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Aeromycological studies of libraries in Purulia district of West Bengal and screening for potential cellulolytic fungi

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Aeromycological survey of the indoor atmosphere of six libraries in the Purulia district of West Bengal was conducted for one year to determine intramural fungal load inside the libraries. The seasonal variation of the fungi in the air of the indoor environment in the stack rooms of libraries was also investigated. Twenty-one fungi of 17 genera were isolated during the study period. *Aspergillus niger* was found to be dominant, followed by *Aspergillus flavus*, *Chaetomium globosum* and *Fusarium oxysporum*. The fungal count was found to change significantly with the changing of the indoor atmosphere during different seasons. The highest counts of fungal colonies appeared in monsoon followed by post-monsoon, winter and summer. The colony count was found to be significantly low in summer in comparison to monsoon and post-monsoon. The cellulolytic potential of dominant mycoflora was evaluated by determining the endoglucanase activity of the cellulase enzyme. The maximum enzyme activity in all four dominant isolates was found in pH 6 at 30ºC in peptone as a nitrogen source during the eighth day except *Aspergillus niger* which showed maximum activity on day four.

Keywords: Airborne fungi, cellulase, *Aspergillus niger, Aspergillus flavus, Chaetomium globosum, Fusarium oxysporum*

INTRODUCTION

Fungi are microorganisms present everywhere and contribute significantly to the biological diversity of this world. Fungi are one of the major bio-pollutants existing in the atmosphere and disseminated through the air. The fungal concentration in the air of a certain locality depends upon the vegetational types, presence of organic and decomposing substrates, agricultural lands, local markets and livestock farms. The sampling of fungal spores in an indoor environment recently draw attention as these spores contaminate the environment and affect the health of residents (Shrikhandia and Sumbali, 2015). Aeromycological study refers to the study of fungal load in a particular environment. Libraries are the main backbone of the educational system and the centre for the collection of resources like books, newspapers, magazines and journals (Ankush and Bhajbhuje, 2014). These collections can cover a wide array of topics and subjects, catering to the diverse interests and needs of their patrons. Additionally, libraries are important institutions that play a crucial role in society by providing access to a wide range of information, knowledge, and resources. Libraries play a major role in knowledge and information dissemination, especially in rural localities. Libraries promote literacy and education by offering reading programs, workshops, and access to educational materials. The physical conditions like relative humidity, temperature, and inorganic and organic substrates influence the load of indoor aeromycoflora. The collection of air sampling data also provides air quality information in the intramural environment of libraries (Swapna and Lalchand, 2016). The fungal spores that remain in the atmosphere of the libraries can cause serious health risks like allergic asthma, rhinitis,

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and atopic dermatitis to the visitors, readers and library staff. Bio-deterioration of materials present in libraries is a global problem as it damages unique books and manuscripts in the library. The cellulosic ingredients of papers are a good source of nutrients for fungi. Environmental factors like humidity, light, and temperature along with biotic factors like fungi, bacteria and insects cumulatively accelerate the deterioration process (Bhattacharya *et al*. 2001). D-glucose units are linked by β-1,4 bonds to form homopolymer cellulose (Islam and Roy, 2018). Cellulose is the utmost abundant natural biopolymer present in the world. Cellulose degradation can be achieved by employing chemical methods, enzymatic methods or both (Reddy *et al*. 2014). Filamentous fungi often colonize on paper and degrade cellulosic fibres by releasing cellulolytic enzymes (El Bergadi *et al*. 2014). These fungi are also responsible for the alteration of the aesthetic and visual of paper by secreting organic acids or pigments, a phenomenon known as foxing (Zotti *et al*. 2011). Papers contain little proportion of hemicelluloses, lignin, and some additives like fillers and pigments in addition to the major component cellulose (El Bergadi *et al*. 2014). The cellulase enzyme catalyzes the hydrolysis of homopolymer cellulose to glucose by the synergistic action of three enzymes endoglucanase, cellobiohydrolase, and β-glucosidase (Sherief *et al*. 2010). Traces of water in a storage environment accelerate the hydrolytic degradation of cellulosic fibres (Meehnian *et al*. 2016).

MATERIALS AND METHODS

Aeromycological study

Purulia is the westernmost district of the state West Bengal and is very close to the Jharkhand state. The aeromycological sampling was conducted in six different libraries present in both rural and urban areas in the Purulia district. The survey and isolation of airborne fungi were conducted over one year, from June 2021 to May 2022. The fungi present in the atmosphere of the intramural environment of libraries were isolated using the Petriplates exposure method (Gorai *et al*. 2022). Potato Dextrose Agar with Chloramphenicol, Rose Bengal Chloramphenicol Agar, and Corn Meal Agar with Chloramphenicol were used for the isolation of indoor aeromycota. The exposed plates were kept in an incubator at 28°C for 5 days. The fungal samples from developing colonies were mounted in slides with lactophenol cotton blue solution for microscopic observation. The identification was done based on microscopic observation and colony morphology from standard literature (Nagamani *et al*. 2006; Watanabe, 2010).

Screening of cellulolytic potential

The screening of cellulose enzyme was based on carboxymethyl cellulose (CMC) assay (Legodi *et al*. 2019). CMC agar with Congo Red (CR) was used as a medium for the determination of cellulolytic activity. The media was composed of CMC (2g), KH_2PO_4(0.5g), $MgSO_4$ (0.25g), gelatin (2g), Congo Red (0.2g), agar (15g), distilled water (1l) and pH was maintained at 6.8 (Gupta *et al*. 2012). The most prevalent fungal isolates were inoculated on CMC agar plate and incubated for 5 days at 28°C. A yellow halo zone around the fungal colony appears due to the release of bound Congo Red after hydrolysis of CMC, confirming the cellulolytic activity of the isolated fungi.

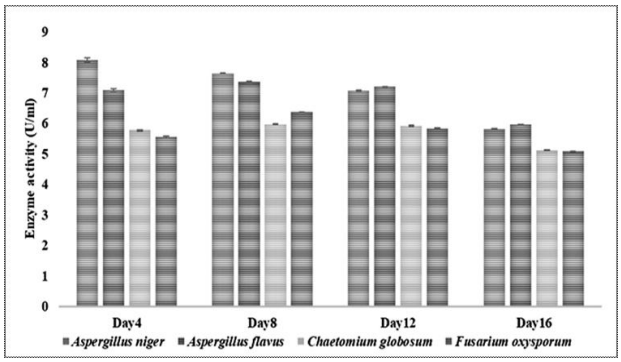

Fig.1. Effect of the incubation period on endoglucanase activity

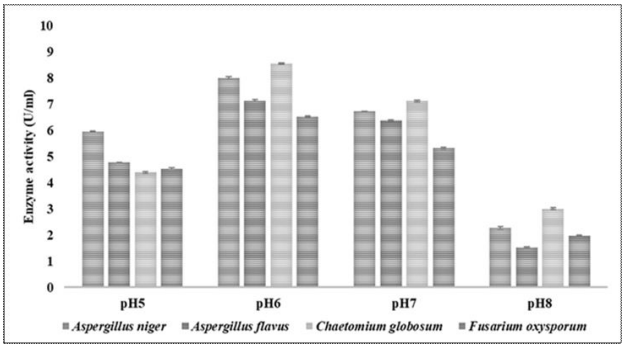

Fig.2. Effect of pH on the endoglucanase activity

Enzyme Production

Carboxymethyl cellulose broth was prepared by dissolving CMC (2g), KH_2PO_4 (0.5g), $MgSO_4$ (0.25g), and peptone (2g) in 1l distilled water to detect enzyme production. The media was autoclaved and inoculated with the fungal isolates. The broths containing fungal mycelia were subjected to centrifugation at 4°C at 5000 rpm for 15 min. The supernatant was collected and stored in the sterilized vials at 4°C for further assays of the cellulase enzyme.

Optimization of culture conditions for cellulase activity

In CMC broth media the effects of different culture parameters like incubation period, temperature, pH and nitrogen source on cellulase production by the dominant fungal isolates were determined. The effects of all the parameters on enzyme activity were assayed by determining the cellulase activity at different temperatures (20ºC, 30ºC, and 40°C), incubation periods (up to 16 days at 3-day intervals), pH (5, 6, 7, and 8), and nitrogen sources (gelatin and peptone).

Assay of Endo-β-1,4-glucanase by DNS method

The activity of Endo-β-1,4-glucanase of cellulase was determined through the DNS (3,5-Dinitrosalicylic acid) method by measuring the quantity of reducing sugar released by the hydrolytic activity of the enzyme (Miller, 1959). The substrate i.e., CMC solution (1%) was prepared using 1N citrate buffer (pH 5.0). Crude enzyme (100 µl) along with 1 ml citrate buffer was mixed with 1 ml CMC solution and the mixture was kept at 45°C for 45 min. The reaction was stopped by adding a DNS solution (Islam and Roy, 2018). The DNS-treated samples were then placed in a boiling water bath for 10 minutes followed by cooling for colour development and stabilization. The absorbance was taken at 540 nm by UV-Vis spectrophotometer (Shimadzu UV1800). One unit of enzyme activity was expressed as the amount of enzyme that could hydrolyze CMC to discharge 1µmol of glucose within 1 min of reaction time (Shanmugapriya *et al*. 2012).

Statistical analysis of airspora

One-way ANOVA test was performed to analyze the effect of seasonal variation in the mean colony counts during the study duration. Post-hoc test was done to identify exactly which seasonal counts significantly differ from each other. The significance was determined at the level of 0.05.

RESULTS AND DISCUSSION

Aeromycological investigation

Fungal spores in the air of libraries play a significant role in the biodeterioration of books and other cellulosic materials. Moreover, the importance of the study of fungal airspora in the indoor atmosphere is increasing due to the manifestation of fungal infection and allergy, as the spores and mycelial fragments enter into our respiratory system during breathing while handling or reading the books in library. The petriplate exposure is the most appropriate method for the study of intramural mycobiota (Lanjewar and Sharma, 2014). The air in the indoor library environment was found to contain a good number of fungi along with their spores for the entire year. A total of 3121 fungal colonies belonging to 21 species under 17 genera along with some sterile mycelial forms were isolated during this one year of aeromycological survey of libraries (Table 1**).** The most dominant fungal isolate was found to be *Aspergillus niger* (15.76%) and the other dominant fungal isolates were *Aspergillus flavus* (11.15%), *Chaetomium globosum* (9.45%) and *Fusarium oxysporum* (9.42%). These four dominant fungi contributed about 46% of total fungal counts throughout the year and the genus *Aspergillus* alone contributed 28% of the total fungal load. *Curvularia lunata* (6.89%), *Cladosporium cladosporioides* (5.64%), *Alternaria alternata* (5.96%), and *Rhizopus stolonifer* (5.06%) were the other significant fungal species traced during the air sampling. Three species of *Aspergillus* viz. *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus* were detected in the intramural environment of libraries, among which *Aspergillus niger* was the dominant species. The predominance of *Aspergillus* in the indoor library environment was reported at Universities such as University of Michigan,

Table.1: Monthly count of fungal colonies in the indoor environment of libraries

Spore types	Month												Total	%
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May		
Curvularia lunata	12	17	20	11	17	21	27	31	18	16	16	09	215	6.89
Cladosporium cladosporioides	-	-	-	-	06	17	32	50	34	18	11	08	176	5.64
Aspergillus niger	42	45	49	43	30	25	34	45	36	54	48	41	492	15.76
Aspergillus flavus	34	48	42	42	34	31	28	25	18	15	12	19	348	11.15
Aspergillus fumigatus	16	12	08	02	-	-	-	-	-	-	-	05	43	1.38
Penicillium chrysogenum	-	-	05	07	07	16	02	08	06	02	-	-	93	2.98
Fusarium oxysporum	32	40	42	37	32	28	23	17	16	08	07	12	294	9.42
Drechslera sp.	16	12	06	02	-	-	-	-	-	-	-	04	40	1.28
Alternaria alternata	19	23	27	32	27	18	12	05	-	-	08	15	186	5.96
Alternaria brassicicola	-	06	10	12	09	09	15	11	13	12	07	-	104	3.33
Nigrospora oryzae	06	14	17	21	11	07	02	-	-	-	-	-	78	2.50
Rhizopus nigricans	08	12	20	26	19	12	15	08	06	-	-	-	126	4.04
Chaetomium globosum	36	48	42	37	30	23	18	15	12	10	10	14	295	9.45
Mucor mucedo	12	14	22	16	10	08	-	-	-	-	-	-	88	2.82
Rhizopus stolonifer	14	18	26	24	21	16	16	12	11	-	-	-	158	5.06
Cunninghamella echinulata	12	11	08	06	02	-	-	-	-	-	-	-	41	1.31
Aureobasidium sp.	10	10	07	06	03	02	-	-	-	-	-	-	38	1.22
Paecilomyces sp.	10	14	16	21	18	12	07	04	-	-	-	-	102	3.27
Stachybotrys sp.	06	09	14	08	05	02	-	-	-	-	-	-	44	1.41
Diplodia sp.	04	07	13	08	03	-	-	-	-	-	-	-	35	1.12
Phoma sp.	04	06	06	08	12	10	15	13	08	-	-	-	62	1.99
Sterile mycelia	07	08	06	08	05	05	06	04	02	03	04	05	63	2.02
Total	312	388	411	377	303	262	260	241	174	138	123	132	3121	
% contribution	10.00	12.43	13.17	12.08	9.71	8.39	8.33	7.72	5.58	4.42	3.94	4.23		

University of Madras, and from Nagpur by Ankush and Bhajbhuje (2014) support this present finding. Five fungal species viz. *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Chaetomium globosum* and *Curvularia lunata* were present in the air of the libraries throughout the year. The common fungal constituents in the indoor atmosphere of libraries at Aurangabad are *Curvularia* sp., *Helminthosporium* sp., *Bispora* sp., *Fusarium* sp., *Torula* sp. and *Cladosporium* sp. as reported by previous authors. *Curvularia lunata*, *Fusarium oxysporum* and *Cladosporium*

Table 2 : ANOVA table for seasonal variation of colony count

Source of Variation	SS	df	MS	F	*P*-value	F crit
Between Groups	13481.76	3	4493.92	3.642168	.01*	2.713227
Within Groups	103644.1	84	1233.859			
Total	117125.9	87				

*Significance at the level of 0.05

Table 3: Results of Post Hoc test for ANOVA

Levels	Mean Difference	*P*-value
Monsoon - Post-monsoon	7.69	.48
Monsoon - Winter	19.82	.08
Monsoon - Summer	32.64	.004*
Post-monsoon - Winter	12.13	.25
Post-monsoon - Summer	24.95	.01*
Winter - Summer	12.82	.21

*Significance at the level of 0.05

Table 4: Seasonal variation of fungi in the indoor atmosphere of libraries

	Season with fungal contribution (in %)			
Fungal isolates	Monsoon	Post-monsoon	Winter	Summer
Curvularia lunata	4.54	5.21	11.18	10.43
Cladosporium cladosporioides	-	2.45	17.06	9.41
Aspergillus niger	12.59	10.43	16.91	36.39
Aspergillus flavus	11.48	11.38	10.44	11.70
Aspergillus fumigatus	3.33	0.21	-	1.27
Penicillium chrysogenum	0.46	3.19	2.35	0.51
Fusarium oxysporum	10.56	10.32	8.24	6.87
Drechslera sp.	3.15	0.21	-	1.02
Alternaria alternata	6.39	8.19	2.50	5.85
Alternaria brassicicola	1.48	3.19	5.74	4.83
Nigrospora oryzae	3.43	4.15	0.29	-
Rhizopus nigricans	3.70	6.06	4.26	-
Chaetomium globosum	11.67	9.57	6.62	8.65
Mucor mucedo	4.44	3.62	-	-
Rhizopus stolonifer	5.37	6.49	5.74	-
Cunninghamella echinulata	2.87	0.85	-	-
Aureobasidium sp.	2.50	1.17	-	-
Paecilomyces sp.	3.70	5.43	1.62	-
Stachybotrys sp.	2.69	1.60	-	-
Diplodia sp.	2.22	1.17	-	-
Phoma sp.	1.48	3.19	5.29	-
Sterile mycelia	1.94	1.91	1.76	3.05

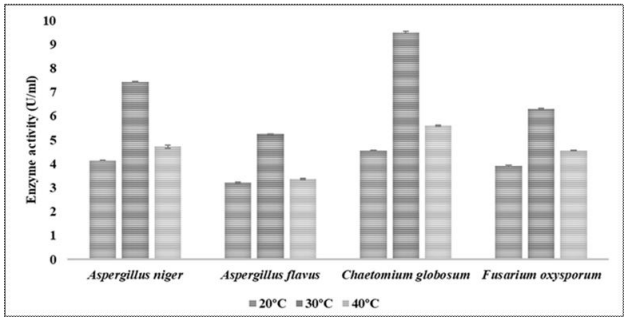

Fig.3. Effect of temperature on the endoglucanase activity

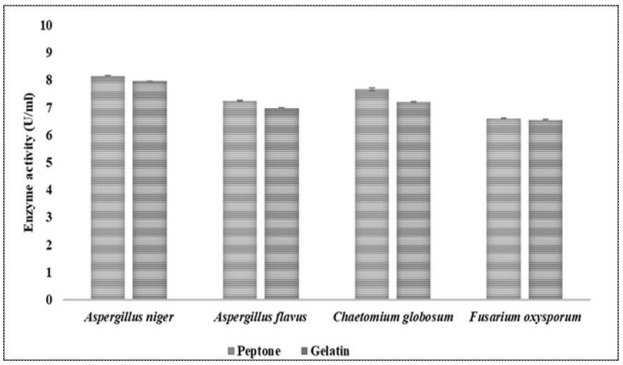

Fig. 4. Effect of nitrogen source on the endoglucanase activity

cladosporioides were also the common constituents of the air mycoflora of libraries in the Purulia district. Along with different species of *Aspergillus*, *Cladosporium* spp., *Penicillium* spp. and several other fungi like *Alternaria alternata*, *Fusarium solani*, *Mucor hiemalis*,*Curvularia lunata*, and *Rhizopus stolonifer* were recorded from indoor air of libraries from different areas like Cuba (Borrego *et al*. 2010), Ethiopia (Hayleeyesus and Manaye, 2014), Poland (Pastuszka *et al*. 2000; Zielinska-Jankiewicz *et al*. 2008), and India (Bhattacharya *et al*. 2001; Shrikhandia and Sumbali, 2015) which corroborate with the findings of current investigation.The highest fungal counts were attributed to August (13.17%) followed by July (12.43%) and September (12.08%) and the lowest number was found in April (3.94%).

Seasonal variation of airborne fungi

The study period was divided into four seasons: monsoon (June-August), post-monsoon (September-November), winter (December-February) and summer (March-May). The highest fungal colony counts were observed during monsoon (35.6%) followed by post-monsoon (30.18%), winter (21.63%), and summer (12.59%). One-way ANOVA (Table 2**)** suggested a significant variation in the seasonal colony numbers ($P<0.05$). Further, post-hoc analysis (Table 3) indicated a significant difference in the colony counts between both monsoon and summer as well as post-monsoon and summer ($P<0.05$). In seasonal variations of fungal appearance, the fungus *Aspergillus niger* (12.59%) was found to be dominant followed by *Chaetomium globosum* (11.67%), *Aspergillus flavus* (11.48%) and *Fusarium oxysporum* (10.56%) in monsoon. In post-monsoon *Aspergillus flavus* (11.38%) was the highest in occurrence followed by *Aspergillus niger* (10.43%), *Fusarium oxysporum* (10.32%) and *Chaetomium globosum* (9.57%). In winter *Cladosporium cladosporioides* (17.06%) was the dominant followed by *Aspergillus niger* (16.91%), *Curvularia lunata* (11.18%), and *Aspergillus flavus* (10.44%). In summer *Aspergillus niger* (36.39%) again appeared as the dominant fungus followed by *Aspergillus flavus* (17.70%), *Curvularia lunata* (10.43%) and *Cladosporium cladosporioides* (9.41%) (Table 4).

Enzymatic activity

Several fungal species can use cellulose as a carbon source for their growth and sporulation. An extensive range of fungi were traced from the atmosphere of libraries due to the existence of different cellulosic materials in libraries which provide a good carbon source for them. Thus, it was felt necessary to determine the cellulolytic potential of isolated dominant fungi as a part of this present investigation. The four dominant fungi viz. *Aspergillus niger*, *Aspergillus flavus*, *Chaetomium globosum* and *Fusarium oxysporum* were tested for cellulose utilization by culturing in the existence of the only carbon source cellulose. These four fungi were shown luxuriant growth by utilizing cellulose through the production of the enzyme cellulase. The cellulase production was found maximum on day 4 for *Aspergillus niger*, and then a gradual decrease was observed. In the case of *Aspergillus flavus*, *Chaetomium globosum* and *Fusarium oxysporum*, the cellulase activity was boosted with the culture duration and the highest activity was noticed on day 8 followed by a sharp decline. On day 8, *Aspergillus flavus* produced the

uppermost activity followed by *Fusarium oxysporum* and *Chaetomium globosum* (Fig.1**).** pH of the medium was another parameter used for determining the effect on enzyme production. The fungi were cultured at different pH ranges (5.0 to 8.0) to determine the optimum pH for the production of cellulase. All the tested isolates were able to produce cellulase from pH 5 to 8 with the maximum at slightly acidic conditions at pH 6,similar to earlier findings where it was reported that the optimum pH for cellulase activity of *Aspergillus niger* is 6.0-7.0. The acidic pH of the medium promotes cellulase production was also claimed by Andrade *et al.* (2011) and Sarkar and Aikat (2014).The activity was significantly decreased when the pH was increased to 8 (Fig.2). The consequence of temperature on enzyme release was tested by incubating in three different temperatures viz. 20ºC, 30ºC and 40ºC at pH 6.0. It is interesting to note that the ideal temperature for the production of the enzyme for all the isolates was found at 30ºC. *Chaetomium globosum* was found to produce the highest activity followed by *Aspergillus niger*, *Fusarium oxysporum*, and *Aspergillus flavus* in their optimum temperature however, in below and above optimum temperature tested fungi are also found to be capable of cellulase production (Fig.3). Sohail *et al.* (2009) also observed sufficient endoglucanase production at 30ºC for *Aspergillus niger*. The maximum cellulase activity was also reported in *Trichoderma reesei* at 30ºC and gradually decreased with the enhancement of temperature (Darabzadeh *et al*. 2019). Temperature significantly affects the growth and metabolism of an organism. Temperature below the optimum was reported to decrease the enzyme activity as a result of low penetration of substrates across the cell, thus reducing cellulase production of test fungi (Dutt and Kumar, 2014). Two nitrogen sources peptone and gelatin were used to analyze the preference of nitrogen source for cellulase enzyme production. In both the nitrogen sources *Aspergillus niger* produced the maximum activity and *Fusarium oxysporum* showed the lowest activity. All the tested fungal isolates showed higher enzymatic activity when grown in peptone as a nitrogen source (Fig.4). The optimum condition for the cellulolytic potential of *Aspergillus niger* was determined on day 4, at pH 6, after incubation at 30ºC with peptone as a nitrogen source. However, *Aspergillus flavus*, *Chaetomium globosum* and *Fusarium oxysporum* were found to reach their highest cellulolytic potential at pH 6 and 30ºC with peptone as a nitrogen source after incubation for 8 days. Reddy *et al.* (2014) got maximum enzyme recovery between 3 to 5 days of incubation and high cellulase activity for *Chaetomium* was reported after 144 hours of fermentation (Al-Kharousi*et al*. 2015). Dutt and Kumar (2014) studied the production of cellulase from *Aspergillus niger* and *Aspergillus flavus* and got the maximum activity at day five. Another study conducted by Ramanathan *et al.* (2010) observed the optimum cellulase production of *Fusarium oxysporum* at 12 days of incubation at pH 6 at 50°C. So, the optimum parameters for the maximum activity of the cellulase enzyme vary from species to species.

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DECLARATION

Conflict of interest: Authors declare no interest conflict.

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