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Using the Petriplate Exposure Approach, Aeromycological Investigations were Conducted in the Outdoor Patient Department of the Rural Healthcare Center Sindewahi

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Introduction

Sindewahi Rural Healthcare Centre is located in India at latitude 20.283220 and longitude 79.6667600. The three seasons of this region's climate—summer, winter, and rainy season—are determined by factors including temperature, humidity, and rainfall. From February to May, the summer season begins, with highs of 45 to 47 degrees Celsius.[1] Rainfall often falls between June and September, while winter begins in October and lasts until January, with lows of 8 to 9 degrees Celsius. The outdoor patient department (O.P.D.) was used for air sampling in order to research the indoor aeromycoflora of the rural health care center.[2] For two years in a row, from August 2014 to July 2016, the air sample was conducted twice a month. The Rural Healthcare Centre Sindewahi has the capacity to admit roughly 70 patients for treatment. O.P.D. began at ten in the morning. [3]The formaldehyde fumigation procedure is used for sterilizing. Regular air samples were taken using the petriplate exposure method from the O.P.D. division of the Rural Health Care Center in Sindewahi.

Fig 1. Latitude and Longitude Position of Rural Healthcare Centre Sindewahi

Results and Discussion

Petriplate exposure method

Indoor aeromycoflora in Rural Healthcare Centre Sindewahi

Fungal aeromycospora were gathered from the O.P.D. section of the Rural Healthcare Center Sindewahi. The most traditional technique for gathering and identifying airborne fungal spores is the petripalte exposure

method.[1] Agar media-containing petriplates are incubated for 4–7 days at room temperature to determine the amount of fungus spores that fall on their surface. Colonies are enumerated and identified up to genera and species after five to seven days. A total of 71 fungal species from 20 distinct fungal genera were collected using the petriplate exposure approach.[4] In addition to these white sterile mycelia, two years of research (August 2014–July 2016) also isolated black and orange sterile mycelia. Two genera—Mucor (7 species) and Rhizopus (4 species)—belong to the Phycomycotina out of the 20 genera that have been identified. The remaining 15 fungal genera, which include *Aspergillus* (12 fungal species), *Penicillium* (7 fungal species), *Alternaria, Cladosporium, Curvularia,* and *Trichothecium* (all 4 fungal species), *Fusarium, Candida, Phoma*, and Torula (all 3 fungal species), *Cercospora, Drechlera, Helminthosporium, Nigrospora*, and *Trichoderma* (all 1 fungal species), and *Chaetomium* (3 fungal species), *Epicoccum* (2 fungal species), and *Geotrichum* (3 fungal species) belong to *Ascomycotina.* (Table 4.1).

Following Phycomycotina with 15.28% (11 fungal species) and Ascomycotina with 7.23% (8 fungal species), Deuteromycotina dominated the two-year study with 66.39% (52 fungal species). During the study period, 11.09 percent of sterile mycelium were found in the rural healthcare facility Sindewahi.

In the first year of the study, Deuteromycotina accounted for 69.30%, Phycomycotina for 14.01%, and Ascomycotina for 7.12%.Deuteromycotina accounted for 64.62%, Phycomycotina for 16.05%, and Ascomycotina for 7.29% of the second-year research.Over the course of the two-year research period, from 2014 to 2016, a total of 4678 fungal colonies were identified at the rural health care center Sindewahi. A total of 1769 fungal colonies were recovered in 2014–2015, and 2909 fungal colonies were isolated in 2015–2016. Aspergillus dominated the study for two years, with 659 colonies (14.08%), followed by Penicillium with 570 colonies (12.18%), Mucor with 458 colonies (9.79%), Alternaria with 432 colonies (9.23%), Rhizopus with 257 colonies (5.49%), Curvularia with 179 colonies (3.82%), and Cercospora with 157 colonies (3.35%). Phoma 139 colonies (2.97%), Epicoccum 136 colonies (2.9%), and Fusarium 145 colonies (3.09%) 134 colonies of Geotrichum (2.86%) Trichoderma 110 colonies (2.35%), Nigrospora 105 colonies (2.24%), Trichothecium 89 colonies (1.90%), Torula 125 colonies (2.62%), Cladosporium 123 colonies (2.62%), and Helminthosporium 113 colonies (2.41%). Chaetomium 68 colonies (1.47%), Drechslera 85 colonies (1.81%), and Candida 75 colonies (1.6%). In addition to this, 366 colonies of White sterile mycelia (7.82%), 145 colonies of Black sterile mycelia (3.09%), and 8 colonies of Orange sterile mycelia (0.17%) were seen over the two-year study.[5]

The most common species in the first year of the study were Aspergillus (235 colonies, 13.28%), Penicillium (223 colonies, 12.6%), Mucor (148 colonies, 8.36%), Alternaria (138 colonies, 7.8%), Rhizopus (100 colonies, 5.08%), Curvularia (93 colonies, 5.25%), and Cercospora (87 colonies, 4.91%). Helminthosporium 71 colonies (4.01%), Geotrichum 62 colonies (3.5%), Fusarium 57 colonies (3.22%), Cladosporium 53 colonies (2.99%), Nigrospora 47 colonies (2.85%), Trichoderma 44 colonies (2.48%), Drechslera 42 colonies (2.37%), Torula, Trichothecium 36 colonies (2.03%), Chaetomium, Phoma 33 colonies (1.87%), Epicoccum, Candida 31 colonies (1.75%), and 125 white sterile mycelia 125 colonies (7%), Black sterile mycelia 36 colonies (2.03%), and Orange sterile mycelia 2 colonies (0.247%) were observed during the two-year study.

Aspergillus dominated the second year of the study as well, with 424 colonies (15.19%), followed by Penicillium with 347 colonies (11.92%), Mucor with 310 colonies (10.65%), Alternaria with 294 colonies (10.10%), Rhizopus with 157 colonies (5.39%), Phoma with 106 colonies (3.5%), Epicoccum with 105 colonies (3.6%), Torula with 89 colonies (3.05%), Fusarium with 88 colonies (3.02%), Curvularia with 86 colonies (2.95%), and Clodosporium

with 70 colonies (2.4%). There were 72 (2.47%) Geotrichum colonies, 70 (2.4%) Cercospora colonies, 66 (2.26%) Trichoderma colonies, 58 (1.99%) Nigrospora colonies, 53 (1.82%) Trichothecium colonies, 44 (1.51%) Candida colonies, Drechslera 43 (1.47%), Helminthosporium 42 (1.44%), and 35 (1.2%) Chaetomium colonies. In addition, during the two-year study, 241 colonies of white sterile mycelia (8.28%), 109 colonies of black sterile mycelia (3.7%), and three colonies of orange sterile mycelia (0.20%) were seen.[6]

During the first year of the study (August 2014–July 2015), a total of 1769 fungal colonies were isolated; the Outdoor Patient Department (O.P.D.) recorded a maximum of 826 fungal colonies.[7]

According to the first-year study, indoor aeromycoflora also exhibits seasonal change. A high of 861 (48.67%) fungal colonies were isolated during the rainy season (June to September), followed by 638 (36.06%) during the winter (October to January) and a minimum of 270 (15.26%) during the summer (February to May).[8] Throughout the entire study, a maximum of 273 fungal colonies were recorded in July, followed by those in August, September, October, November, December, January, June, February, March, and April, while a minimum of 44 fungal colonies were reported in May.[9]

A total of 2909 fungal colonies were identified during the second year of the study (August 2015–July 2016), with the Outdoor Patient Department (O.P.D.) recording the highest number of fungal colonies at 1489.[10]

According to a second-year study, seasonal variation affects the amount of fungal spores in the air. There were a maximum of 1284 (44.13%) fungal colonies isolated during the rainy season (June to September), 1069 (36.74%) during the winter (October to January), and a minimum of 556 (19.11%) during the summer (February to May). During the entire study, a maximum of 382 fungal colonies were recorded in July, followed by those in August, September, October, November, December, January, June, February, March, and April. A minimum of 77 fungal colonies were documented in May.[11]

Only five genera—Aspergillus, Penicillium, Alternaria, Mucor, and Rhizopus—as well as white sterile mycelia were detected in the indoor air atmosphere of all three sections—the O.P.D. of the Rural Healthcare Center Sindewahi—during the first years of the study (Aug. 2015–July 2016). In addition to these five genera, Curvularia was also identified during the second year of investigation. [12]

Indoor aeromycoflora inOutdoor Patient Department (O.P.D.)

Using the petriplate exposure method, air samples were taken from O.P.D. at the rural healthcare center Sindewahi. A total of 71 fungal species from 20 distinct genera have been identified.[13] In addition to these white, sterile mycelia that were black and orange were also isolated during the two years of research (August 2014–July 2016). Phycomycotina is represented by the fungal genera Rhizopus (four fungal species) and Mucor (seven fungal species) out of 20. Ascomycotina is represented by the fungal genera Chaetomium (3 fungal species), Epicoccum (2 fungal species), and Geotrichum (3 fungal species), while Deuteromycotina is represented by the fungal genera Aspergillus (12 fungal species), Penicillium (7 fungal species), Alternaria, Cladosporium, Curvularia, and Trichothecium (all 4 fungal species), Fusarium, Candida, Phoma, and Torula (each containing three fungal species), Cercospora, Drechslera, Helminthasporium, Nigrospora, and Trichoderma (all 01 fungal species).[14]
The O.P.D. of the rural health center Sindewahi documented 2315 fungal colonies over the course of the two-year study period, which ran from August 2014 to July 2016.[15]
During the first year of the study (August 2014–July 2015), 826 fungal colonies were isolated. Seasonal change demonstrates the influence on airborne fungal spore concentration.[16]A maximum of 405 (49.03%) fungal

colonies were isolated during the rainy season (June to September), followed by 296 (35.83%) during the winter (October to January) and at least 125 (15.11%) during the summer (February to May).[17] Throughout the entire study, a maximum of 128 fungal colonies were reported in July, followed by those in August, September, November, October, December, January, February, June, March, and April, and at least 18 fungal colonies in May.[18]

Between August 2015 and July 2016, 1489 fungal colonies were isolated during the second year of the study. A maximum of 627 (42.10%) fungal colonies were isolated during the rainy season (June to September), followed by 555 (37.27%) during the winter (October to January) and a minimum of 307 (20.61%) during the summer (February to May).[19]Throughout the entire study, a minimum of 56 fungal colonies were recorded in May, and a maximum of 316 fungal colonies were recorded in July, followed by August, September, October, November, December, January, February, March, June, and April, respectively.[20]

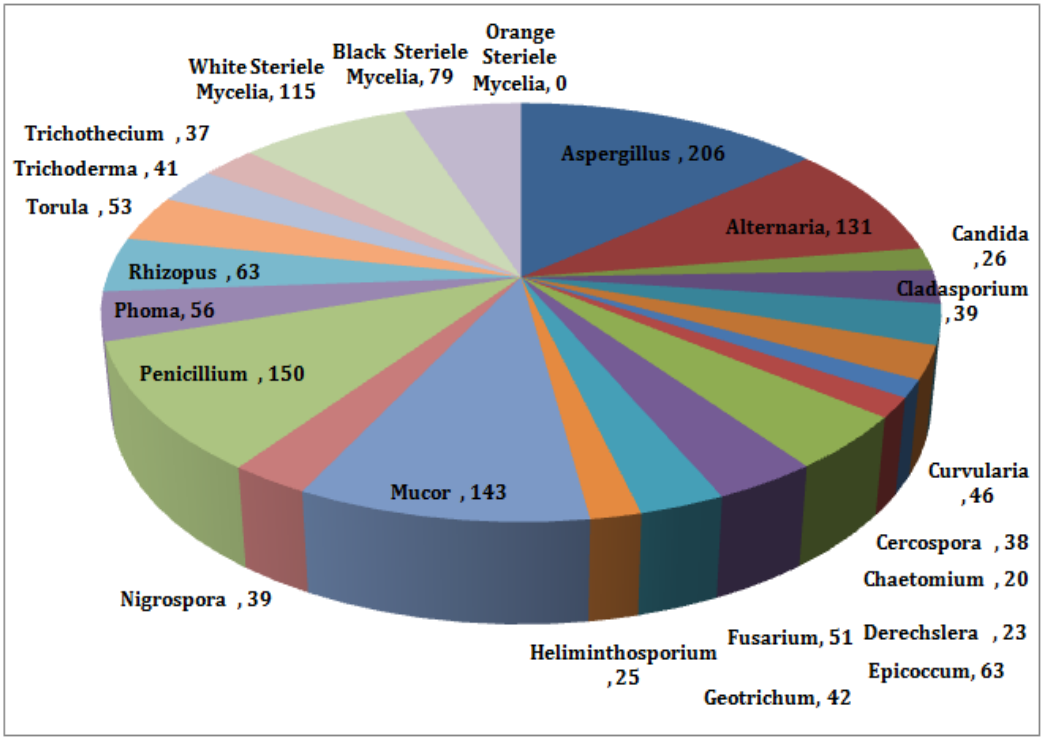
With 52 fungal species, Deuteromycotina (65.5%) dominated the study for two years. Phycomycotina (14.16%) and Ascomycotina (8.76%) came next with 11 and 8 fungal species, respectively. Additionally, 12% of these sterile mycelium were found in the O.P.D. of the rural healthcare facility Sindewahi. while conducting research.[21]

Deuteromycotina, Phycomycotina, and Ascomycotina were documented at 69.61 percent, 11.13%, and 9.44%, respectively, in the first year of the study. Deuteromycotina and Phycomycotina were 64.53% and 13.83%, respectively, in the second year of the study. Ascomycotina accounted for 8.39%. During a study period, however, 12% of sterile mycelium was found in the O.P.D. of the rural healthcare center Sindewahi.

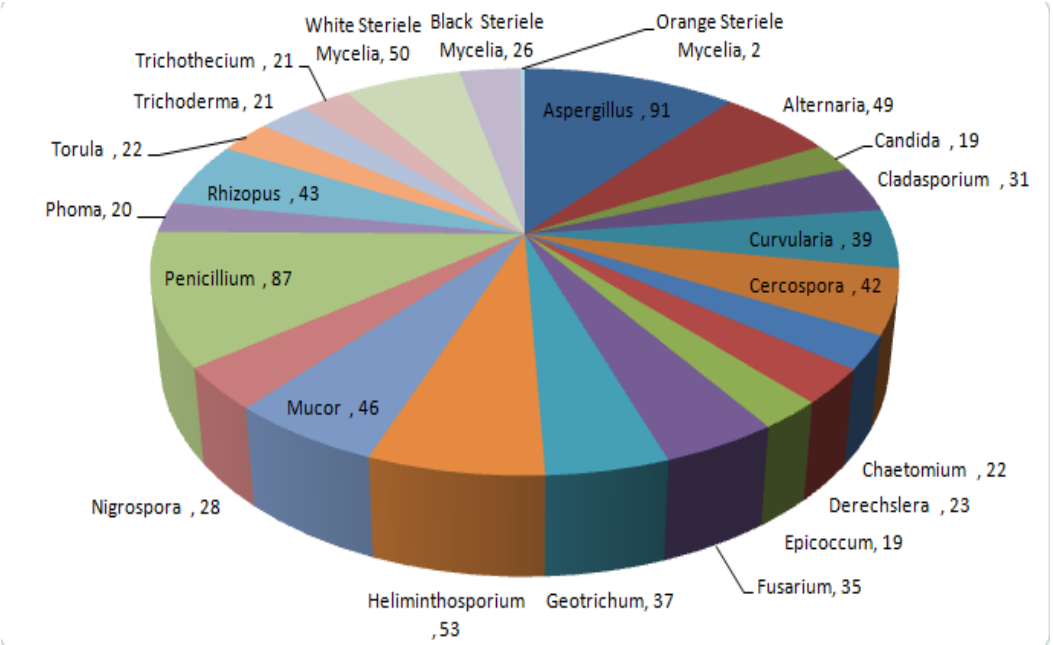
Rural Healthcare CenterSindewahi 2014-2016					
Petriplate Exposure Method /Settle Plate Method					
Total No of Fungal Colonies & Their Percentage Contribution in Outdoor Patient Department (O.P.D.)					
Year August 2014- July 2016					
Sr. No	**Month**	**Total No. of Colonies**			**%**
		O. P. D.		**Total**	
		2014-2015	**2015-2016**		
1	August	55	89	144	6.220302376
		60	86	146	6.306695464
2	September	58	85	143	6.177105832
		55	83	138	5.96112311
3	October	50	80	130	5.615550756
		46	78	124	5.35637149
4	November	41	75	116	5.010799136
		37	71	108	4.665226782
5	December	35	67	102	4.406047516
		31	64	95	4.103671706
6	January	29	61	90	3.887688985
		27	59	86	3.714902808

7	February	24	55	79	3.412526998
		21	54	75	3.239740821
8	March	21	50	71	3.066954644
		16	46	62	2.678185745
9	April	13	39	52	2.246220302
		12	25	37	1.598272138
10	May	10	21	31	1.339092873
		8	17	25	1.079913607
11	June	19	39	58	2.505399568
		30	57	87	3.758099352
12	July	65	95	160	6.911447084
		63	93	156	6.738660907
Total		**826**	**1489**	**2315**	

With 297 colonies (11.96%), Aspergillus dominated the study for two years. Penicillium came in second with 237 colonies (9.54%), followed by Mucor (8.16%), Alternaria 180 (7.25%), Rhizopus 106 (4.27%), Curvularia 85 (3.42%), Fusarium 86 (3.46%), Epicoccum 82 (3.30%), Geotrichum 79 (3.18%), and Cercospora 77 (3.10%). Cladosporium 70 colonies (2.82%), Nigrospora 67 colonies (2.69%), Trichoderma 62 colonies (2.49%), Trichothecium 58 colonies (2.33%), Phoma 76 colonies (3.02%), and Torula 72 colonies (3.02%) During two years of research, 50 colonies of Helminthosporium (2.01%), 46 colonies of Drechslera (1.85%), 45 colonies of Candida (1.81%), 42 colonies of Chaetomium (1.69%), and 165 colonies of White sterile mycelia (6.642%), Black sterile mycelia (4.23%), and Orange sterile mycelia (5 colonies, 0.34%) were observed.



Total No of Fungal Colonies in O.P.D. Sections & % Contribution to Total Aeromycoflora for the year 2015-2016



Total No of Fungal Colonies recorded in OPD & their % contribution of totalaeromycoflora for the year 2014-2015

In 1st year of investigation (Aug2014- July 2015),*Aspergillus* were dominant having 91 colonies (11.01%) followed by *Penicillium 87 colonies (9.32%), Helminthosporium* 53 colonies (6.41%), *Mucor* 46 colonies (5.56%) ,*Alternaria* 49colonies (5.93 %), *Rhizopus**43** colonies* (4.60 %), *Cercospora 42 colonies* (3.51%), *Curvularia* 39 colonies (4.1%),Geotrichum 37 colonies (3.96%), *Fusarium 35 colonies* (3.75%), *Cladosporium 31colonies* (3.75%),*Nigrospora* 28 colonies (3.00 %),*Drechslera* 23 colonies (2.46%),*Chaetomium, Torula 22 colonies each (*2.35%), *Trichoderma, Trichothecium 21 colonies each*(2.259%), *Phoma* 20 colonies (2.14%), Candida, Epicoccum 19 colonies (2.03%). Alonge with these *White sterile mycelia* 50 colonies (5.35%), Black *sterile mycelia* 26 colonies (2.78%) and *Orange sterile mycelia 2 colonies* (0.24%)were recorded.

In 2nd year investigation (Aug2015- July 2016), Aspergillus were dominant having 206 colonies (23.77%) followed by *Penicillium 150 colonies (9.68%), Mucor* 143 colonies (9.23%) ,*Alternaria* 131 colonies (8.45 %), *Rhizopus* ,Epicoccum***63*** *colonies each*(4.06 %),Phoma 56 colonies (3.61%), *Torula 53 colonies (*3.42%),*Cercospora 38 colonies* (2.45%), *Trichothecium 37 colonies* (2.38%), *Fusarium 51 colonies* (3.29%), *Curvularia* 46 colonies (2.97%),Geotrichum 42 colonies (2.71%), *Trichoderma 41 colonies (2.67%) , Cladosporium, Nigrospora 39 colonies* (2.51%),*Cercospora 38 colonies* (2.45%), *Trichothecium 37 colonies* (2.38%), Candida 26 colonies (1.67%), *Helminthosporium* 25 colonies (1.61%), *Drechslera* 23 colonies (2.46%), *Chaetomium*20 colonies (1.29%). Along with these *White sterile mycelia* 115 colonies (7.42%), Black *sterile mycelia* 79 colonies (5.1%) and *Orange sterile mycelia 3 colonies* (0.20%)were recorded in two years of investigation.

Discussion

Using the petriplate exposure method and volumetric air sampling with the Hi Media Air Sampler Mark II, an aeromycological survey was carried out every two weeks for two years (Aug 2014-July 2016) from the indoor environment of the rural health care center Sindewahi, Chandrapur District. In order to sample aeromycoflora in

an indoor environment, Tilak (1982) proposed combining two distinct air sampling techniques: volumetric air sampling using the Hi Media Air Sampler Mark II and the petriplate exposure method.[22]

According to a two-year study conducted at the rural health care center Sindewahi between August 2014 and July 2016, 71 fungal species from 20 different fungal genera were recovered. In addition, sterile mycelia that were white, black, and orange were isolated.[23] In which Deuteromycotina were dominant with 66.39 % (52 fungal species), followed by Phycomycotina with 15.28% (11 fungal species) and Ascomycotina with 7.23 % (8 fungal species). 11.09 percent sterile mycelium was found.According to the findings of Kotwal S.G. and Gosavi S.V. (2010), Deuteromycotina predominated in the indoor environment of the healthcare facility, followed by Phycomycotina and Ascomycotina.[24]

Rhizopus 257 colonies (5.49%), Curvularia 179 colonies (3.82%), Cercospora 157 colonies (3.35%), Mucor 458 colonies (9.79%), Alternaria 432 colonies (9.23%), and Aspergillus dominated with 659 colonies (14.08%). Phoma 139 colonies (2.97%), Epicoccum 136 colonies (2.9%), and Fusarium 145 colonies (3.09%) 134 colonies of Geotrichum (2.86%)125 colonies of Torula (2.62%), 123 colonies of Cladosporium (2.62%), 113 colonies of Helminthosporium (2.41%), 110 colonies of Trichoderma (2.35%), 105 colonies of Nigrospora (2.24%), and 89 colonies of Trichothecium (1.90%) Chaetomium 68 colonies (1.47%), Drechslera 85 colonies (1.81%), and Candida 75 colonies (1.6%). In addition to this, 366 colonies of White sterile mycelia (7.82%), 145 colonies of Black sterile mycelia (3.09%), and 8 colonies of Orange sterile mycelia (0.17%) were seen over the two-year study. [25]The most prevalent aeroallergens in indoor air are Aspergillus and Penicillium spores. The fundamental element of the air mycoflora was Aspergillus. Harzara and Majumdar (2005).

During the first year of the study (August 2014–July 2015), 1769 fungal colonies were isolated using the petriplate exposure method; the Outdoor Patient Department (O.P.D.) recorded the most fungal colonies, 826.[26]

A total of 2909 fungal colonies were identified during the second year of the study (August 2015–July 2016), with the Outdoor Patient Department (O.P.D.) recording the highest number of fungal colonies at 1489.The results of a study carried out at a university hospital in Rotterdam, Netherlands, were connected with the current findings.[27] In contrast to wards and operating rooms, the concentration of fungal spores was higher in indoor open area sections.by Leenders, A.C., and others (1999)

Seasonal Variation

The table shows the monthly contribution of all the colonies counted in three distinct areas of the rural health care center Sindewahi for the years 2014–2015 and 2015–2016, respectively.[28] During the first year of the study, the number of colonies in each month ranged from 44 to 273, and during the second year, it ranged from 77 to 382. In 2014-2015, the months of July (273) and August (242) had the highest colony counts. During the following year of the study, the highest number of colonies was recorded in July (382) and August (350).[29]Between 2.64% and 13.13% in both years, and between 2.48% and 15.43% on average, the monthly colony counts' percentage contribution to the total colony counts fluctuated. August (13.68%), September (12.88%), October (11.02%), November (99.83%), December (8.3%), January (6.89%), June (6.67%), February (5.25%), March (4.4%), April (3.1%), and May (2.48%) had the highest colony counts in the first year (15.43%). The months with the highest colony counts for the second year of the study (2015–2016) were July (13.13%), August (12.03%), and September (11.44%). November (9.62%), December (8.62%), January (7.8%), and October (10.69%) The lowest percentage was in May (2.64%), followed by June (7.52%), February (6.66%), March (5.77%), and April (4.02%).When the study's two years were compared season-wise using the exposure petriplate method—that is, summer, rainy, and winter—the highest number of colonies was found during the rainy season as opposed to the winter and summer seasons. Rainy season prevailed in 2014–2015, contributing 48.67 percent

with 861 colony counts, whilst in 2015–2016, it contributed 44.13% with 1284 colony counts.[30] The winter (October to January) followed the dominance of the rainy season. In 2014–2015, 638 colonies were found, with a frequency of 36.05%. With a colony count of 1069 in the second year of the study, or 2015–2016, that was 36.74%. Summertime makes up a smaller portion. It was 270 (15.26%) in 2014–2015 and 556 (19.11%) in 2015–2016.

In 2014–2015, the rainy season contributed 41.74% with 7765 CFUS/m3, and in the second year of the study, it contributed 40.81% with 8935 CFUS/m3. In the first and second years of the study, the winter season contributed roughly the same amounts—34.6% with 5705 CFUS/m3 and 35.58% with 7790 CFUS/m3, respectively. In 2014–2015, the summer season contributed at least 17.97% (2960 CFUS/m3), but in 2015–2016, it contributed 23.82% (5151 CFUS/m3).

Because of the ideal climate, which includes considerable rainfall with rising humidity and moderate temperatures that are conducive to fungal growth and development, the highest number of fungal colonies were seen during the rainy season. And high temperature with decreasing humidity (dry condition) arrests the growth and development of fungi.[28-27] The correlation between fungal spore concentration and metrological parameter was reported by Sudharsanam S. and Srikanth P. (2008), Pandey (1992), Tilak and Vishwe (1975).

Conclusion

The Rural Healthcare Centre Sindewahi in India is conducting aeromycological studies on its indoor environment using the Petriplate exposure method. The study focuses on the indoor aeromycoflora of the center's outdoor patient department (O.P.D.) and air sampling conducted twice a month for two years from Aug. 2014-July 2016. In two years, 71 fungal species belonging to 20 different genera were recovered, including white, black, and orange sterile mycelia. Deuteromycotina was the dominant fungal species, followed by Phycomycotina with 15.28% and Ascomycotina with 7.23%. Sterile mycelium was recorded at 11.09%. During the two-year research period, 4678 fungal colonies were recorded in the center. In the first two years, only five genera and white sterile mycelia were recorded from the indoor air atmosphere of all three sections. In the second year, Deuteromycotina (65.5%) was dominant with 52 fungal species, followed by Phycomycotina(14.16%) and Ascomycotina (8.76%). The maximum fungal colonies were observed during the rainy season, with the highest recorded in 2014-2015 and the lowest in 2015-2016.

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