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Petriplate Exposure Method for Aeromycological Studies of the General Ward's Indoor Environment at 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.[1] From February to May, the summer season begins, with highs of 45 to 47 degrees Celsius. Rainfall often falls between June and September, while winter begins in October and lasts until January, with lows of 8 to 9 degrees Celsius. Sections of the General Ward (G.W.) were used for air sampling in order to research the indoor aeromycoflora of the rural health care center. Regular air sampling was conducted twice a month for the two years in a row, from August 2014 to July 2016.[2] Formaldehyde fumigation is the procedure used for sterilizing. Air samples were routinely taken using both the petriplate exposure method and from the G.W. portions of the rural health care center Sindewahi.

Fig. Latitude and Longitude Position of Rural Healthcare Centre Sindewahi

Results and Discussion

Petriplate exposure method

Indooraeromycoflora in Rural Healthcare Centre Sindewahi

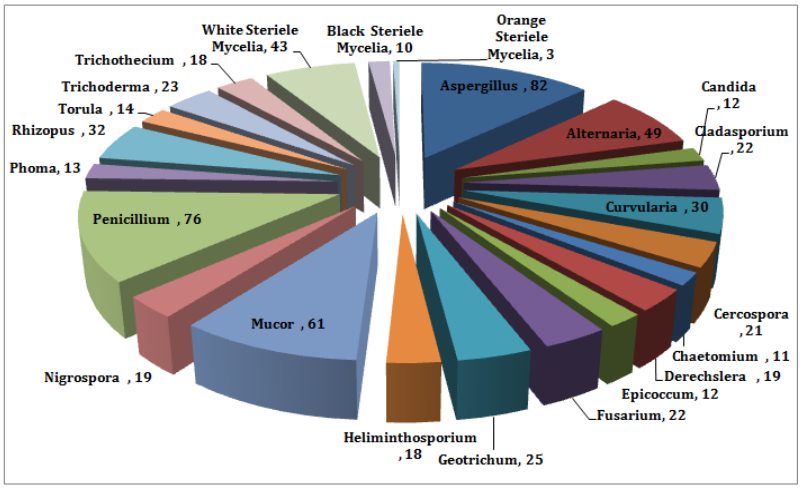
The G.W. Petripalte exposure method, the earliest technique for gathering and identifying airborne fungal spores, is where the fungus aeromycospora were gathered from the rural healthcare centerSindewahi.[3] The quantity of fungus spores that land on the petriplate surface with agar media is cultured at room temperature at intervals of 4–7 days. Colonies are enumerated and identified up to genera and species after five to seven

days.[4] A total of 71 fungal species from 20 distinct fungal genera were collected using the petriplate exposure approach. In addition to these white sterile mycelia, two years of research (August 2014–July 2016) also isolated black and orange sterile mycelia.[5] Of the 20 genera that have been identified, two belong to the *Phycomycotina:Rhizopus* (4 species) and Mucor (7 species). 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.Deuteromycotina* accounted for 66.39% of the 52 fungal species in the two-year study, while *Phycomycotina*came in second with 15.28% of the 11 fungal species and *Ascomycotina* with 7.23% of the 8 fungal species. During the study period, 11.09 percent of sterile mycelium were found in the rural healthcare facility Sindewahi.[7] 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 centreSindewahi. A total of 1769 fungal colonies were recovered in 2014–2015, and 2909 fungal colonies were isolated in 2015–2016.[8] *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.[9] 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 7.5 percent were observed during the two-year study.[10] In the second year of the study, *Aspergillus* also dominated 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%), *Cladosporium*with 70 colonies (2.4%), *Geotrichum* with 72 colonies (2.47%), *Cercospora* with 70 colonies (2.4%), *Trichoderma* with 66 colonies (2.26%), *Nigrospora* with 58 colonies (1.99%), *Trichothecium*with 53 colonies (1.82%), *Candida* with 44 colonies (1.51%), *Drechslera*with 43 colonies (1.47%), *Helminthosporium* with 42 colonies (1.44%), and *Chaetomium* with 35 colonies (1.2%). 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.[11] A total of 1769 fungal colonies were

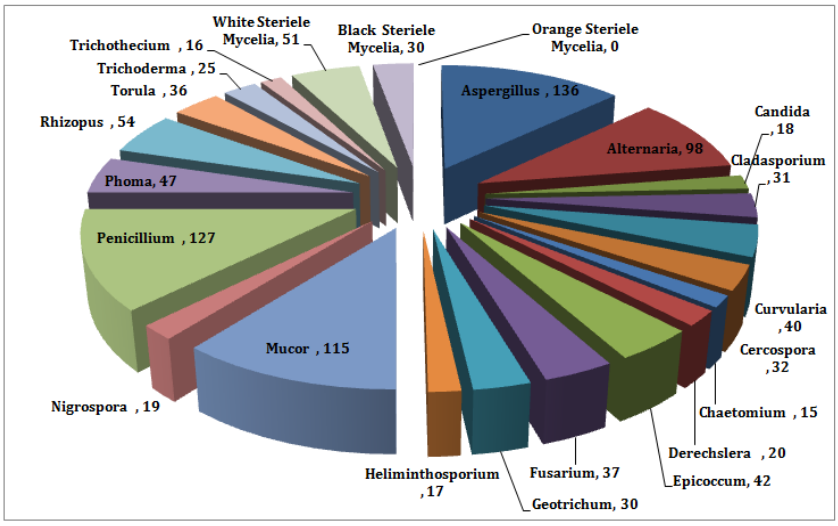
identified throughout the first year of the study (August 2014–July 2015), with 635 of those colonies coming from General Ward (G.W.).According to the first-year study, indoor aeromycoflora also exhibits seasonal change.[12] A maximum of 861 (48.67%) fungal colonies were isolated during the rainy season (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).[13] 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.[14] A total of 2909 fungal colonies were identified during the second year of the study (August 2015–July 2016), with 1036 of those colonies coming from General Ward (G.W.).[14–15] 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).[16] Over the course of 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, while a minimum of 77 fungal colonies were reported in May.[17] Only five genera—*Aspergillus, Penicillium, Alternaria, Mucor*, and *Rhizopus*—as well as white sterile mycelia were detected in the indoor air atmosphere of the sections G.W. of the Rural Healthcare CenterSindewahi during the first years of the study (Aug. 2015–July 2016).[18] In addition to these five genera,*Curvularia* was also identified during the second year of investigation.

Aeromycoflora inside in the General Ward (G.W.)

Air samples were taken from G.W. at the Rural Healthcare Center in Sindewahi. A total of 71 fungal species from 20 distinct fungal genera were isolated using the petriplate exposure method.[19] Over the course of two years of research (August 2014–July 2016), in addition to these white sterile mycelia, black and orange sterile mycelia were also isolated. Two fungal genera, *Rhizopus* (4 fungal species) and *Mucor*(7 fungal species), out of 20 fungal genera, represent *Phycomycotina.* 15 fungal genera, including *Aspergillus* (12 fungal species), Penicillium (7 fungal species), *Alternaria, Cladosporium, Curvularia,* and *Trichothecium*(each containing 4 fungal species), *Fusarium, Candida, Phoma,* and *Torula* (each containing 3 fungal species), *Cercospora, Drechslera, Helminthosporium*, *Nigrospora,* and*Trichoderma* (each containing 1 fungal species), and *Chaetomium* (3 fungal species), *Epicoccum* (2 fungal species), and *Geotrichum* (3 fungal species) represent the *Ascomycotina.[*20]



Total No of Fungal Colonies recorded in G.W. & their % contribution of total aeromycoflora for the year 2014-2015



Total No of Fungal Colonies recorded in G.W. & their % contribution of total aeromycoflora for the year 2015-2016

During the two-year study period, which ran from August 2014 to July 2016, 1671 fungal colonies were found in the general vicinity of the rural health center Sindewahi. During the first year of the study, which ran from August 2014 to July 2015, 635 fungal colonies were isolated. A maximum of 318 (50.07%) fungal colonies were isolated during the rainy season (June to September), followed by 234 (36.85%) during the winter (October to January) and a minimum of 83 (13.07%) during the summer (February to May). Throughout the entire study, a maximum of 102 fungal colonies were reported in July, followed by those in August, September, October, November, December, January, June, February, March, and April, and at least 12 fungal colonies in May.[21] During the second year of the study, which ran from August 2015 to July 2016, 1036 fungal colonies were isolated. A maximum of 460 (44.40%) fungal colonies were isolated during the rainy season (June to September), followed by 382 (36.87%) during the winter (October to January) and a minimum of 194 (18.72%) during the summer (February to May).[22] During the entire study, a maximum of 138 fungal colonies were recorded in July, followed by those in August, September, October, November, June, December, January, February, March, and April, while at least 30 fungal colonies

were documented in May.[24] *Phycomycotina* (15.67%) had 11 fungal species, *Ascomycotina* (8.07%) had 8 fungal species, and *Deuteromycotina* (68.04%) had 52 fungal species, making it the leading group over the two years of study. In addition, during the study period, 8.19% of sterile mycelium was found in the rural healthcare facility Sindewahi.[25] *Deuteromycotina, Phycomycotina,* and *Ascomycotina*were recorded at 68.97%, 14.64%, and 7.55%, respectively, in the first year of the study. *Deuteromycotina* accounted for 67.47%, *Phycomycotina* for 16.31%, and *Ascomycotina* for 8.39% in the second year of the study. *Aspergillus* dominated the study for two years, with 218 colonies (13.04%), followed by *Penicillium* with 203 colonies (12.14%), *Mucor* with 176 colonies (10.53%), *Alternaria* with 147 colonies (8.79%), *Rhizopus* with 86 colonies (5.14%), *Curvularia* with 70 colonies (4.18%), *Phoma* with 60 colonies (3.59%), *Fusarium* with 59 colonies (3.53%), *Geotrichum* with 55 colonies (3.29%), *Epicoccum* with 54 colonies (3.23%), *Cladosporium,* and *Cercospora* with 53 colonies (3.17%). 50 colonies of Torula (2.99%), 48 colonies of *Trichoderma*(2.87%), 39 colonies of *Drechslera* (2.33%), 38 colonies of *Nigrospora* (2.27%), 34 colonies of *Trichothecium* and *Helminthosporium* (2.03%), 30 colonies of Candida (1.79%), and 26 colonies of *Chaetomium* (1.55%), During the course of the two-year experiment, 94 colonies of White sterile mycelia (5.62%), 40 colonies of Black sterile mycelia (2.39%), and three colonies of Orange sterile mycelia (0.17%) were also seen. [26] *Aspergillus* dominated the first year of the study with 82 colonies (12.92%), followed by *Penicillium* with 76 colonies (11.98%), *Helminthosporium* with 18 colonies (2.67%), *Mucor* with 61 colonies (9.61%), *Rhizopus* with 32 colonies (5.71%), *Cercospora* with 21 colonies (3.31%), *Curvularia* with 30 colonies (4.73%), *Geotrichum* with 25 colonies (3.94%), *Cladosporium* and *Fusarium* with 22 colonies each (3.47%), *Drechslera*and *Nigrospora*with 19 colonies each (2.99%), *Chaetomium* with 11 colonies (1.72%), *Torula* with 14 colonies (2.20%), *Trichoderma* with 23 colonies (3.62%), *Trichothecium* with 18 colonies (2.82%), *Phoma*with 13 colonies (2.05%), and Candida with 12 colonies (1.89%). In addition to these 43 White sterile mycelial colonies (6.78%), during the two years of the study, 10 Black sterile mycelial colonies (1.57%) and 3 Orange sterile mycelial colonies (0.474%) were found.[27]
Aspergillus dominated the second year of the study (Aug 2015–July 2016), with 136 colonies (13.12%), followed by *Penicillium* (127 colonies, 12.25%), *Mucor*(115 colonies, 11.10%), *Alternaria* (98 colonies, 9.45%), *Rhizopus*(54 colonies, 5.21%), *Phoma* (47 colonies, 4.53%), *Epicoccum* (42 colonies, 4.05%), *Geotrichum* (30 colonies, 2.89%), *Trichoderma* 25 colonies (2.41%), *Drechslera* 20 colonies (1.93%), *Cladosporium, Nigrospora* 19 colonies each (1.83%), Candida 18 colonies (1.79%), *Helminthosporium* 17 colonies (1.64%), *Trichothecium* 16 colonies (1.54%), and *Chaetomium* 15 colonies (1.44%). In addition, during the two-year study, 50 colonies of White sterile mycelia (4.92%) and 30 colonies of Black sterile mycelia (2.89%) were observed.[28]

Discussion

For two years (August 2014–July 2016), an aeromycological survey was carried out every two weeks using the petriplate exposure method with a Hi Media Air Sampler Mark II in the indoor environment of the rural health care center Sindewahi in the Chandrapur District (Tilak, 1982).

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.[29] In addition, sterile mycelia that were white, black, and orange were isolated. Where *Deuteromycotina* accounted for 66.39% of the fungal species, followed by *Phycomycotina* with 15.28% and *Ascomycotina* with 7.23% and 8 fungal species, respectively.[30]

Rural Healthcare Center Sindewahi 2014-2016							
Petriplate Exposure Method /Settle Plate Method							
Total No of Fungal Colonies recorded in G.W. & their % Contribution of Total Aeromycoflora							
Year August 2014- July 2016							
Sr.No	Genera	Total No. of Colonies					%
		General Ward					
		2014-2015	%	2015-2016	%	Total	
1	Aspergillus	82	12.91	136	13.1	218	13.04
2	Alternaria	49	7.717	98	9.46	147	8.79
3	Candida	12	1.89	18	1.74	30	1.79
4	Cladosporium	22	3.465	31	2.99	53	3.1718
5	Curvularia	30	4.724	40	3.86	70	4.1891
6	Cercospora	21	3.307	32	3.09	53	3.1718
7	Chaetomium	11	1.732	15	1.45	26	1.556
8	Drechslera	19	2.992	20	1.93	39	2.3339
9	Epicoccum	12	1.89	42	4.05	54	3.2316
10	Fusarium	22	3.465	37	3.57	59	3.5308
11	Geotrichum	25	3.937	30	2.9	55	3.2914
12	Helminthosporium	18	2.835	17	1.64	35	5.2161
13	Mucor	61	9.606	115	11.1	176	10.53
14	Nigrospora	19	2.992	19	1.83	38	2.2741
15	Penicillium	76	11.97	127	12.3	203	12.14
16	Phoma	13	2.047	47	4.54	60	3.5907
17	Rhizopus	32	5.039	54	5.21	86	12.817
18	Torula	14	2.205	36	3.47	50	2.9922
19	Trichoderma	23	3.622	25	2.41	48	2.8725
20	Trichothecium	18	2.835	16	1.54	34	2.0347
21	White Sterile Mycelia	43	6.772	51	4.92	94	5.6254
22	Black Sterile Mycelia	10	1.575	30	2.9	40	2.3938
23	Orange Sterile Mycelia	3	0.472	0	0	3	0.1795
Total		635		1036		1671	

Sterile mycelium 11.09 % was recorded (table No.4.1 & 4.2). *Deuteromycotina* were dominant in indoor environment of health care center followed by *Phycomycotina* and *Ascomycotina* , these results are in agreement with the finding of Kotwal S.G. and Gosavi S.V.(2010).*Aspergillus* were dominant having 659colonies (14.08%) followed by *Penicillium*570 colonies (12.18%), *Mucor*458 colonies (9.79 %), *Alternaria* 432 colonies (9.23 %), *Rhizopus*257colonies (5.49 %), *Curvularia* 179 colonies (3.82 %),*Cercospora* 157colonies

(3.35 %) *Fusarium*145 colonies (3.09%), *Phoma* 139 colonies (2.97%), *Epicoccum*136 colonies (2.9%) *Geotrichum* 134 colonies (2.86%) *Torula*125colonies (2.62%), *Cladosporium*123 colonies (2.62%),*Helminthosporium* 113 colonies (2.41%), *Trichoderma* 110 colonies (2.35%), *Nigrospora*105 colonies (2.24 %), *Trichothecium*89 colonies (1.90%)*Drechslera*85colonies (1.81%),*Candida* 75 colonies (1.6 %), *Chaetomium* 68 colonies (1.47%). Along with these White sterile mycelia 366 colonies (7.82%), Black sterile mycelia 145 colonies (3.09%) and Orange sterile mycelia 8colonies (0.17%) was noted in two years investigation.[28-30] *Aspergillus, Penicillium* spores represent most abundant aeroallergens in the indoor air. *Aspergillus* was the basic component of the atmospheric *mycoflora.* Majumdar and Harzara (2005). In 1st year investigation (Aug2014- July 2015)by petriplate exposure method, total 1769 fungal colonies were isolated, out of which 635 fungal colonies from General Ward (G.W.) were recorded.[17-19] In 2nd year of investigation (Aug2015- July 2016) total 2909 fungal colonies were isolated, out of which 1036 fungal colonies from General Ward (G.W.).The present finding was correlated to the result of study conducted at university hospital in Rotterdam, Netherlands. Where the concentration of fungal spores was higher in indoor open area sections as compared to wards and operation theaters .by A.C Leenders et al. (1999)[25-27] Seasonal Variation The monthly contribution of total colonies enumerated in general ward of rural health care center Sindewahi, during 2014-2015 and 2015-2016 were illustrated in table respectively.[24-26] The colony counts in different months varied from 44-273 in first year of study, while it was from 77 to 382 in the second year of study period. The higher colony counts were observed in the month of July (273) followed by August (242) in 2014-2015. Also in the next year of investigation the maximum colony count were observed in month of July (382) followed by August (350).[17-21] On the average percentage contribution of monthly colony counts to the total colony counts varied from 2.48% to 15.43% and 2.64 % to 13.13% in both years respectively.[15] In the first year the highest colony counts was recorded in month of July (15.43%) followed by 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 least was reported in May (2.48%). In second year of investigation (2015-2016), the highest colony counts were recorded in month of July (13.13%) followed by August (12.03%), September (11.44%) October (10.69%), November (9.62%), December (8.62%), January (7.8%) June (7.52%), February (6.66%), March (5.77) April (4.02%) and least in May (2.64%). When both the year of study was analyzed comparatively according to season wise by exposure petriplate method i.e. summer, rainy and winter, the maximum colony count were observed in rainy season compared with that of winter and summer in both the year of investigation.[28] In the 2014-2015, rainy season dominated with the colony counts of 861 which contribute (48.67%) where as in 2015-2016 it was (44.13%) with colony count 1284. Dominance of rainy season was followed by the winter (Oct. -Jan).[5-15] The colony count 638 was observed in 2014-2015 with the frequency (36.05%). While that was 36.74% with the colony count 1069 in the second year of investigation i.e. 2015-2016. Summer season contribute less. In 2014-2015 it was 270 (15.26%) while in 2015-2016 it was 556 (19.11%) .[10-14]

Conclusions

The Rural Healthcare Centre Sindewahi in India, located in the Chandrapur District, has been studying its indoor aeromycoflora using the petriplate exposure method. The study found 71 fungal species, including white, black, and orange sterile mycelia, in the center's indoor environment. *Deuteromycotina* was the dominant fungal species, followed by *Phycomycotina* with 15.28% and *Ascomycotina* with 7.23%. Sterile

mycelium was recorded at 11.09%. Between 2014-2016, 4678 fungal colonies were recorded, with *Aspergillus* being the dominant species. The study highlights the importance of understanding the indoor aeromycoflora of rural healthcare centers in India. Seasonal variation affected the concentration of fungal spores in the air. In the first two years, only five genera and white sterile mycelia were recorded from the indoor air atmosphere. In the second year, *Deuteromycotina* (68.04%) was dominant with 52 fungal species, followed by *Phycomycotina* (15.67%) and *Ascomycotina* (8 fungal species). The highest colony counts were recorded in July and August. The study emphasizes the importance of understanding the indoor air environment for preventing fungal growth and health.

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