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Studies on Diversity of Cellulolytic fungi in Gorakhpur, with special reference to cellulase activity of most Frequent fungi

Shailendra Yadav, Supriya Pandey and *Neeraj Srivastava

Applied Mycology Lab, Department of Botany,
St. Andrew's Post-Graduate College,
GORAKHPUR – 273001 (UP), INDIA
*Corresponding Author
E-mail : mycology1999@gmail.com

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ABSTRACT

Microorganisms, especially fungi and bacteria cause biodeterioration of our cultural heritage including paper, cloths, wood and leather. Cellulolytic Fungi cause decomposition of papers and cloths present as waste material. In the present investigation, fungi invading books of Rajkiya Jila Pustkalaya (Govt. District Library), Gorakhpur and Central Library of St. Andrew's College, Gorakhpur have been collected, cultured and identified. Isolations from a total number of eight samples and four isolates from each sample have been done. Fifteen species of these cellulolytic fungi belonging to seven genera have been reported. The two most abundant species found are *Aspergillus niger and A. flavus,* which have been isolated from all the 4 isolates of 8 samples. These two species have been selected for detailed studies of their cellulolytic activities. Enzymatic Index (EI) of these two species have been calculated using CMC Agar Medium and Gram Iodine Solution as indicator. EI of *Aspergillus niger* is reported to be 2.50 and of *A. flavus* as 1.3125.

Figures : 07 References : 31 Table : 01

KEY WORDS : *Aspergillus*, Cellulase activity, Cellulolytic fungi, Enzymatic index (EI), Government Library, St. Andrew's College Library.

Introduction

The humid tropical and sub-tropical countries are facing problem of biodegradation of important commodities as waste material. Our cultural heritages are made up mainly of paper, cloth, wood and leather. The hot and humid climate supports growth of microbes including cellulolytic fungi. Movable and immovable, both objects of our cultural heritage are destroyed by these microbes, including fungi and bacteria. Fungi have been reported as the most active microbes and cause maximum damage to these objects[16]. Fungi degrading objects containing cellulose, such as papers and cloths, are known as Cellulolytic Fungi. They produce cellulase enzyme and cause biodeterioration of objects in our cultural heritage, libraries *etc*.[18, 30]. High relative humidity and moderate temperature and light intensity of July to October months support abundant growth and decomposition action of these fungi. Cellulolytic fungi like *Alternaria, Aspergillus*, *Chaetomium*, *Cladosporium*, *Penicillium*, and *Trichoderma etc.* have been reported to cause decomposition of antique documents[10, 14, 20, 21, 29].

Fungal biodeterioration of properties made of papers and archival materials is very high in India[2]. Decomposition of papers have been reported to be caused by a large number of cellulolytic fungi[5]. Infestation of these objects containing cellulose has been reported to be caused by Xerophilic fungi even under very low

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TABLE– 1 : Frequencies of Cellulolytic Fungi ilsolated from 8 Samples of Papers

S. No.	Cellulolytic Fungi	* Frequencies			
		Isolation 1	Isolation 2	Isolation 3	Isolation 4
1.	*Alternaria alternata*	√	√√	x	x
2.	*Aspergillus nidulans*	x	√√	√√	x
3.	*A. flavus*	√√√√	√√√	√√√	√√√
4.	*A. fumigatus*	√	√√	x	√√
5.	*A. terreus*	√	√√	x	x
6.	*A. ochraceous*	√	√	x	x
7.	*A. niger*	√√√	√√√	√√√	√√√
8.	*A. versicolor*	x	x	x	√
9.	*Chaetomium globosum*	√√	√√√	√√	√√√
10.	*Cladosporium cladosporioides*	√√	√	x	√
11.	*C. herbarum*	√	√√	√	x
12.	*Curvularia lunata*	√√	√√√	√√	√√√
13.	*Helminthosporium sp.*	x	√√	x	x
14.	*Penicillum chrysogenum*	√√	x	√	√
15.	*P. citrinum*	√	x	√	√

*** x = Absent (No fungal Species), √= Mild FungalGrowth, √√=Moderate Fungal Growth, √√√=Rich FungalGrowth**

Fig. 1 : Collection of Cellulolytic Fungi from infested papers

humid conditions. In earlier reports also, rich fungal diversity has been found in library books in Gorakhpur[27, 28].

These cellulolytic fungi produce cellulase enzymes to cause biodeterioration of objects like papers and textiles containing cellulose. Nine fungal species have been reported to cause biodegradation of Nineteenth -Century Islamic and Koranic books, with dominance of *Aspergillus niger*, *A. oryzae* and *Hypocrea lixii*. These fungal species showed their ability to degrade Carboxy Methyl Cellulose (CMC)[13]. Workers[19,21] described four species of these fungi, named *Aspergillus versicolor*, *A. nidulans*, *A. terreus* and *Chaetomium globosum* causing damage of old documents. The cellulase producing ability of these fungi has been exploited for treatment of agro-industrial residues[4, 6]and production of cellulase enzymes[9]. Similarly, exploitation of micro-organisms/their enzymes for degradation of lignocellulosic wastes is the need of time[20]. Protection and preservation of ancient documents from biodegradation is a big challenge[26]. However, isolation of these fungi causing biodeterioration and their clear identity is the first step in this direction. Only then their cellulolytic activities can be determined using the parameter of Enzymatic Index (EI), and proper and effective control measures can be proposed.

Keeping these facts in view, the present work was undertaken and samples of papers infested with fungi have been investigated and fungal samples have been collected. Various species of cellulolytic fungi have been identified and cellulolytic activities of the most frequent species have been calculated by calculating their Enzymatic Index (EI).

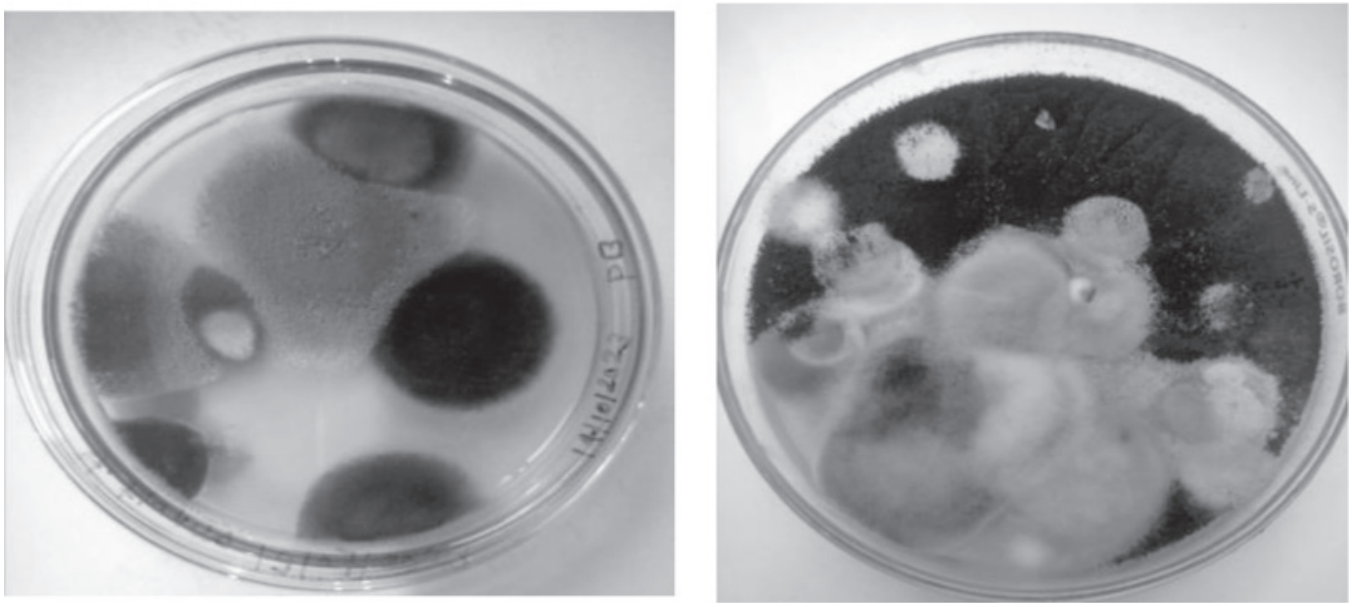


Fig. 2 : Mixed culture obtained from collected samples

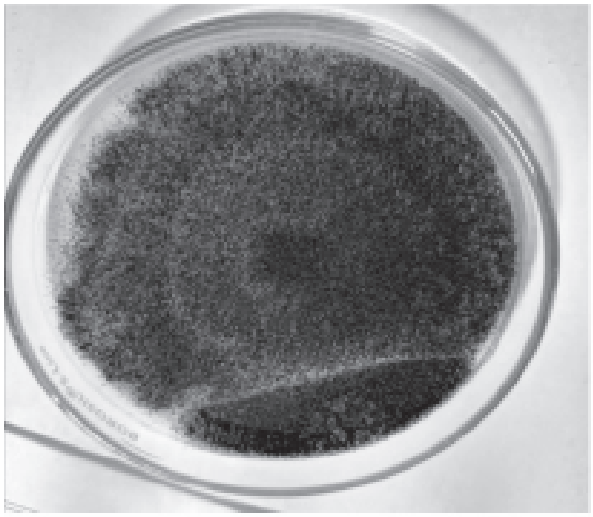


Fig. 3 (A) : *Aspergillus niger* (Accession No. NFCCI 5688)

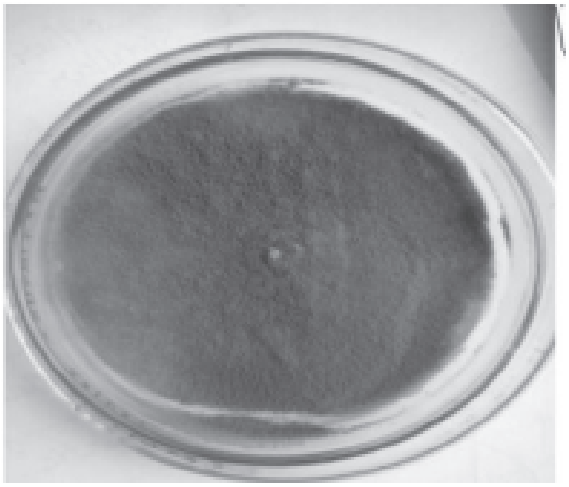


Fig. 3 (B) *: Aspergillus flavus* (Accession No. NFCCI 5710) (Pure Culture/ITCC No. 8691)

Pure cultures obtained from mixed culture / ITCC, IARI, New Delhi

Materials and Methods

(1). **Collection and Identification of Cellulolytic Fungi**

Eight samples (with four isolations from each sample) of cellulolytic fungi were collected from deteriorated Glazed and Unglazed papers in the areas under investigations *i.e*., Rajkiya Jila Pustkalay (Government District Library), Gorakhpur and Central Library of St. Andrew's Post-Graduate College, Gorakhpur, using sterilized inoculation needle and cotton swab. These fungi were mounted in Lactophenol-Cotton Blue and were examined by direct observation under compound microscope. *In - vitro* culture of these fungi was done on PDA medium. The mixed culture so obtained was purified by streaking and fungi were identified by observing under light microscope and using various taxonomic criteria. The literatures were used for identification[3,4,8,11,15,17,23].

(2). Screening of Cellulolytic Activity

Of all the isolated cellulolytic fungi, the two most frequently occurring fungi were *Aspergillus niger* and *A. flavus*. Accession Numbers of these two fungal species were obtained from National Fungal Culture Collection of India(NFCCI), Pune, Maharashtra (*A. niger* : NFCCI5688; *A. flavus* : NFCCI 5710). The pure culture of *A. flavus* was also obtained from Indian Type Culture Collection (ITCC), IARI, New Delhi (ITCC No. 8691).

These two *Aspergillus* species producing Cellulase Enzyme were selected for screening of their cellulolytic activities, which was done using 1% Carboxy Methyl Cellulose (CMC) Agar Medium. This Carboxy

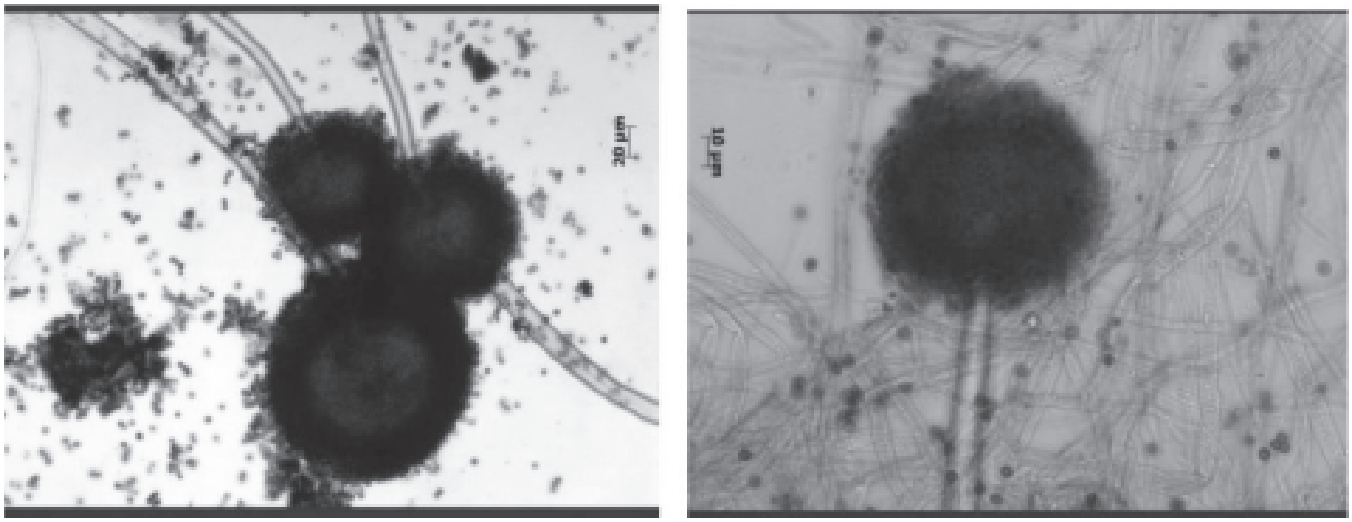
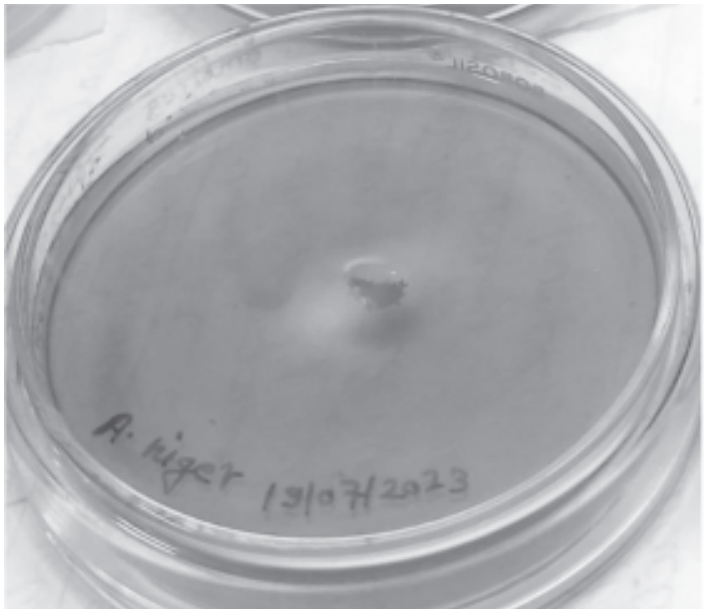
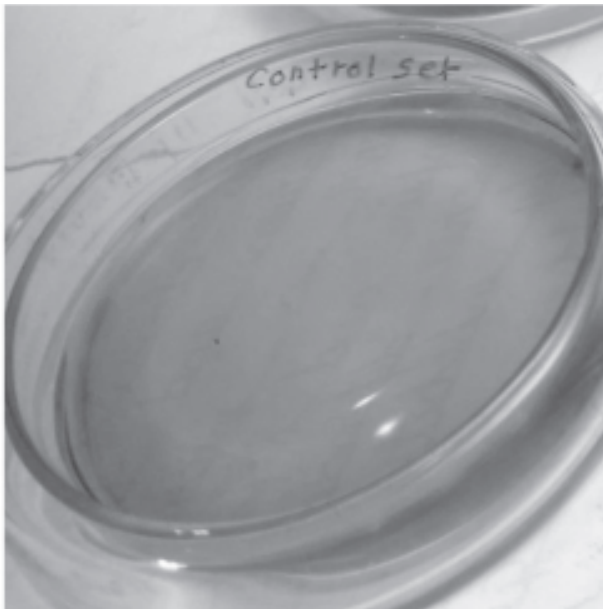


Fig. 4 : *Aspergillus niger* (Mycelium, Conidiophore and Conidia)





Treatment Set (Inoculated) **Control Set (Uninoculated)**

Fig. 5 : Culture of *Aspergillus niger* on 1% CMC Agar Medium (Flooded with Gram's Iodine Solution)

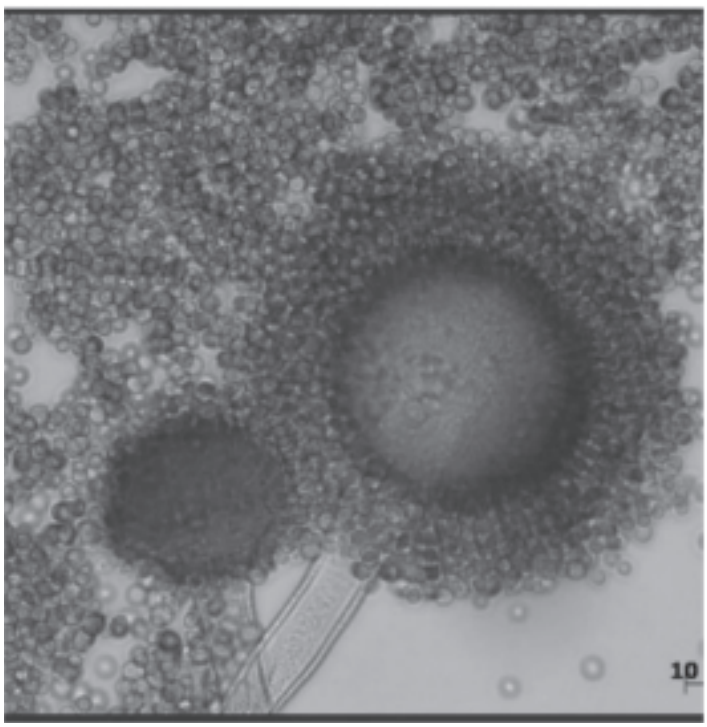
Enzymatic Index (EI) of *Aspergillus niger*

Hydrolysis Zone = 25 mm. Diameter Colony = 10 mm. Diameter

Enzymatic Index of *Aspergillus niger* = 25/10 = 2.50

Methyl Cellulose was added as source of Carbon and indicator used was Gram's Iodine Solution[10].

The theory behind this qualitative determination of cellulolytic activities is that, the Gram's Iodine reacts with both, the cellulose and its degraded components. The integral and unhydrolyzed Cellulose holds the colour of Gram's Iodine dye, whereas the cellulose hydrolyzed into its components by fungal enzymes shows clear zone or pale hallow zone. This clear/pale halo zone was



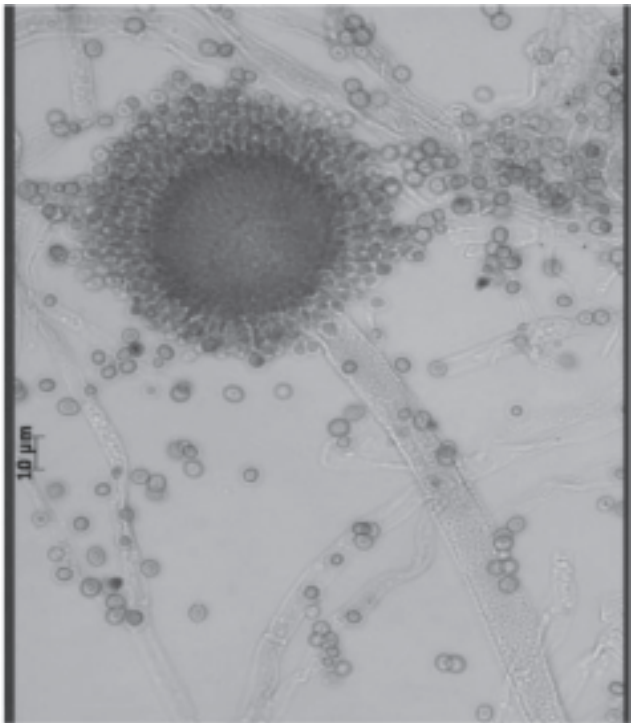
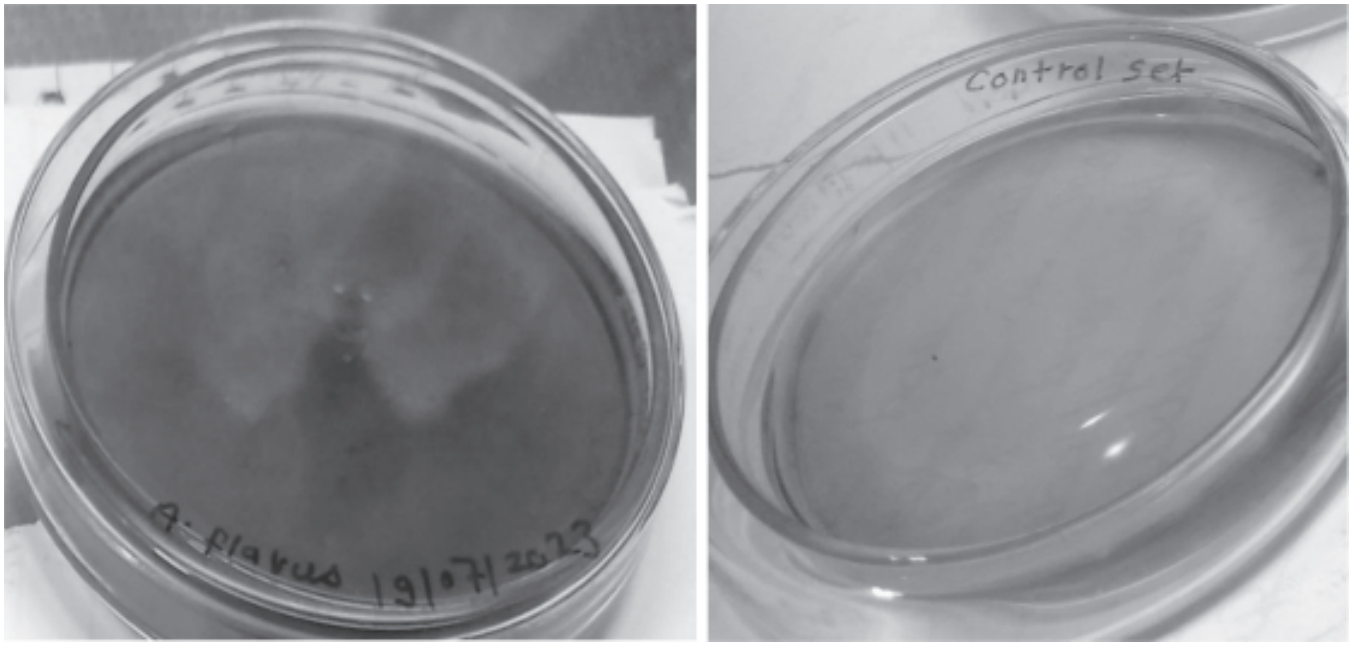


Fig. 6 : *Aspergillus flavus* (Mycelium, Conidiophore and Conidia)



Treatment Set (Inoculated) **Control Set (Uninoculated)**

Fig. 7 : Culture of *Aspergillus flavus* on 1% CMC Agar Medium (Flooded with Gram's Iodine Solution)

Enzymatic Index (EI) of *Aspergillus flavus*

Hydrolysis Zone = 21 mm. Diameter Colony = 16 mm. Diameter

Enzymatic Index of *Aspergillus flavus*= 21/16 = 1.3125

measured and Enzymatic Index (EI) was calculated using the following formula:

$$EI = \frac{\text{Eiameter of Hydrolysis Zone}}{\text{Diameter of Colony}}$$

The fungal discs of 4 mm diameter were cultured on 1% CMC Agar Medium containing Streptomycin and were incubated for 7 days at 30 ± 2°C temperature. After 7 days incubation period, these cultures were treated with Gram's Iodine indicator. Before observation, these cultures were washed with water. The clear zone around the fungal growth was measured and photographs were taken for reporting.

The experiments were repeated twice with one fungal culture used as control set[10].

Observations

Fifteen species of cellulolytic fungi were isolated from infested glazed and unglazed papers (Table – 1).

Result and Discussion

Fifteen species of cellulolytic fungi have been isolated from 8 samples of deteriorated papers (Table -1). *Aspergillus niger* and *Aspergillus flavus* have been isolated from all the 8 samples and 4 isolates, and therefore, have been reported as the most frequently occurring species. *Chaetomium globosum* and *Curvularia lunata,* show moderate growth and others show mild to moderate growth.

Enzymatic Index (EI) of *Aspergillus niger* is 2.50 and of *Aspergillus flavus* is 1.3125.

These cellulolytic fungi causing biodegradation of papers mostly belong to Fungi Imperfecti of Division – Deuteromycotina. Growth of these fungi is supported by high relative humidity and moderate temperature and light intensity. Also, these fungi show great adaptability under changing environment and can survive under unfavorable environmental conditions.

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