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Airborne Culturable Fungi from Outdoor Environment of College Premises

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Abstract:

Exposure to mould significantly increased risks of respiratory symptoms such as rhinitis, sore throat, cough and common cold in adults. There is no doubt that fungi produce allergens, mycotoxins, neurotoxins and also cause a number of human diseases and adversely affect human health. Present study was carried out to find out the airborne culturable fungi from the outdoor environment of college premises. The study was conducted from November- 2013 to February-2014 at fortnight intervals by using Hi-Air sampler (LA-002). Altogether 17 fungal species were isolated and identified from the college premises by using two different media strips viz. RBS-640 and PS-290 one sampling after the next simultaneously. The dominant fungi isolated from the campus includes *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Curvularia*, *Trichoderma*, *Mucor*, *Rhizopus*, *Fusarium*, *Nigrospora*, *Drechslera*, *Trichoderma* and non-sporulated fungi. The fungal isolates varied from 625 to 2181 CFU/m³ on the RBS-640 and PS-290 media strips respectively. The highest colony forming units was recorded in the month of January-2014 which is 2181 CFU/m³ on RBS-640 media strips and lowest 625 CFU/m³ recorded in the month of December-2013 on PS-290 media strips. On an average of total CFU counts of four months, the maximum 5,144 CFU/m³ was recorded on RBS-640 media strips, while lowest was recorded 3,731 CFU/m³ on PS-290 media strips. This difference of colony forming units was recorded might be due to culture medium which favors the growth of fungi i.e. RBS-640 is Rose Bengal Agar for Yeasts and Moulds and PS-290 is TSA- Agar for total count. The results of this study provide basic information about the prevalence of airborne culturable fungi which are highly allergenic, toxigenic and producing many different types' of health complaints.

Key words: Airborne, Culturable fungi, Outdoor Environment, College Premises, *Aspergillus*.

Introduction:

Fungi are ubiquitous in our daily environments. The campus of college became relatively crowded due to number students admitted in classes of undergraduates, graduates and post graduates courses. Our college was established in 1949, which was old and prime institution in the heart of Nagpur city. As an educational place, attentions should be focused on the finding of airborne culturable fungi and their percent contribution in the environment of college campus. However, problems of public health in colleges were not concerned enough in Nagpur. In fact, activities of disease prevention and control like infectious diseases, food-borne pathogens, airborne fungal, and pollen allergens and other microbial infections are not taken into consideration at government level. The health related problems and the promotion of health prevention and control awareness among the students of college was a prime focus on environmental health issues considered here. Environmental problems, especially the air quality in the crowded places in colleges should be significantly concerned of, since many allergenic and infectious diseases are correlated with microorganisms in the air Alan et al., (2012).

Fungi are ubiquitous in the atmosphere, and often constitute the main biological component of the air. They are considered to be closely related with air pollution and human health. Exposure to bio-aerosols containing airborne microorganisms and their by-products can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions Lacey (1991). More than 80 genera of fungi were associated with symptoms of respiratory tract allergies Horner et al., (1995). There are over 100 species of fungi were involved with serious human and animal infections, while many other species caused serious plant diseases Cvetnic and Pepelnjak (1997). It was reported that the species of *Cladosporium*, *Curvularia*, *Alternaria*, *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus*, *Trichoderma*, *Fusarium* and numerous non-sporulated fungi in the atmosphere and these are most common and their concentrations differed from place to place because of local environmental variables, fungal substrates, and human activities Fang et al., (2005). The potential of health risk caused by exposure to airborne fungi could occur in workplaces and residential spaces at any time. The concern about adverse health effects from bioaerosol inhalation has led to consideration of permissible exposure limits for fungi. However, currently there are seldom specific standards or directives or other exposure limits for fungi Hedayati et. al., (2007).

As an important indicator, airborne fungi investigation in college campus was considered to be necessary for their impacts on the student's health. Many studies were carried out about the fungal presence and their contribution in extramural and intramural environments in relation to human health in Nagpur city Giri (1996); Saoji & Giri (1997); Giri (2012 & 2015); Giri & Matey (2015). However, little is known about the prevalence of airborne culturable fungi in extramural environment of college campus. Nagpur is one of the most suitable to live cities in India, and generally speaking, its environment is pretty well. The present study was conducted to evaluate the concentration and the types of airborne culturable fungi in the environment of college campus for a part of environmental monitoring for the human health issues.

Material and Methods:

Physiography of Nagpur

Nagpur (Latitude: 21° 8'N, Longitude: 79° 9'E) is situated at an approximate centre of India and it is 307.4 meter above the sea level. The city of Nagpur is having population of about 2-2.7 million which is spread over an area of about 150 sq. kilometer. Most of the land is occupied by business, industrial or residential buildings and natural vegetation is found on the fringe of the city. Northwest position of the '0' milestone of Nagpur is occupied by seminary hills, west to '0' milestone, Laxminarayan Institute of Technology hill and just near the '0' milestone, in the centre of the city, Sitabuldi fort is situated. Nag River flows towards the east through the middle of the Nagpur.

Climate and Season:

The climate of Nagpur is of typical monsoon type with total rainfall of nearly 1974 mm. per annum. The temperature may reach maximum of 47°C in May, while it may come down to 8°C. Similarly the relative humidity ranges from 12.33% to 91.74%. The weather in general can be described as dry and moderately extreme. The minimum and maximum temperature and relative humidity were recorded during the sampling period (Table 1).

Sampling site:

Nagpur is the second capital of Maharashtra (India) with a registered population of 26-27 million. It has a subtropical climate with four distinctive seasons, characterized by rainy, winter and summer. Shri Mathuradas Mohota College of Science, Sakkardara square, Umred road, Nagpur, was selected for the study of culturable airborne fungi. Total college campus area is 5.79 acres and 12142 sq/mts.

Sampling methods:

Hi-Air sampler (Hi-Media Ltd. LA-002) was used to isolate culturable fungi from the college campus. Fortnightly sampling was conducted from November-2013 to February-2014 (4 months). Air sampling was conducted in between the 11.30 am to 1.30 pm for 4 minutes. Two different media strips were used i.e. RBS-640 (Rose Bengal Agar for Yeasts and moulds) and PS-290 (TSA-agar for total count), (Plate 1).

CFU Count and Identification:

Exposed media strips were incubated in an inverted position at $27 \pm 2^{\circ}\text{C}$; after 5-7 days of incubation, the colony forming units (CFUs) were visually counted and the total fungal count was expressed as colony forming units per cubic meter of air (CFU/m^3). Isolated genera/species were identified by macroscopic and microscopic analysis with the help of standard published literature (Plate 3). Nagamani, I., K. Kunwar, and C. Manoharachary (2006); Raper, K. B. & Fennell, D. I. (1977); Ellis, M. B. (1971); Raper, K. B. and Thom, C. (1949). The fungus detected per unit volume of air was calculated as under:

$$\text{CFU}/\text{m}^3 = \frac{\text{Colonies on agar strip} \times 25}{\text{Sampling time in minutes (4)}}$$

Results and Discussion:

Altogether 17 fungal genera/species of culturable types were identified from the outdoor environment of college premises (Table 2 & Figure 1). The dominant fungi isolated were includes *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Curvularia*, *Trichoderma*, *Mucor*, *Rhizopus*, *Fusarium*, *Nigrospora*, *Drechslera*, *Trichoderma* and non-sporulated fungi. The fungal isolates varied from 6-275 CFU/m³ on the RBS-640 and 6-206 CFU/m³ noted on the PS-290 media strips. Total 12 genera/species were isolated in the month of November-2013 on RBS-640 media strips, while 8 fungal genera/species were isolated on the PS-290 media strips. These are *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium herbarum*, *Cladosporium spp.*, *Curvularia geniculata*, *Curvularia tetramera*, *Fusarium spp.*, *Drechslera spp.*, *Nigrospora spp.*, *Torula spp.*, *Trichoderma spp.*, and non-sporulated fungi (Table 3 & Figure 2).

In the month of December-2013 total eleven (11) fungal genera/species were isolated on RBS-640 media strips. 9 fungal species were isolated and identified from PS-290 media strips. The total CFU counts on media strips RBS-640 was ranges from 6-138 CFU/m³ and 6-150 CFU/m³ noted on PS-290 media strips. These are *Alternaria spp.*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Cladosporium spp.*, *Curvularia geniculata*, *Curvularia tetramera*, *Drechslera spp.*, *Mucor spp.*, *Nigrospora spp.*, *Penicillium spp.*, *Rhizopus spp.*, and Non-sporulating fungi (Table 4 & Figure 2).

While in the month of January-2014 altogether 10 fungal genera/species were isolated on RBS-640 media strips and 9 fungal genera/species were isolated on the PS-290 media strips. The CFU counts was found to maximum on RBS-640 media strips, which is ranges from 13-963 CFU/m³ and 6-794 CFU/m³ on PS-290 media strips. The dominant fungi were isolated from the college premises are *Alternaria spp.*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium herbarum*, *Cladosporium spp.*, *Curvularia geniculata*, *Curvularia tetramera*, *Fusarium spp.*, *Torula spp.*, and Non-sporulating fungi (Table 5 & Figure 2).

In the month of February-2014 total 13 fungal genera/species were isolated on RBS-640 media strips. Whereas 12, fungal species was isolated from PS-290 media strips. The CFU counts recorded on the RBS-640 media strips was varies from 13-363 CFU/m³ and 13-244 CFU/m³ on PS-290 media strips. These are *Alternaria spp.*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium herbarum*, *Cladosporium spp.*, *Curvularia geniculata*, *Curvularia tetramera*, *Fusarium spp.*, *Mucor spp.*, *Penicillium spp.*, *Rhizopus spp.*, and Non-sporulating fungi (Table 6 & Figure 2).

The main cause of higher fungi concentration in outdoor environment of college premises might be attributed to its climatic characteristics, since it has a subtropical climate with four distinctive seasons (humid rainy, winter chilly, and hot summer). Nearby college have vegetable & fruit market place and play ground of colleges, which is partly covered by grasses, seasonal plants and large size trees also. Nagpur city has relatively high vegetation coverage, and the vigorous growth of plants which might be provided much fungal growth substrates in the air Giri (1996).

Adhikari et. al., (2004) recorded culturable molds from the large cattle shed of Kolkata West Bengal, they isolated dominant types *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Absidia blakesleeana*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizopus nigricans* and *Syncephalastrum racemosum*. In

present investigation total 17 fungal genera species were isolated and are already reported highly allergenic, toxin producing, carcinogenic, and producing infections in human beings. Nagpur city have great variations in environmental conditions such as climate characteristics, vegetation coverage, and human activities and nearby vegetable and fruit markets. However, to date in Nagpur city (M.S.) India, the network for microbiological measurements in indoor and outdoor air is not installed by government authority to find out the load of airborne microorganisms like airborne bacteria, viruses, fungi and also for the plants pollen grains which are highly allergenic to individuals. Airborne fungi of the indoor and outdoor environments have attracted much public attention especially in India: Calcutta (WB), Delhi, Chennai (TN), Mumbai, Pune, Aurangabad, Nagpur (MS), Pondicherry, Shimla (HP), Gwalior, Bhopal & Jabalpur (MP), and Raipur (CG).

High concentration of *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia* and *Penicillium* in indoor and outdoor environments could cause allergic diseases Alan et. al., (2012). In the previous studies Giri (2012) reported 36 fungal types from intramural environment of a college laboratory. *Aspergillus* was the most dominant component of the airspora followed by *Penicillium*, *Curvularia*, *Cladosporium*, *Alternaria*, *Chaetomium*, *Trichoderma*, *Mucor*, *Rhizopus*, *Phoma spp.*, *Scopulariopsis spp.*, *Torula herbarum* and *Helminthosporium spp.* in indoor air of laboratories and in control (outdoor). Brian Shelton et. al., (2002) reported the median outdoor fungal concentration was approximately 500 CFU/m³. The fungi most commonly recovered from outdoor air were *Cladosporium*, *Penicillium*, *Aspergillus* and the non-sporulating fungi. *Cladosporium* was usually found at a median concentration of approximately 200 CFU/m³; *Penicillium* 50 CFU/m³, *Aspergillus* was found 20 CFU/m³ and the non-sporulating fungi was found 100 CFU/m³. This result also correlated to the present investigation of colony forming unit's which measure in all the four months sampling periods (Table 3, 4, 5 & 6) and (Figure 2).

Xiuqin Lou et al., (2012) reported the culturable airborne fungi in a university campus in Hangzhou, Southeast China. They reported the *Penicillium* was the most common fungal group, and contributed 36% of the total fungal concentration, followed by *Cladosporium*, *Alternaria*, *Aspergillus* isolates and non-sporulating fungi. Present results provided the first hand data of airborne fungi in college campus of Nagpur city, and could efficiently help to evaluate the students and public health risks from exposure to these atmospheres.

Conclusion:

In the present study, it has been found that the airborne fungal concentration was relatively high in outdoor environment of college premises in southeast of Nagpur city. The culturable airborne fungi isolated from the college campus has dominant types *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria alternata*, *Alternaria spp.*, *Cladosporium herbarum*, *Curvularia geniculata*, *Curvularia tetramera*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizopus nigricans*, *Mucor spp.*, *Trichoderma spp.*, *Nigrospora spp.*, *Fusarium spp.*, *Drechslera spp.*, *Torula spp.*, and Non-sporulated fungi. The evaluation of air sampling results is currently based on the comparison of two different types of media strips were used one sampling after the next simultaneously in present investigation (RBS-640 & PS-290). The isolated fungi were identified and counted as colony forming units in per meter cube and also measure the levels of fungi detected in extramural environment of college campus. This study finds great variations amongst the fungi isolated and recorded in college campus by using two different culture media strips.

Present study provides data on monthly basis and their compositions of fungal populations isolated from the outdoor environment of college premises.

Table 1. Minimum & Maximum temperatures, and relative humidity recorded during the sampling periods by Temperature-Hygrometer

Sr. No.	Date of Samplings	Time of exposure in minutes	Temperatures		Relative humidity
			Minimum	Maximum	
1.	4 th November-2013	4 minutes	24.8	33.4	31
2.	20 th November-2013	4 minutes	22.4	27.5	43
3.	4 th December-2013	4 minutes	30.4	36.4	41
4.	22 nd December-2013	4 minutes	16.6	21.5	57
5.	15 th January-2014	4 minutes	24.8	27.5	43
6.	27 th January-2014	4 minutes	22.4	25.4	41
7.	13 th February-2014	4 minutes	27.3	30.9	32
8.	28 th February-2014	4 minutes	29.9	32.1	34

Table 2. Total monthly fungal species isolated from the college premises

Sr. No.	Fungal genera/species isolated	November-2013		December-2013		January-2014		February-2014	
		Media strips RBS-640	Media strips PS-290	Media strips RBS-640	Media strips PS-290	Media strips RBS-640	Media strips PS-290	Media strips RBS-640	Media strips PS-290
1.	<i>Alternaria spp.</i>	-	-	+	+	+	+	+	+
2.	<i>Aspergillus flavus</i>	+	-	-	-	+	+	+	+
3.	<i>Aspergillus fumigatus</i>	+	+	+	+	-	-	+	+
4.	<i>Aspergillus niger</i>	+	+	-	-	+	+	+	-
5.	<i>Cladosporium herbarum</i>	+	+	+	+	+	+	+	+
6.	<i>Cladosporium spp.</i>	+	+	+	+	+	+	+	+
7.	<i>Curvularia geniculata</i>	+	+	+	+	+	+	+	+
8.	<i>Curvularia spp.</i>	-	-	-	-	+	+	-	-
9.	<i>Curvularia tetramera</i>	+	+	+	+	+	+	+	+
10.	<i>Fusarium spp.</i>	+	-	-	+	+	-	+	+
11.	<i>Drechslera spp.</i>	+	-	+	+	-	-	-	-
12.	<i>Mucor spp.</i>	-	-	+	-	-	-	+	+
13.	<i>Nigrospora spp.</i>	+	-	+	-	-	-	-	-
14.	<i>Penicillium chrysogenum</i>	-	-	+	-	-	-	+	-
15.	<i>Rhizopus spp.</i>	-	-	+	+	-	-	+	+
16.	<i>Torula spp.</i>	+	+	-	-	+	+	-	-
17.	<i>Trichoderma spp.</i>	+	+	-	-	-	-	-	-
18.	<i>Non-sporulating fungi</i>	+	+	+	+	+	+	+	+
Total monthly fungal species count		12	08	11	09	10	09	12	10

Sr. No.	Table 3. Fungal species isolated from the college premises in the month of November-2013, total colony counts, their CFU/m ³ and percentage contribution						
	Fungal genera/species isolated	No. of Colonies on RBS-640 media strips	CFU/m ³	%	No. of Colonies on PS-290 media strips	CFU/m ³	%
1.	<i>Aspergillus flavus</i>	5	31	3	0	0	0
2.	<i>Aspergillus fumigates</i>	7	44	5	1	6	1
3.	<i>Aspergillus niger</i>	6	38	4	3	19	3
4.	<i>Cladosporium herbarum</i>	44	275	29	33	206	30
5.	<i>Cladosporium spp.</i>	21	131	14	13	81	12
6.	<i>Curvularia geniculata</i>	12	75	8	7	44	6
7.	<i>Curvularia tetramera</i>	28	175	18	27	169	25
8.	<i>Fusarium spp.</i>	1	6	1	0	0	0
9.	<i>Drechslera spp.</i>	2	13	1	0	0	0
10.	<i>Nigrospora spp.</i>	2	13	1	0	0	0
11.	<i>Torula spp.</i>	6	38	4	2	13	2
12.	<i>Trichoderma spp.</i>	1	6	1	1	6	1
13.	Non-sporulating fungi	18	113	12	22	138	20
Total counts & Percentage		153	956	100	109	681	100

Sr. No.	Table 4. Fungal species isolated from the college premises in the month of December-2013, total colony counts, their CFU/m ³ and percentage contribution						
	Fungal genera/species isolated	No. of Colonies on RBS-640 media strips	CFU/m ³	%	No. of Colonies on PS-290 media strips	CFU/m ³	%
1.	<i>Alternaria spp.</i>	16	100	12	9	56	9
2.	<i>Aspergillus fumigatus</i>	14	88	11	8	50	8
3.	<i>Cladosporium herbarum</i>	22	138	17	18	113	18
4.	<i>Cladosporium spp.</i>	18	113	14	24	150	24
5.	<i>Curvularia geniculata</i>	11	69	8	17	106	17
6.	<i>Curvularia tetramera</i>	16	100	12	12	75	12
7.	<i>Drechslera spp.</i>	4	25	3	1	6	1
8.	<i>Mucor spp.</i>	2	13	2	0	0	0
9.	<i>Nigrospora spp.</i>	3	19	2	1	6	1
10.	<i>Penicillium chrysogenum</i>	2	13	2	0	0	0
11.	<i>Rhizopus spp.</i>	1	6	1	1	6	1
12.	Non-sporulating fungi	21	131	16	9	56	9
Total counts & Percentage		130	813	100	100	625	100

Sr. No.	Table 5. Fungal species isolated from the college premises in the month of January-2014, total colony counts, their CFU/m ³ and percentage contribution						
	Fungal genera/species isolated	No. of Colonies on RBS-640 media strips	CFU/m ³	%	No. of Colonies on PS-290 media strips	CFU/m ³	%
1.	<i>Alternaria spp.</i>	17	106	5	12	75	5
2.	<i>Aspergillus flavus</i>	3	19	1	3	19	1
3.	<i>Aspergillus niger</i>	4	25	1	4	25	2
4.	<i>Cladosporium herbarum</i>	36	225	10	13	81	5
5.	<i>Cladosporium spp.</i>	47	294	13	17	106	7
6.	<i>Curvularia spp.</i>	22	138	6	29	181	11
7.	<i>Curvularia geniculata</i>	25	156	7	19	119	7
8.	<i>Curvularia tetramera</i>	34	213	10	21	131	8
9.	<i>Fusarium spp.</i>	5	31	1	11	69	4
10.	<i>Torula spp.</i>	2	13	1	0	0	0
11.	<i>Non-sporulating fungi</i>	154	963	44	127	794	50
Total counts & Percentage		349	2181	100	256	1600	100

Sr. No.	Table 6. Fungal species isolated from the college premises in the month of February-2014, total colony counts, their CFU/m ³ and percentage contribution						
	Fungal genera/species isolated	No. of Colonies on RBS-640 media strips	CFU/m ³	%	No. of Colonies on PS-290 media strips	CFU/m ³	%
1.	<i>Alternaria spp.</i>	18	113	9	11	69	8
2.	<i>Aspergillus flavus</i>	12	75	6	18	113	14
3.	<i>Aspergillus fumigates</i>	5	31	3	9	56	7
4.	<i>Aspergillus niger</i>	7	44	4	0	0	0
5.	<i>Cladosporium herbarum</i>	37	231	19	22	138	17
6.	<i>Cladosporium spp.</i>	2	13	1	2	13	2
7.	<i>Curvularia geniculata</i>	23	144	12	12	75	9
8.	<i>Curvularia tetramera</i>	58	363	30	39	244	30
9.	<i>Fusarium spp.</i>	3	19	2	7	44	5
10.	<i>Mucor spp.</i>	7	44	4	7	44	5
11.	<i>Penicillium chrysogenum</i>	2	13	1	0	0	0
12.	<i>Rhizopus spp.</i>	2	13	1	2	13	2
13.	<i>Non-sporulating fungi</i>	15	94	8	3	19	2
Total counts & Percentage		191	1194	100	132	825	100

PLATE 1. AIR SAMPLING IN COLLEGE PREMISES BY HI-AIR SAMPLER (HI-MEDIA - LA002).



PLATE 2. FUNGAL COLONIES GROWN ON EXPOSED MEDIA STRIPS (RBS-640 & PS-290) IN COLLEGE PREMISES.

MEDIA STRIPS (RBS-640) – COVERED STRIPS	MEDIA STRIPS (PS-290) – COVERED STRIPS
MEDIA STRIPS (RBS-640) – OPEN STRIPS	MEDIA STRIPS (PS-290) – OPEN STRIPS

Plate 3. Micro-photographs of isolated fungal species from the college premises


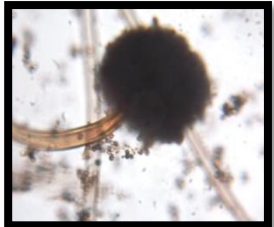

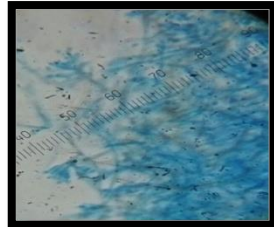
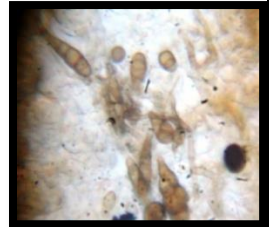
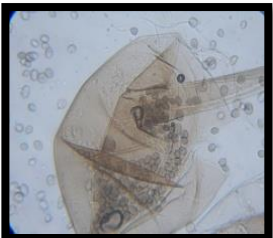
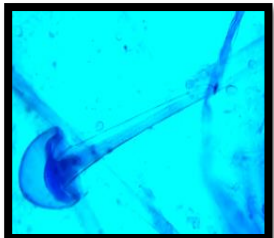




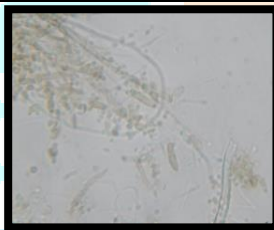
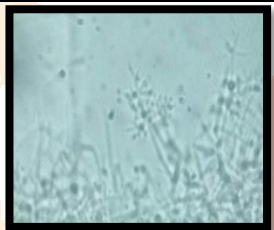
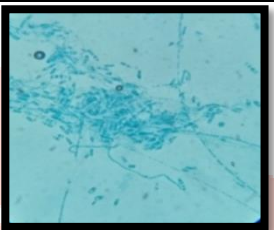

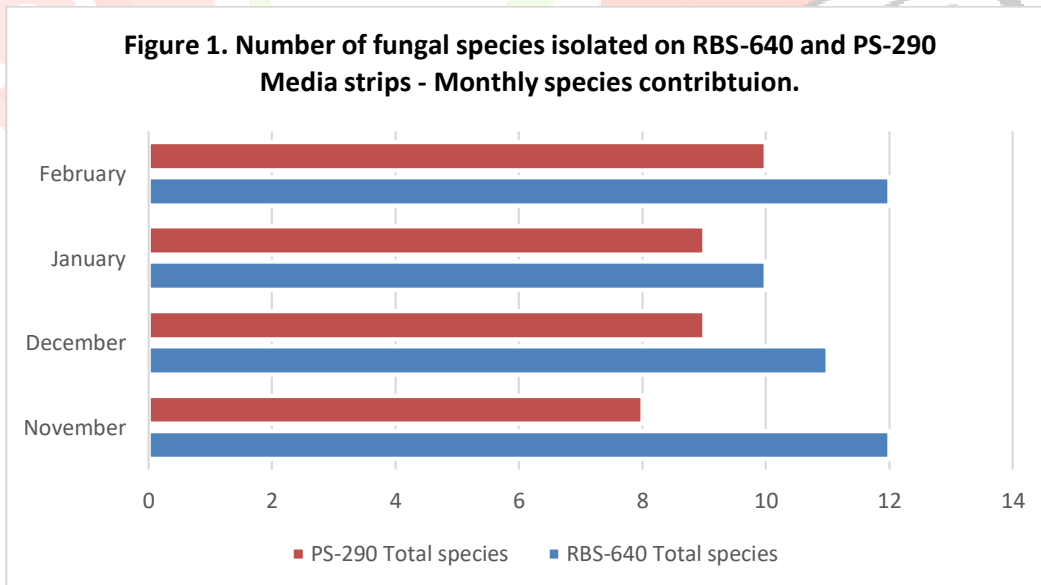
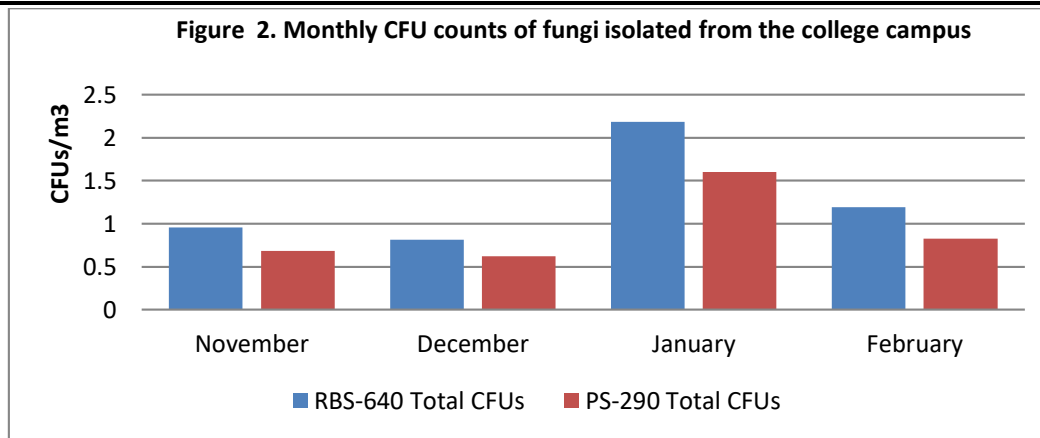
				
<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Penicillium chrysogenum</i>	<i>Alternaria & Nigrospora spp.</i>
				
<i>Rhizopus spp.</i>	<i>Mucor spp.</i>	<i>Curvularia geniculata</i>	<i>Curvularia spp.</i>	<i>Curvularia tetramera</i>
				
<i>Drechslera spp.</i>	<i>Cladosporium herbarum</i>	<i>Trichoderma spp.</i>	<i>Fusarium spp.</i>	<i>Torula spp.</i>

Figure 1. Number of fungal species isolated on RBS-640 and PS-290 Media strips - Monthly species contribution.





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