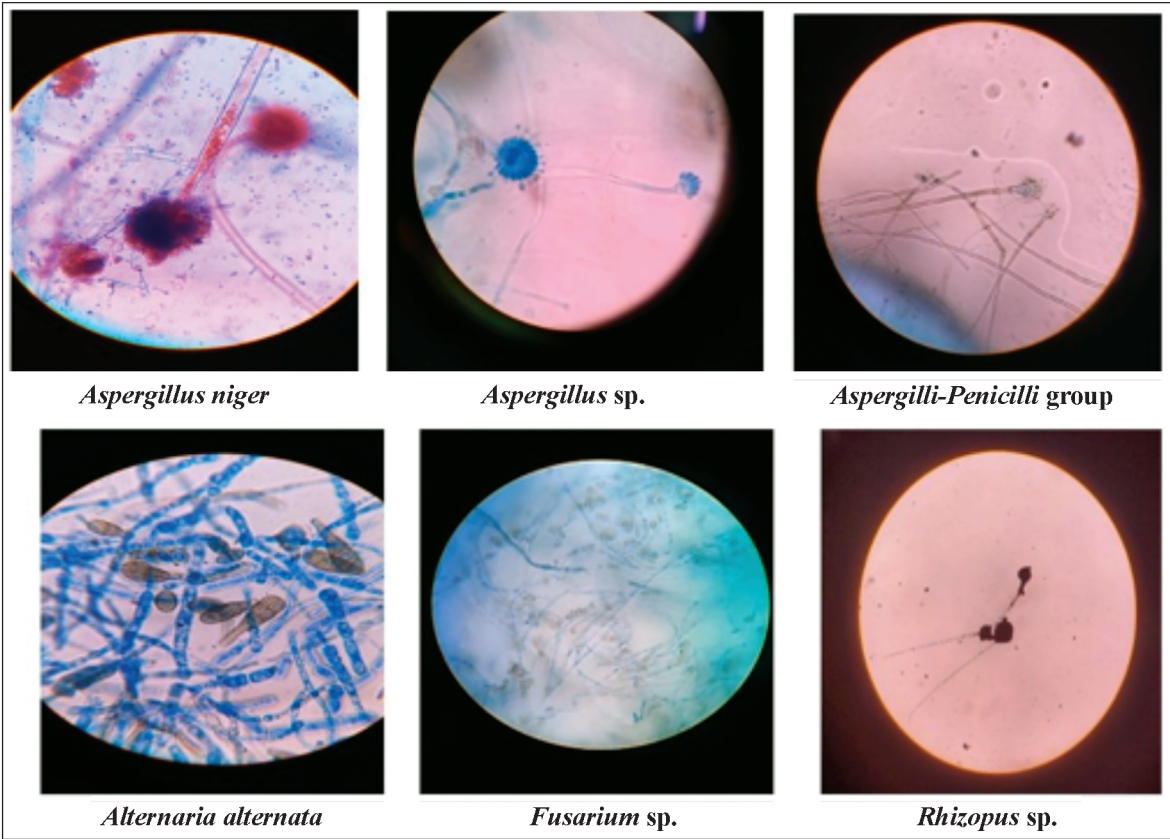


Fig. 3.10: Staining and microscopic identification of fungal species

Table 3.11: Statistical analysis of microbial CFU with different parameters				
CFU	Correlation with	Significance level	r value	Remarks
Bacteria of site 1	Temperature (°C)	P>0.05	-0.09265	non-significant relationship
	Average relative humidity (%)		0.05002	non-significant relationship
	Total rainfall (mm)		-0.342	non-significant relationship
	NO ₂ (μg/m ³)		0.5763	non-significant relationship
	SO ₂ (μg/m ³)		0.1055	non-significant relationship
	PM ₁₀ (μg/m ³)		0.1824	non-significant relationship
	PM _{2.5} (μg/m ³)		0.5013	non-significant relationship
Bacteria of Site 2	Temperature (°C)		0.3729	non-significant relationship
	Average relative humidity (%)		-0.0857	non-significant relationship
	Total rainfall (mm)		-0.08518	non-significant relationship
	NO ₂ (μg/m ³)		0.0204	non-significant relationship
	SO ₂ (μg/m ³)		-0.4911	non-significant relationship
	PM ₁₀ (μg/m ³)		-0.4057	non-significant relationship
	PM _{2.5} (μg/m ³)		-0.09366	non-significant relationship
Fungi of Site 1	Temperature (°C)	P>0.05	-0.9754	significant negative relationship
	Average relative humidity (%)		-0.2323	non-significant relationship
	Total rainfall (mm)		0.04903	non-significant relationship
	NO ₂ (μg/m ³)		0.8793	significant positive relationship
	SO ₂ (μg/m ³)		0.9119	significant positive relationship
	PM ₁₀ (μg/m ³)		0.9751	significant positive relationship
	PM _{2.5} (μg/m ³)		0.8124	significant positive relationship
Fungi of Site 2	Temperature (°C)		-0.3922	non-significant relationship
	Average relative humidity (%)		0.4643	non-significant relationship
	Total rainfall (mm)		-0.3908	non-significant relationship
	NO ₂ (μg/m ³)		0.2795	non-significant relationship
	SO ₂ (μg/m ³)		0.7556	significant positive relationship
	PM ₁₀ (μg/m ³)		0.6242	non-significant relationship
	PM _{2.5} (μg/m ³)		0.4886	non-significant relationship

correlation between fungal CFU and SO₂ of study site 2. Value of correlation coefficient (r) for concentration of NO₂/SO₂/PM₁₀/PM_{2.5}/temperature with fungal CFU of site 1 were +0.8793, +0.9119, +0.9751, +0.8124, -0.9754. Value of correlation coefficient (r) for concentration of SO₂ with fungal CFU was +0.7556.

DISCUSSION

The current study was conducted to analyses the diversity, biochemical properties, antibiotic sensitivity and correlation between meteorological parameters and

concentration of airborne microbes in the study site 1 and study site 2. Among all the bacterial species, Gram negative Bacillus was dominant in both the study site 1 and study site 2 with the percentage of 62.5% and 50% respectively (Fig. 3.11).

Mean CFU of bacterial community in study site 1 is 213.75 whereas mean CFU in study site 2 was 237.75, which was slightly higher (Table 3.1). As the bacteria got favourable condition and sufficient nutrient in hospital area to propagate in high amount, there was always a high chance for getting a greater number of bacteria.

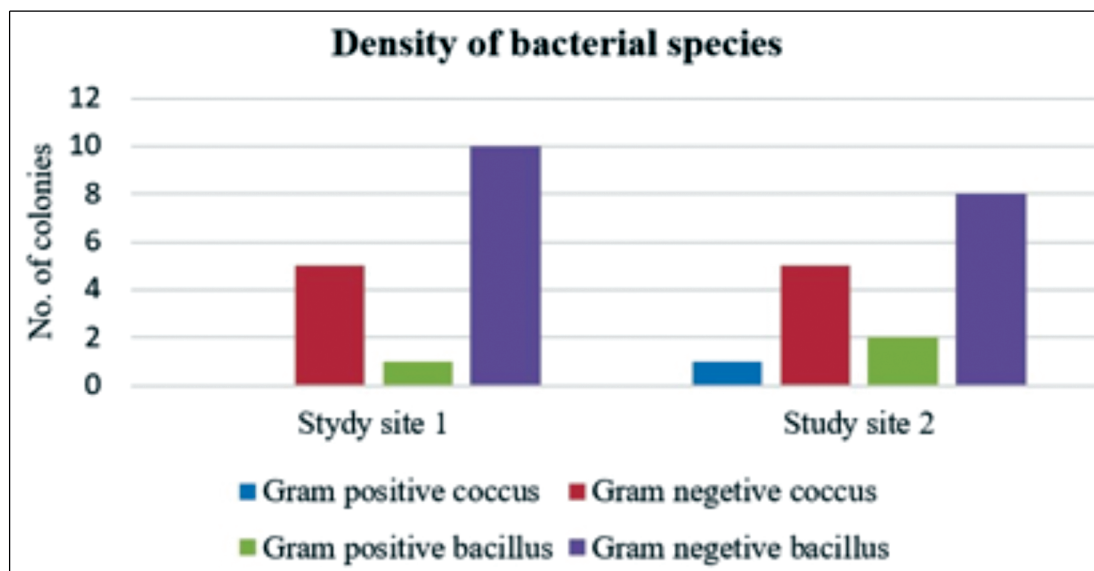


Fig. 3.11: Density of bacterial species

Study site 2 was always filled up with patients, who visited the place having various contagious and airborne disease in contrast to study site 1.

Mean level of fungal community in study site 1 was 43.75 CFU/m³ whereas the value in study site 2 was 34.75 CFU/m³, which is slightly lesser (Table 3.2). Study site 1 comes under a green campus, populated with big trees, shrubs and bushes. On the other hand,

most of the areas of study site 2 was covered with concrete constructions. Chance of getting more nutrients and suitable host for colonization of fungal spores was relatively higher in study site 1 compared to study site 2.

Several fungal members like *Aspergillus niger*, *Aspergillus* sp., *Aspergilli-Penicilli* group, *Rhizopus* sp., *Cladosporium* sp., *Trichoderma* sp. and *Cryptococcus*

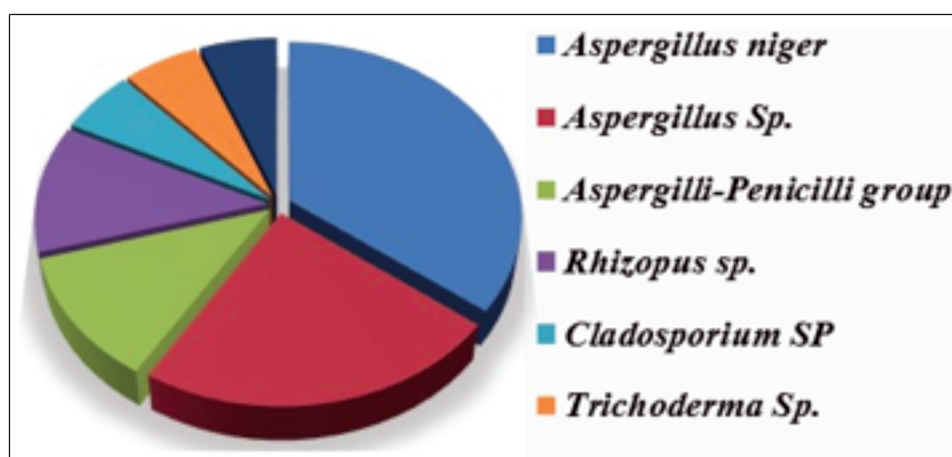


Fig. 3.12: Total number of fungal species in study site 1

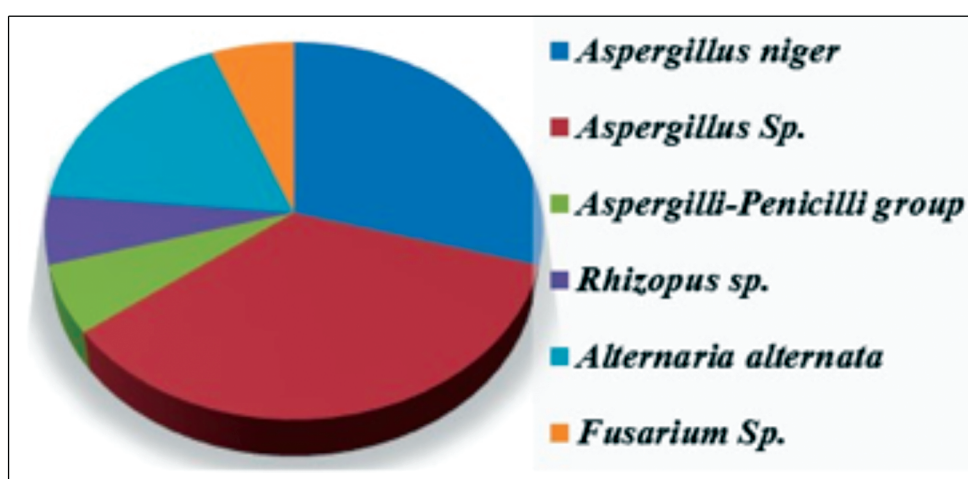


Fig. 3.13: Total number of fungal species in study site 2

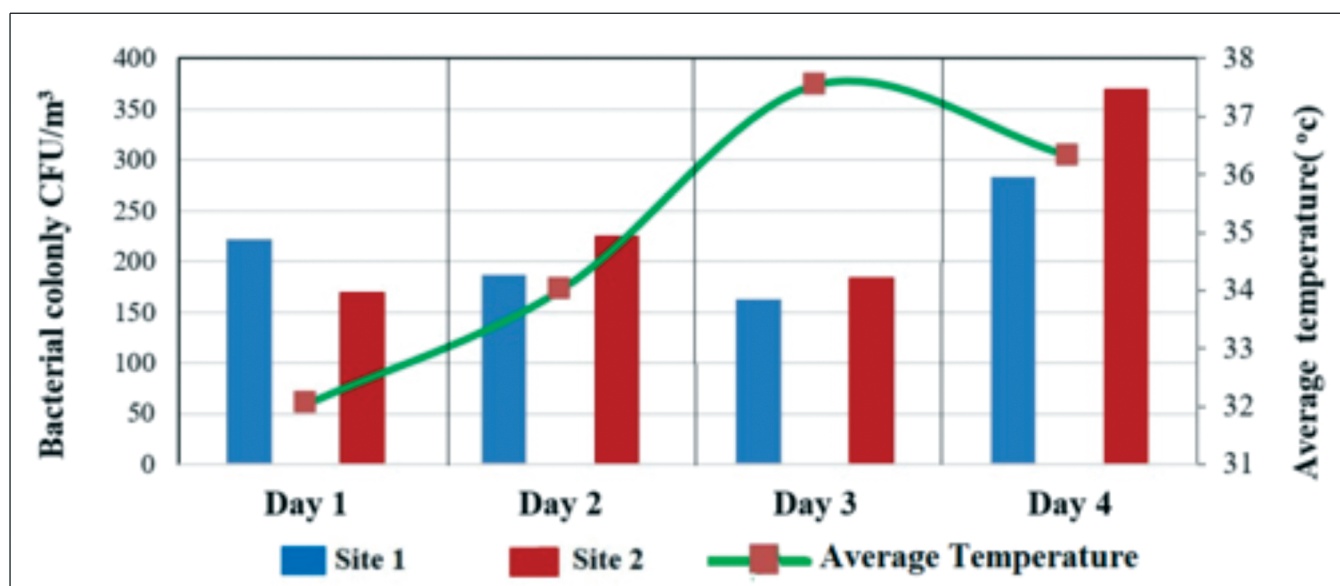


Fig. 3.14: Bacterial colony count (CFU/m³) and average temperature (°C) in study area

neoformans were present in study site 1 (Fig. 3.12), whereas *Aspergillus niger*, *Aspergillus* sp., *Aspergilli-Penicilli* group, *Alternaria alternata* and *Fusarium* sp. were found in study site 2 (Fig. 3.13) which are common in different aerobiological studies⁵⁻⁷. Variety in fungal species was more in study site 1.

The change in bacterial and fungal density in the study sites depending upon meteorological parameters was observed in the study. It was found that concentration of bacteria and fungus were decreasing with the increasing temperature, as reported previously⁸⁻¹⁰. The highest average temperature was 37.54°C on day 3 (Fig. 3.14), so a smaller number of microbial CFU was counted on that day in study site 1 and study site 2.

It was observed that air quality affected the occurrence of airborne microbes significantly as already observed

by Raisi *et al.*¹¹. Microbial population was increased with respect to the increasing concentration of pollutants, which was corroborative to the observation of Andualem *et al.*¹². The bacterial load was higher on Day 4 (Fig. 3.15), whereas, on the other hand, fungal load was high in the air on Day 1 (Fig. 3.16). Presence of more pollutants and particulate matters in the air seems to facilitate the microbes to flow freely.

In sugar fermentation test, bacterial samples from study site 2 gave more positive results in lactose sugar (Fig. 3.6). Different types of clinical, surgical wastes and other contagious agents are present in hospital area. Due to the contamination and rapid fluctuation in the surroundings, a greater number of bacterial populations in hospital area are capable to live under stress and ferments lactose sugar in spite of simple sugar as already reported in previous studies¹³⁻¹⁵.

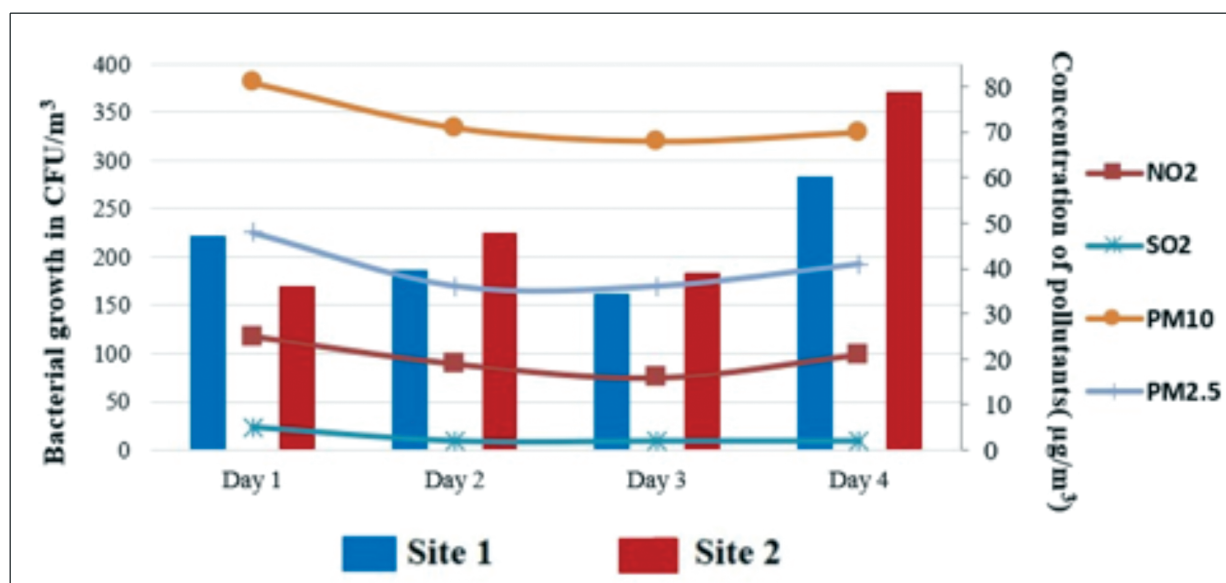


Fig. 3.15: Bacterial CFU/m³ vs Concentration of pollutants (µg/m³)

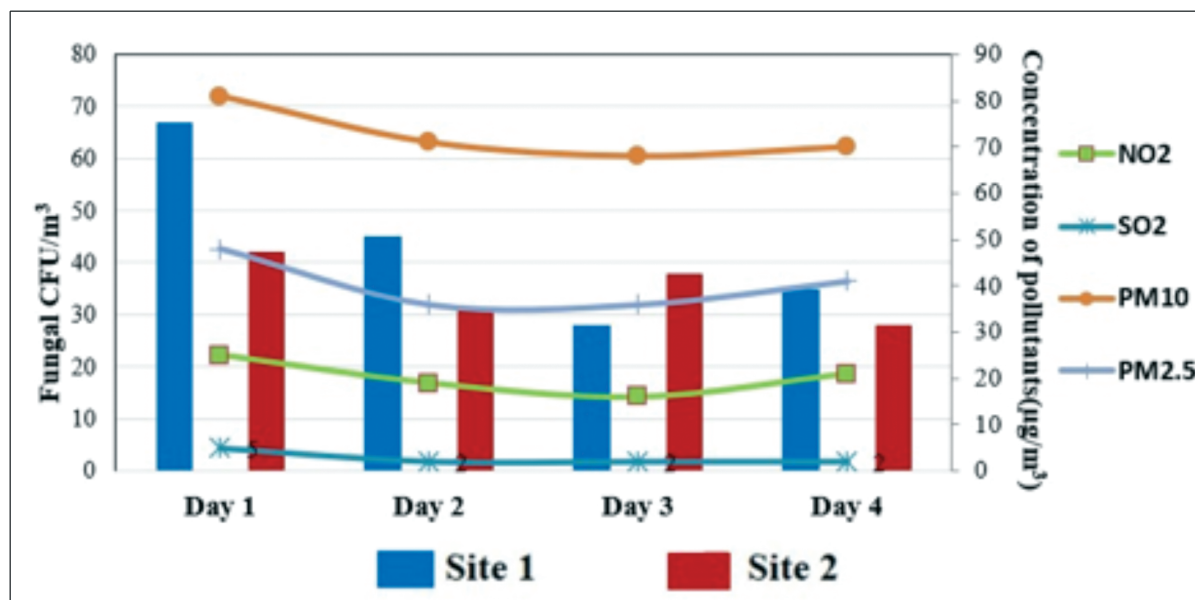


Fig. 3.16: Fungal CFU/m³ vs Concentration of pollutants (µg/m³)

Although most of the bacterial colonies were antibiotic sensitive, there were some colonies with resistant nature too. Colony D1-C2 from study site 2 (Table 3.5) showed the resistant property against chloramphenicol and bacitracin. Sometimes such resistance is observed as the characteristic of airborne microbes specifically from healthcare units¹⁶. Actually in hospital, various antibiotics are used for the treatment, which may come to the outer environment from the patient body. Some of the exposed bacterial population of hospital areas slowly gains the resistant property against such antibiotics¹⁷. After living so long under unfavorable condition and getting exposed in antibiotics they develop their escaping mechanism slowly¹⁸.

Based on Pearson's correlation test between fungal CFU and the concentration of pollutants/temperature, it was determined that the occurrence of fungal colonies at study site 1 and study site 2 was significantly correlated with pollutant concentrations (Table 3.11). The number of fungal colonies increased with rising pollutant concentrations, indicating a positive correlation. Conversely, fungal CFU decreased as temperature increased.

CONCLUSION

The present study provided an insightful information about the diversity of airborne microbial population in Vidyasagar University campus and Midnapore Medical College and Hospital area. Both of these sites are significantly important places of Midnapore town. University area, although sparsely populated, is crowded in day time. It is populated by mostly healthy young adults. On

the other hand, hospital area is densely populated. Everyday numerous patients visit this place having various contagious and airborne diseases. Moreover, it is situated in the heart of the town, whereas university area is surrounded by forest plantations. From that point of view, it is very important to know and to compare the airborne microflora of these two sites.

Meteorological parameters and air quality seems to have effects on the occurrence of bacterial and fungal communities in the study areas. Increasing temperature negatively influenced the concentration of airborne microbial population, whereas higher concentration of pollutants had positive effect on the bacterial and fungal occurrence in the air. Various factors like presence of contaminating agents, drugs, antibiotics, healthcare waste were responsible for gaining new characteristics of bacteria in study site 2. Due to the stressful condition some of the bacterial species adopted themselves and gained resistant properties against antibiotics like Chloramphenicol and Bacitracin. In future, these resistant characteristics of bacterial species can lead to rise of MDR strain, which cannot be controlled by our conventional antibiotics. Density of fungal population was higher in study site 1 because of the green campus.

In the present study, an overview on airborne bacterial and fungal communities of study site 1 and 2 were depicted which may be helpful to know about the possible risks of these microbes on human health.

Although identification of bacterial population at genus and species level was not possible in present study, the

future look up is their metagenomic study, which will depict more accurate and detail information about the species.

ACKNOWLEDGEMENT

The study was conducted under the enthusiastic guidance of the late Dr. Nandini Ghosh, Faculty, Department of Microbiology, Vidyasagar University, Midnapore, West Bengal who tragically passed away in a car accident in August 2023.

The authors dedicate this article to the cherished memory of their respected teacher and guide, Dr. Nandini Ghosh.

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Research Article

ASSESSMENT OF ENVIRONMENTAL BIOPARTICLES AND IMPACT ON PUBLIC HEALTH IN KAMPTEE, NAGPUR, MAHARASHTRA

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Aerobiology is concerned with the nature and behaviour of a suspension of bioparticles in the atmosphere—whether viable or not—whose movement from one location to another is governed by atmospheric conditions. These airborne particles, primarily of biological origin, include microorganisms such as pollen grains, fungal spores, mites, bacteria, viruses, algae, leaf and animal hairs, small seeds, and fragments of plants, among others. Their concentration in the environment varies depending on climatic conditions, height above ground level, indoor or outdoor location, altitude, and proximity to both large and small water bodies. When dispersed in the air, these biological particles are referred to as aerosols. Pollen and fungal spores are perhaps the most common sources of aeroallergens.

A five-year aerobiological survey for pollen and spore monitoring was conducted using a volumetric Rotorod Air Sampler (Model-40), installed approximately 10m above ground level on the rooftop terrace of S. K. Porwal College, Kamptee, Nagpur, Maharashtra, from June 2014 to May 2019. A total of 60 types of pollen grains were recorded, along with the identification of 46 fungal species. Respiratory conditions such as asthma and chronic obstructive pulmonary diseases are largely associated with exposure to pollen and fungal bioparticles. At the Sub-district Government Hospital in Kamptee, a health survey involving 3,678 individuals was undertaken over the same five-year period. A questionnaire-based method was employed to facilitate the assessment and monitoring of airborne bioaerosols and their impact on human health.

Key Words: Aerobiology, rotorod sampler, fungal spores, pollen grains, questionnaire-based survey, public health.

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INTRODUCTION

Our environment has a great influence on public health. The atmosphere is loaded with essential and non-essential particles of biological origin. Pollen grains released from the anthers of flowering plants and spores from lower plants constitute the airborne bioparticles/aerosol. In the atmosphere, the fungal spores are most predominant. The ratio of pollen to fungal spore goes up to 1:30 in certain seasons. It has been proved substantially that environmental bio-pollution has a significant role to play in human health hazards. The connection between airborne pollen and fungal spores with allergy symptoms has been convincingly established¹⁻⁶.

Public health is achieved through surveillance of cases and the promotion of healthy behaviors, communities and environments. Analyzing the determinants of health of a population and the threats it faces is the basis for public health. Many diseases are preventable through simple, non-medical methods. For example, research has shown that the simple act of hand washing with soap can prevent the spread of many contagious diseases².

The health of susceptible and sensitive individuals can be impacted even on low air pollution days. Short term exposure to bio-pollutants is closely related to COPD (Chronic Obstructive Pulmonary Disease), cough, shortness of breath, wheezing, asthma, respiratory disease, and high rates of hospitalization. The long-term effects associated with air pollution are chronic asthma, pulmonary insufficiency, cardiovascular diseases, and cardiovascular mortality³.

A five year long aerobiological survey of pollen/spore monitoring was carried out by operating a volumetric Rotorod Air Sampler at the Nagpur Maharashtra from June 2014 to May 2019 to record atmospheric pollen grains and fungal species and also to make a correlation with respiratory diseases like asthma and chronic pulmonary obstructive diseases using questionnaire survey.

MATERIALS AND METHODS

Aerobiological survey of pollen/spore was carried out by operating a volumetric Rotorod air sampler (Model-40) installed at about 10 m above the ground level on

the rooftop of the terrace of S. K. Porwal College, Kamptee, Nagpur, Maharashtra for the period of five year from June-2014 to May-2019. Kamptee is a satellite town of Nagpur and is known for its Big Friday market, many types of small, mediate industries of tanning and dying, bakeries, and central India’s largest butchery. The sampler was operated every 10 minutes for a day i.e. 24 hours. Airborne pollen, fungal spores and other particulate matter get impacted on the silicone grease-coated rods. The rods are brought to the laboratory mounted in the grooved plastic slide holder and thoroughly screened microscopically. The slides were prepared and observed under the microscope in low and high magnification and identified with reference slides and standard literature⁴⁻¹¹. A total 60 types of pollen grains and 46 fungal species were identified.

The primary aim of the study was to monitor airborne bioparticles. The secondary aim, relevant to public health, was achieved by preparing a survey report based on responses from individuals of various social classes within the locality, using a questionnaire method. At the

Sub-district Government Hospital in Kamptee, a survey was conducted over a period of five years involving 3,678 individuals, utilising a questionnaire approach to support the assessment and monitoring of airborne bioaerosols and their impact on human health.

RESULTS AND DISCUSSIONS

Percentages of allergically significant pollen and fungal spores were determined (Table 4.1) and depicted in a pie diagram (Fig. 4.1 and 4.2).

The health survey was undertaken for about 5 years visiting the patients at the Government Sub-district hospital, Kamptee. The questionnaire was developed with 26 questions, in Hindi-English language directly addressed to the patients who came for their symptoms mostly of cough and cold at the Government sub-district hospital. The questions were mostly referred to patients with respiratory diseases or symptoms suggesting respiratory allergies, mostly allergic rhinitis, asthma, and skin-related. We collected 3678 on-site completed questionnaires in five years. Data sets, graphs and statistical analysis using chi-square for more than 2 variables were

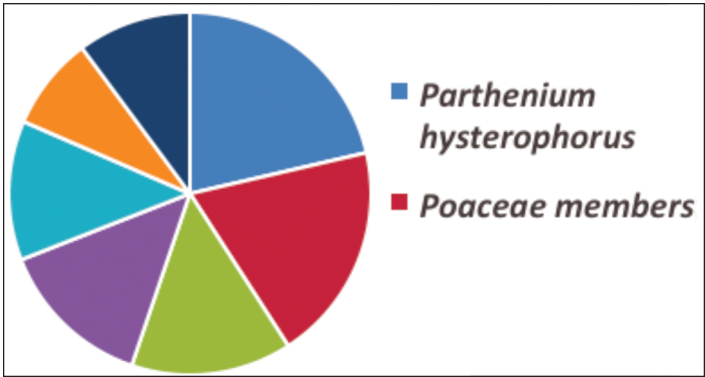


Fig. 4.1: Percentage of allergically significant pollen

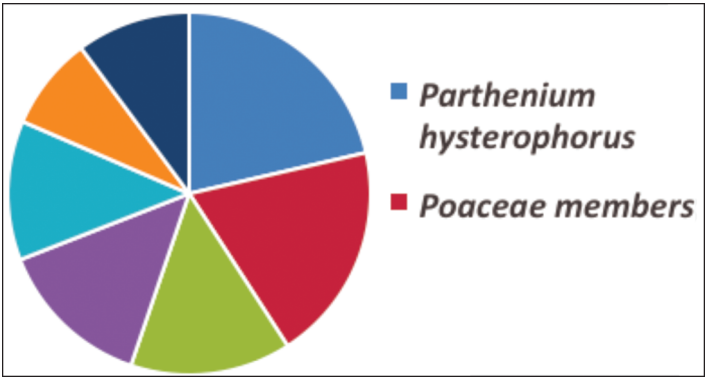


Fig. 4.2: Percentage of allergically significant fungal spores

Table 4.1: Percentages of allergically significant pollen and fungal spores recorded from June-2014 to May-2019

Pollen grains		Fungal spores	
<i>Parthenium hysterophorus</i>	21.37%	<i>Cladosporium</i> sp.	17.88%
Poaceae members	19.5%	Smut/Rust spores	15.15%
<i>Cassia</i> pollen	14.34%	<i>Aspergillus/Penicillium</i> sp.	14.37%
<i>Casuarina equisetifolia</i>	13.85%	<i>Periconia</i> sp.	11.72%
<i>Eucalyptus</i> sp.	12.45%	<i>Nigrospora</i> sp	9.02%
Amaranth, Chenopod pollen	8.33%	<i>Helminthosporium</i> sp.	8.96%
Other pollen types	10.16%	<i>Sterilia mycelia</i> (black, white, pink)	5.80%
		Other fungal types	17.1%

done using Microsoft Office Excel 2021. A p-value of < 0.05 was considered statistically significant.

Responder's data: 2014-2015

The mean age is 36.8 years (range between 8 years to 71 years old), with female predominance 56.2% and 43.8% over the men. Lower middle-class people were around 40%, low-income group people around 36% and below poverty-line visitors/patients around 24%. By critically evaluating the data (n = 1132) obtained after the survey, the most frequent chronic diseases of registered patients are Respiratory chronic diseases (asthma, COPD, airway blockages nasal and throats, lung diseases, etc.) 361 (31.89%) followed by Cardiovascular diseases (Chest pain, shortness of breath, cardiac diseases, high blood pressure, coronary artery disease, dizziness, and fatigue) for 216 (19.08%) followed by Metabolic disorders (Diabetics and endocrinological diseases) 206 (18.19%), Osteoarticular diseases(bones and joints related, osteoporosis, rheumatoid arthritis, etc.) 155 (13.69%), Pediatric and Gynecological disorders 103 patients were recorded with 8.7%, Skin/eye infections 68 (6%), Cancer 12(1.06%), and 11 patients recorded with other ailments which was 0.97% (Fig. 4.3).

Responder's data: 2015-2016

The mean age of the surveyed individuals was 29.1 years, ranging from 6 to 63 years, with a male predominance of 53.4% compared to 46.6% female. Approximately 50% of the participants belonged to the lower middle-class, 26% were from low-income groups, and 24% were living below the poverty line. A critical evaluation of the data (n = 563) obtained from the survey revealed that the most frequently reported chronic illnesses among registered patients were respiratory diseases—including asthma, COPD, airway obstructions (nasal and throat), and other lung conditions—affecting 143 individuals (25.39%). These were followed

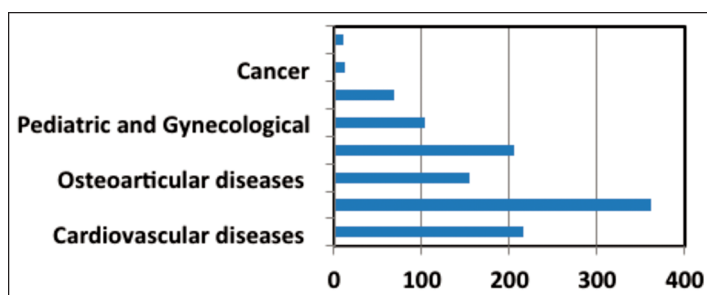


Fig. 4.3: Various ailments recorded in patients visited the Government hospital in study period June 2014 to May 2015 (n = 1132)

by osteoarticular diseases, such as osteoporosis and rheumatoid arthritis, in 103 patients (18.29%). Cardiovascular conditions—such as chest pain, breathlessness, heart disease, hypertension, coronary artery disease, dizziness, and fatigue—were identified in 87 patients (15.45%). Metabolic disorders, including diabetes and other endocrinological conditions, were observed in 115 patients (20.42%). Paediatric and gynaecological disorders were recorded in 59 patients (10.47%), while skin and eye infections were reported by 41 individuals (7.28%). Additionally, three patients (0.53%) were diagnosed with cancer, and 12 patients (2.13%) presented with other ailments (Fig. 4.4).

Responder's data: 2016-2017

The average age of the participants was 32.2 years, ranging from 8 to 75 years, with a female predominance of 69.4% compared to 30.6% males. Socioeconomically, approximately 41% of individuals belonged to the lower middle class, 35% to the low-income group, and 24% were living below the poverty line. A critical evaluation of the survey data (n = 643) revealed that the most common chronic illnesses among registered patients were cardiovascular diseases—including chest pain, shortness of breath, heart conditions, high blood pressure, coronary artery disease, dizziness, and fatigue—affecting 180 individuals (27.99%). This was followed by respiratory disorders such as asthma, COPD, airway obstructions, and lung diseases, affecting 141 individuals (23.63%). Osteoarticular conditions, including osteoporosis and rheumatoid arthritis, were reported by 137 patients (21.30%), while metabolic disorders such as diabetes and endocrine conditions affected 95 individuals (14.77%). A further 56 patients (8.70%) presented with paediatric and gynaecological issues, 21 (3.26%) with skin or eye infections, 13 (2.02%) with cancer, and 10 (1.55%) with other ailments (Fig. 4.5).

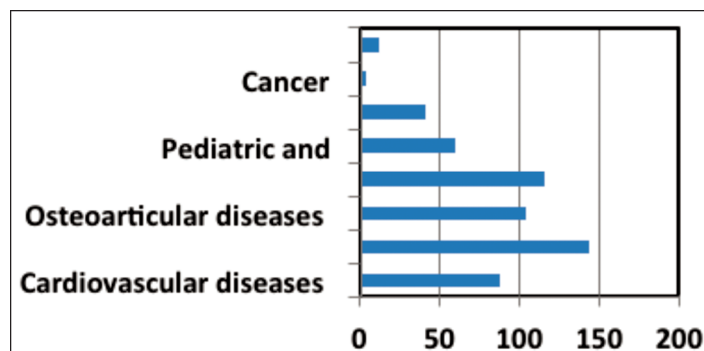


Fig. 4.4: Various ailments recorded in patients visited the Government hospital in study period June 2015 to May 2016 (n = 563)

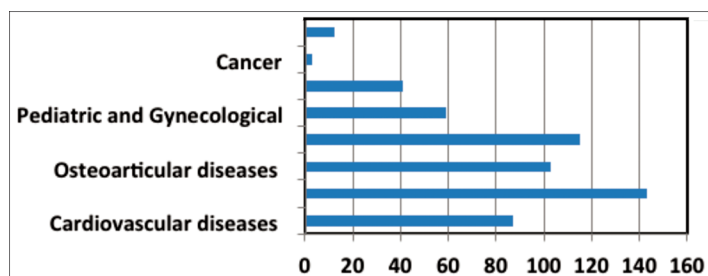


Fig. 4.5: Various ailments recorded in patients visited the Government hospital in study period June 2016 to May 2017 (n = 643)

Responder's data: 2017-2018

The mean age of the patients was 26.4 years, ranging from 8 to 59 years, with a female predominance of 63.4% compared to 36.6% male. Approximately 38% of the participants belonged to the lower middle class, 35% to the low-income group, and 27% were below the poverty line. A critical evaluation of the survey data (n = 569) revealed that the most common chronic conditions among registered patients were cardiovascular diseases—including chest pain, shortness of breath, cardiac conditions, hypertension, coronary artery disease, dizziness, and fatigue—reported in 163 patients (28.64%). This was followed by chronic respiratory diseases—such as asthma, COPD, airway obstructions (nasal and throat), and other lung conditions—affecting 128 patients (22.49%). Metabolic disorders, including diabetes and endocrinological conditions, were observed in 105 patients (18.45%). Osteoarticular disorders, including conditions related to bones and joints such as osteoporosis and rheumatoid arthritis, were recorded in 66 patients (11.59%). Paediatric and gynaecological disorders were reported in 42 patients (7.38%), while skin and eye infections were noted in 51 patients (8.96%). Cancer was diagnosed in 2 patients (0.35%), and 12 patients (1.55%) presented with other ailments (Fig. 4.6).

Responder's data: 2018-2019

The mean age of the participants was 35.4 years, ranging from 5 to 80 years, with a female predominance of 69.4% compared to 30.6% male. Socioeconomic classification, based on the type of ration card, revealed that approximately 10% of individuals belonged to the middle class, 31% to the lower-middle class, 35% to the low-income group, and 24% were below the poverty line. A critical evaluation of the survey data (n = 895) indicated that the most frequently reported chronic conditions among registered patients were: Cardiovascular

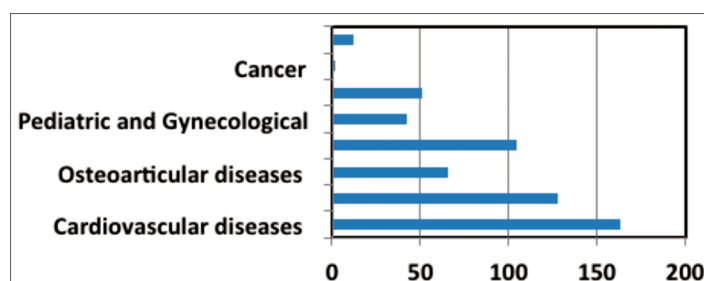


Fig. 4.6: Various ailments recorded in patients visited the Government hospital in study period June 2017 to May 2018 (n = 569)

diseases (including chest pain, shortness of breath, heart disease, hypertension, coronary artery disease, dizziness, and fatigue), affecting 227 individuals (25.36%); Chronic respiratory diseases (such as asthma, COPD, airway obstructions of the nasal and throat passages, and other lung conditions), reported by 204 individuals (22.79%); Osteoarticular disorders (including bone and joint conditions, osteoporosis, and rheumatoid arthritis), noted in 163 individuals (18.21%); Metabolic disorders (such as diabetes and endocrine conditions), affecting 143 individuals (15.97%); Paediatric and gynaecological disorders, reported by 75 individuals (8.37%); Skin and eye infections, noted in 54 individuals (6.03%); Cancer, diagnosed in 18 individuals (2.01%); and other ailments, reported by 11 individuals (1.22%) (Fig. 4.7).

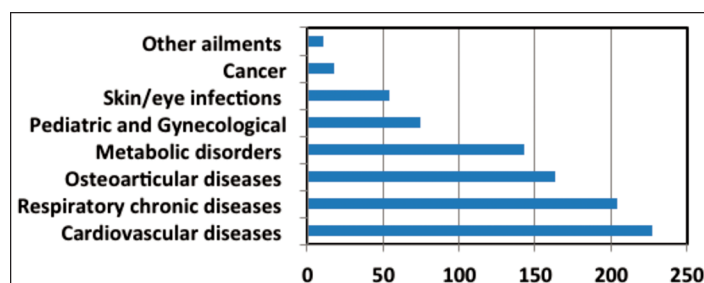


Fig. 4.7: Various ailments recorded in patients visited the Government hospital in study period June 2018 to May 2019 (n = 895)

Skin and eye infection and Respiratory chronic disease patient questionnaires were further studied in detail (n = 1212). The most evocative signs and symptoms of allergic manifestation in those patients was sneezing for 23 % (n = 279) respondents, rhinorrhoea (running nose) for 17% (n = 206), nasal obstruction (nasal passage swollen with excess fluid) for 12 % (n = 145), nasal pruritis (itching inside the nose) 10 % (n = 121), Ocular symptoms (itching and involuntary movement of eye-balls) for 7 % (n = 85), cough for 14 % (n = 170), asthma symptoms in 9 % (n = 109), skin itching and irritation in 8 % (n = 97) respondents.

Table 4.2: Data recorded for 5 years June 2014 to May 2019 (n = 3678)

Sl. No.	Categories of diseases	No. of patients observed	% of patients having the disease symptoms/ diagnosed
1.	Respiratory chronic diseases (Asthma, COPD, airway blockages nasal and throats, lung diseases, etc.)	977	26.56%
2.	Cardiovascular diseases (Chest pain, shortness of breath, cardiac diseases, high blood pressure, coronary artery disease, dizziness, and fatigue etc.)	873	23.73%
3.	Metabolic disorders (Diabetics and endocrinological diseases)	664	18.05%
4.	Osteoarticular diseases (Bones and joints related, osteoporosis, rheumatoid arthritis, etc.)	624	19.96%
5.	Pediatric and Gynecological disorders	344	9.35%
6.	Skin/eye infections	226	6.14%
7.	Other ailments	56	1.52%
8.	Cancer	48	1.38%

Table 4.3: Respiratory chronic disease patient and Skin and eye infection patient's data of 5 years

Sl. No.	Survey year	Average age of the patients	Total number of Respiratory chronic disease and Skin and eye infection patients
1.	June 2014-May 2015	36.8 yrs	429
2.	June 2015-May 2016	29.1 yrs	184
3.	June 2016-May 2017	32.2 yrs	162
4.	June 2017-May 2018	26.4 yrs	179
5.	June 2018-May 2019	35.4 yrs	258
	Total		1212

Regarding epidemiological aspects of respiratory allergies, 88% of responders considered an increased incidence of allergic or respiratory in our country. 12% considered it as stable. The possible factors responsible for the increased prevalence of respiratory allergies were air pollution for 29% of the responders, climate /environment change for 17%, smoking for 12%, pollen and mould for 32%, atopy for 5%, and lifestyle for 5%. The most important aeroallergen considered responsible for respiratory allergies was pollen and dust mites that were also diagnosed by doctors by 390 and 141 responders respectively, moulds by 287, pets by 67 and most of the responders didn't know.

Aerobiological studies carried out so far have brought to light several facts, which are very useful from the point of creating awareness among the public about environmental biopollution. Predominant types of pollen grains and fungal spores in the Kamptee, Nagpur atmosphere compiled recently indicate several types of allergically significant pollen and fungal spores¹²⁻¹⁴.

Aerobiological studies carried out so far have brought to light several facts, which are very useful from the point of creating awareness among the public about environmental biopollution. Predominant types of pollen grains and fungal spores in the Kamptee, Nagpur atmosphere compiled recently indicate several types of

allergically significant pollen and fungal spores. Weed pollens of plant species encountered in the air include Amaranth, Chenopod, *Mimosa pudica*, members of Poaceae and *Parthenium hysterophorus*. Tree pollens like Caesalpiniaceae members like *Cassia* sp., *Caesalpinia* sp., *Casuarina equisetifolia*, *Eucalyptus* sp., Moraceae members, and Poaceae members achieve the peak of pollen in the atmosphere.

Particles suspended in air are called aerosols. These pose a threat to human health mainly through respiratory intake and deposition in nasal and bronchial airway. In addition, soil or dust particles can act as a “raft” for biological entities known as bioaerosols. Smaller aerosols travel further into the respiratory system and generally cause more health problems than larger particles¹⁵.

Atmospheric monitoring of mould spores showed that there are several predominant types of fungal spores viz. *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Helminthosporium* sp., *Nigrospora* sp., *Penicillium* sp., *Periconia* sp., Smut spores, Ascospores and Basidiospores. The peak of mould spores in the atmosphere is achieved by spores of *Cladosporium* (17.88%) and Smut and rust spores (15.15%) followed by other typical fungal spores, *Periconia* (11.72%), *Nigrospora* sp. (9.02%), *Helminthosporium* sp. (8.96%), and *Aspergillus-Penicillium* spores (14.37 %). Fungal spores are ubiquitous in nature and they are at least 1:30 times more abundant than pollen in the atmosphere. Types and abundance of fungal spores are influenced by meteorological factors. Precipitation accompanied by low temperature is highly favourable for the liberation of fungal spores. Particles suspended in the air pose a threat to human health mainly through respiratory intake and deposition in nasal and bronchial airway. In addition, soil or dust particles can act as a “raft” for bio-particles deposition. Smaller aerosols travel further into the respiratory system and generally cause more health problems than larger particles. Fungal spores are also clinically important as some of them cause respiratory allergy¹⁶, respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions. In addition, long-term contact of people with bioaerosols can influence personal’s mental power and learning ability^{17,18}. Different environmental conditions such as temperature, UV light, dryness and humidity, play a role in controlling the growth of airborne particles.

Moreover, frequent causes of asthma, respiratory allergies, hypersensitive rhinitis and chronic obstructive pulmonary disease (COPD), and others are associated with airborne fungal pathogenic exposure owing to their omnipresence. *A. fumigatus* is implicated in numerous well-being difficulties in people compromised by immune deficiencies or bronchopulmonary aspergillosis. *Stachybotrys chartarum*, on the other hand, colonizes in damp and produces mycotoxins. Fungal bioaerosols considerably influence the oxidative ability and chemical composition of the ambient PM in the presence of *A. fumigatus* spores¹⁹. Also, bioaerosols accelerate reactive oxygen species (ROS) in human lungs in response to different microorganisms and concentrations. For example, fungal spores generated up to 10 times more ROS than bacterial cells²⁰. Pathogenic bioaerosols in outdoor environments are responsible for various allergens and respiratory diseases among children and adults, and the response is not identical. For public health safety, it is essential to detect and control the pathogenic bioaerosol loads, along with the spatio-temporal distribution in Asian megacities. Particular emphasis should be given to developing on-site automated bioaerosol monitoring systems and bioaerosol deactivation or reactivation mechanisms.

After critically analyzing the survey report, the missing factors like the way of living, poor knowledge of sanitation, non-awareness about health hazards, use of mosquito coils, smokers in the family, previous/family history of any kind of allergy etc. led to promotion of severe level of hypersensitive symptoms to the people. There are also pollen grains in high concentration in the air as bio-pollutants causing human health hazards. The survey and bio-monitoring results ultimately prove that the pollen grains were also promoting respiratory troubles and allergic symptoms in the Kamptee locality.

CONCLUSION

A large number of airborne pollen, come in contact with the eyes, nose, mouth and skin of susceptible/sensitive individuals and cause allergic manifestations. The allergy symptoms produced due to airborne pollen and fungal spores include itching of the nose, and skin, watering of the eyes, choking of the nose and blocking of tracheal tubes resulting in asthma and breathlessness. The primary objective of aerobiologists is to monitor air continuously for airborne pollen and spores. On the

basis of family history, atopic and skin prick tests with allergenic extracts from pollen and fungal spores will be performed. Once the offending allergen is established the patient has to undergo immunotherapy, which involves decentralization of allergy patients to get relief from airborne allergically significant pollen and fungal spores. For Palynological and Mycological surveys and assessment of allergenicity, continuous monitoring of atmospheric pollen and fungal spores is essential.

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The present work is an extension of Major Research Project which was granted to the Author by University Grants Commission, New Delhi and She also thankful to the Principal and Staff of Seth Kesarimal Porwal College, Kamptee, and also grateful to the staff of Sub district Government Hospital, Kamptee for their kind support and timely help.

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Short Communication

A PRELIMINARY MELISSOPALYNOLOGICAL ANALYSIS OF HONEY SAMPLES FROM VARIOUS AREAS IN KOTHAMANGALAM TALUK OF ERNAKULAM DISTRICT, KERALA

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A preliminary melissopalynological study was conducted to determine the floral sources and identify pollen types indicative of the ecological origins of honey samples. The study involved the analysis of seven honey samples, including one commercial sample, collected between February and April 2023 from various locations in and around Kothamangalam Taluk, Ernakulam district, Kerala. Pollen from a total of eight plant families was identified. Each taxon was categorised as a major or minor source of nectar and pollen. All collected samples were found to be multifloral, as no single pollen type was predominant. The commercial sample exhibited a notable lack of pollen grains. Secondary pollen from *Hevea brasiliensis* and *Cassia* spp. was observed in some samples, along with minor pollen from several families, including *Azadirachta indica*, Chenopodiaceae, Fabaceae, and Urticaceae. A significant variation in honey colour was recorded, reflecting differences in pollen content. The study identified a diverse range of foraging plant sources for honeybees, highlighting the considerable potential for the expansion and sustainability of beekeeping in the region. Notably, apiaries situated amidst rubber plantations were identified as a major source of honey production.

Key Words: Melissopalynology, honey, pollen grain, unifloral and multifloral honey, honeybee, apiculture.

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INTRODUCTION

Honey is produced by mutual interactions between bees and nectariferous plants. From ancient times man knows about honey and its uses. Till date, honey, a major bee and plant product made from the nectar of flowers, is one of the most widely used natural medicine¹. It has been observed that bees select grains that are rich in nutrient content for superior honey production. Earlier studies have proven beyond doubt that bees are highly discriminative about selecting the plant for nectar collection²⁻⁴. Pollen is the bee's major sources of protein, fatty substances, minerals and vitamins. In most cases the primary foraging areas for pollen collection are insect-pollinated plants as bees visit for nectar. Adulterations of market honey, along with its poisonous nature can be easily ascertained from this information⁵. Pollen analysis of honey facilitates understanding of the distribution and abundance of foraging source in a region, which enables the assessment of the potential of the area for the produc-

tion of honey at a commercial level. The methods recommended by the International Commission of Bee Botany were followed for the recovery, analysis and quantification of the contents of pollen in the sample⁵.

Kothamangalam, a small town area in the gateway of Western Ghats, has tropical evergreen forest and ample rainfall owing to south-west and north-east monsoon. Despite hosting one of the richest floras in India, the region's palynological potential remains largely unexplored⁶. Pollen analysis of honey also helps in the identification of the geographical origin of honey samples because local flora have characteristic plant associations that are reflected in the corresponding spectrum of pollen types represented in the local honeys. It deals with the study of pollen grains present in the honey samples and pollen loads collected by honeybees. The present study is very essential to assess the beekeeping potentials of the studied place, and also to compile a bee floral calendar.

METHODOLOGY

In the present study, the honey samples were brought to the laboratory and first examined through the acetolysis method¹. Acetolysis of the microscopic elements in honey is not a necessity since the pollen grains in the honey are recently produced and well preserved in the honey. Further it destroys some pollen grains which are thin walled and other particles which may be useful for evaluating honey. The acetolysis method would be helpful in special cases of microscopical examination of honey as a complementary method.

Collection of Samples

In the present study, honey samples were collected from Kothamangalam Taluk for pollen identification. The samples were collected over a period from February to April 2023 from various apiaries in and around Kothamangalam Taluk. A total of 6 honey samples were collected from different apiaries from Kothamangalam taluk. One commercial sample was also collected from the Market. The honey samples were classified in the following way: Sample I - Forest 1(Kallimedu), Sample II - Commercial Honey, Sample III - Nellikuzhy, Sample IV - Karukadam, Sample V - Forest 2 (Urulanthanni), and Sample VI - Kozhipilly.

The collected honey was stored in transparent glass bottles and kept in room temperature. The analysis of honey sample was done in accordance with known methods^{1,5}.

Preparation of honey samples for vegetation study

Two ml of honey sample was dissolved in 4 ml of distilled water and then shaken well for preparation of honey sample. The sample was centrifuged for 10 minutes at 2500 rpm and supernatant was decanted out carefully without disturbing the pollen sediments^{1,5}. Water is mixed with honey to remove the sugar content present in honey samples so that we get pollen only. The

entire sediments was put on a neat glass slide using a Pasteur pipettes and evenly spread on the slide to avoid clumping of pollen. The slide was left for a few minutes in room temperature so that the left moisture contents would be vapourized. If the moisture content is so high, slight heating of the slide at 40°C would be advisable.

Preparation of reference pollen grains by Acetolysis method

Reference pollen slides were prepared to study pollen morphology for identification following the standard methods^{1,5}. The reparation of reference pollen though acetolysis method was done in the laboratory of Dr. Swati Gupta Bhattacharya at Bose Institute Kolkata.

Study of pollen slides

After preparation of slides, they were placed under light microscope and viewed at 40 X magnification. The picture of pollen viewed under light microscope was photographed by a trinocular microscope at phase contrast (Nikon Eclipse Ci-L 706586).

Identification of Pollen grains

Two slides were prepared for each honey sample and pollen grains were identified based on the pollen morphological characters and also by comparing with the reference slides prepared from the local flora. Considerable spatial and seasonal fluctuations were apparent in the samples collected from different locations. The colour and moisture content also revealed a variation depending upon the metal content⁵. However, colour in honey samples also varies according to season, temperature conditions and pollen grain content.

RESULTS

Pollen analysis in honey

Pollen grains of *Cassia* spp. (Fabaceae) were present in Samples I, III and VII. These grains could originate

Table 5.1: Distribution of pollen grains in different honey samples

Sl. No.	Types of pollen	Family	Percentage of pollen	Pollen types
Sample I. Forest 1(Kallimedu):				
1.	<i>Cassia</i> spp.	Fabaceae	16-45%	Secondary pollen
Sample II. Commercial Sample:				
1.	Nil	—	—	—

Sl. No.	Types of pollen	Family	Percentage of pollen	Pollen types
Sample III. Nellikuzhy:				
1.	<i>Alternanthera</i> sp.	Amaranthaceae	<3%	Minor pollen
2.	—	Asteraceae	<3%	Minor pollen
3.	—	Cheno-Amaranthaceae	<3%	Minor pollen
4.	—	Urticaceae	<3%	Minor pollen
5.	<i>Azadirachta indica</i>	Meliaceae	<3%	Minor pollen
6.	<i>Cassia</i> spp.	Fabaceae	<3%	Minor pollen
7.	—	Fabaceae	<3%	Minor pollen
Sample IV. Karukadam:				
1.	—	Fabaceae	<3%	Minor pollen
Sample V. Forest 2 (Urulanthanni):				
1.	<i>Hevea brasiliensis</i>	Euphorbiaceae	16-45%	Secondary pollen
2.	<i>Cassia</i> spp.	Fabaceae	16-45%	Secondary pollen
Sample VI. Inchoor:				
1.	<i>Hevea brasiliensis</i>	Euphorbiaceae	16-45%	Secondary pollen
Sample VII. Kozhipilly:				
1.	<i>Hevea brasiliensis</i>	Euphorbiaceae	16-45%	Secondary pollen
2.	<i>Cassia</i> spp.	Fabaceae	<3%	Minor pollen

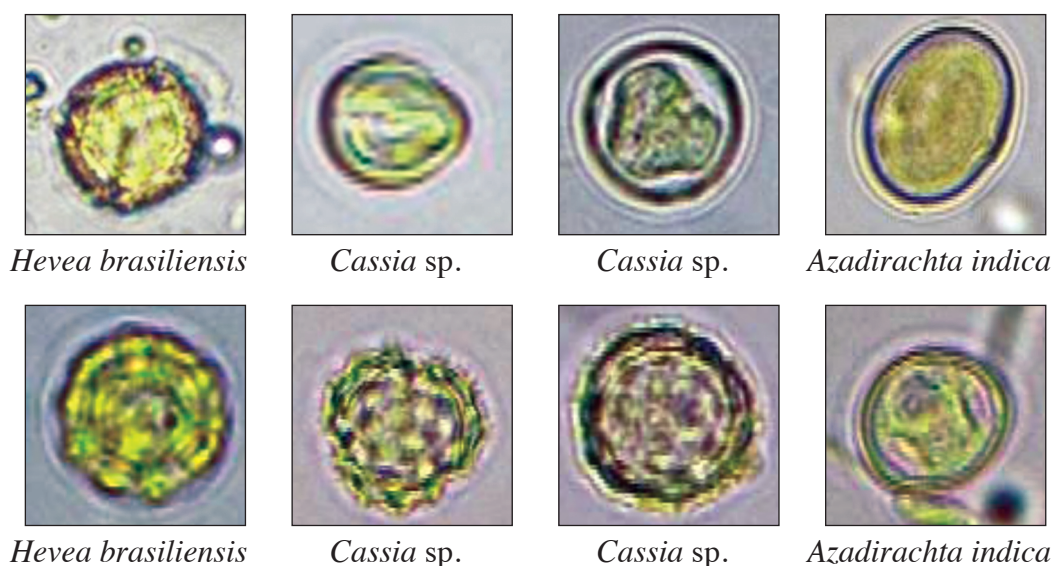


Fig. 5.1: Major pollen grains retrieved from honey samples

from either *Cassia fistula* or *Cassia siamea*, and are classified as either secondary or minor pollen. Their occurrence between February and April coincides with the flowering of *Cassia fistula*, which marks the Vishu

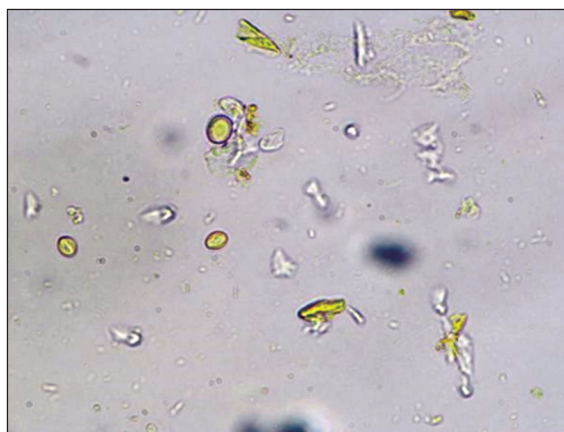
festival in Kerala. *Hevea brasiliensis* (para rubber; Euphorbiaceae) appears as secondary pollen in Samples V, VI, and VII. Apiaries located within rubber plantations were found to be a significant source of

Hevea brasiliensis honey. Pollen of *Azadirachta indica* (Meliaceae) was identified as minor pollen solely in Sample III. The remaining pollen types appear as minor constituents across various samples (Table 5.1, Plate 5.1). Notably, the commercial honey sample contained no pollen grains, suggesting it is spurious (Table 5.1 and Fig. 5.1).

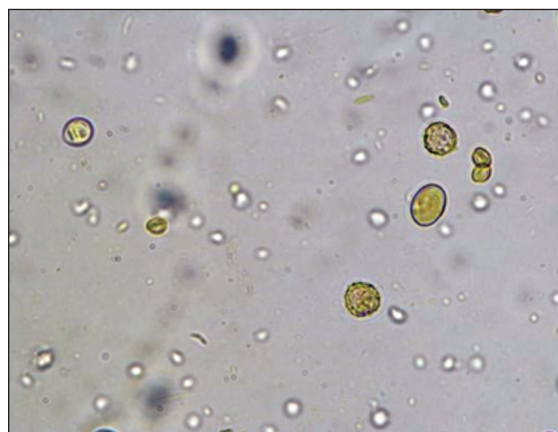
The amount and diversity of pollen present in honey is usually related to vegetation, climate and geographical

location of beehive. The pollen composition of the honey samples studied have revealed important information on the local flora. Thus we can conclude that all samples were of multifloral in nature. Significant variation was observed in the colour of honey among the various collected samples (Fig. 5.2). Colour of honey varies with botanical origin, age and storage conditions, but transparency or clarity depends on the amount of suspended particles like pollen.

Plate 5.1: Pollen grains in different honey samples



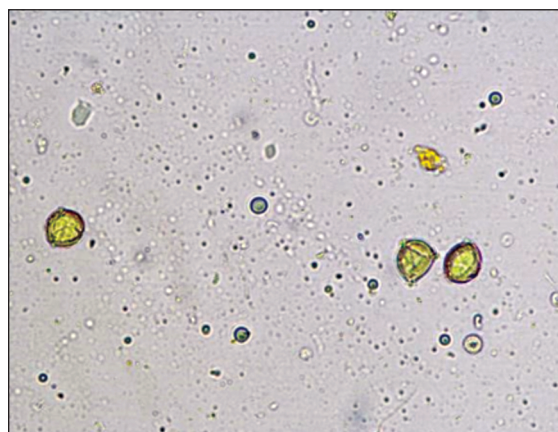
Sample I



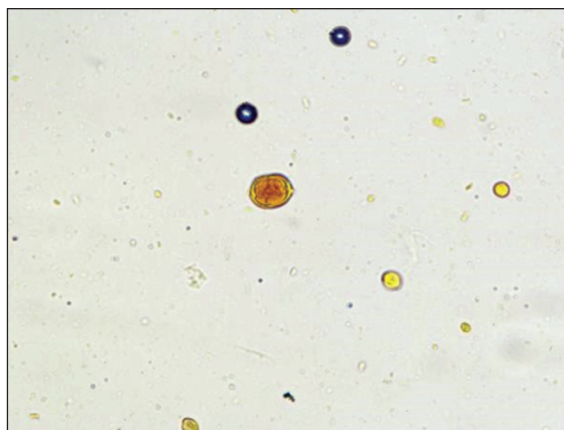
Sample III



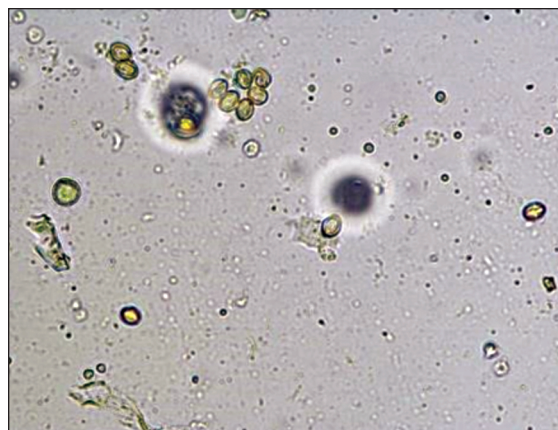
Sample IV



Sample V



Sample VI



Sample VII

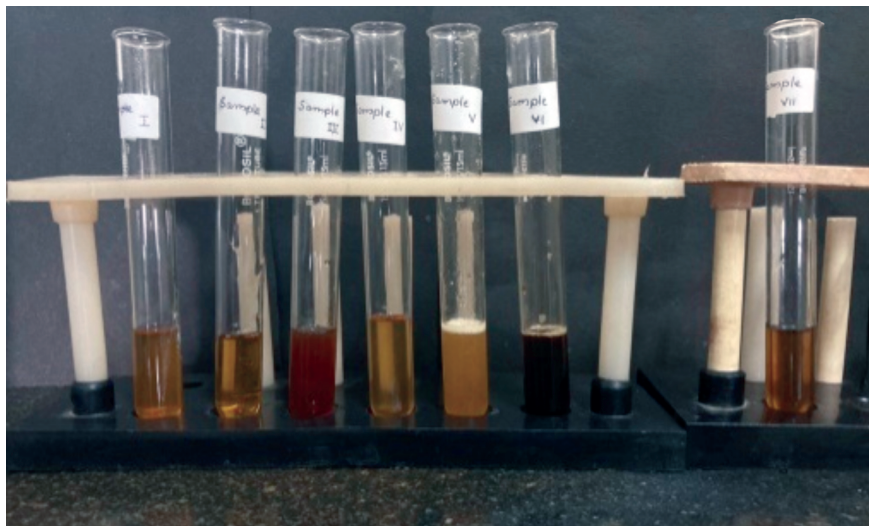


Fig. 5.2: Colour variation of different honey samples

CONCLUSION

The pollen analysis of the honey samples provided vital insights into the local flora. None of the samples displayed a dominance of any single pollen type, confirming that all were multifloral in origin. This study recommends that the Forest Department and relevant agencies take proactive measures to cultivate bee forage plants in and around agricultural fields, wastelands, and forest areas, ensuring bees have a continuous food supply throughout the year.

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Erickson, E. H. & Buchmann, S.L. 1983. Electronics and pollution – In Handbook of Experimental Pollination Biology (Eds. C. E. Johns & R. P. Little), pp 173 van Nostrand Reinhold, New York, U.S.A.

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