Plant Archives Volume 20 No. 2, 2020 pp. 7013-7020 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ISOLATION AND SCREENING OF CELLULOLYTIC FUNGI FROM DEGRADING LEAF LITTER OF *SACCHARUM OFFICINARUM* L.

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Abstract

Cellulases are the group of hydrolytic enzyme capable to hydrolyzing the most abundant lignocellulosic biomass to smaller sugar components. The present study has been carried out to isolate and screening of cellulolytic fungi from degrading leaf litter of *Saccharum officinarum* L. collected from Sonagir area of Datia district M.P. For this study total 47 fungal species have been isolated and identified. Identified genera have been classified according to their classes. Two genera belongs to class zygomycetes e.g. *Mucor* and *Rhizopus,* nine genera *i.e. Ceratocystis, Chaetomium, Cochliobolus*, *Corynascus, Emericella, Emericellopsis, Eurotium*, *Neosartorya* and *Sordaria* are belongs to class ascomycetes, two genera *i.e. Rhodotorula* and *Trichosporon* belongs to class basidiomycetes and remaining thirteen genera *e.g. Acremonium, Alternaria*, *Aspergillus*, *Candida*, *Cladosporium, Colletotrichum*, *Curvularia*, *Fusarium, Myrothecium, Paecilomyces, Penicillium, Phoma* and *Trichoderma* are members of class deuteromycetes. Out of which 28 species showed cellulolytic activity.

Key words: Cellulase, Cellulolytic fungi, leaf litter, *Saccharum officinarum* L.

Introduction

Cellulose is the primary structure of the plant cell wall. It is a linear, unbranched homopolymer of glucose subunits linked with β 1, 4 glycosidic bonds (Rathore *et al.,* 2014). It is also one of the most important carbon sources on the earth. So its degradation and subsequent utilization are important for global carbon source (Lekhram *et al.,* 2014). Degradation of these cellulosic materials is achieved by many chemical as well as enzymatic or by the combination of both chemical and enzymatic process (Bailey and Poutanen 1987, Christov *et al.,* 1999, Xia and Cen 1999 and Reddy *et al.,* 2014).

Cellulase is an extra cellular enzyme produced by many microorganisms, brings hydrolysis of cellulose by synergistic action of its constituent enzymes (Bhat and Bhat 1997). Cellulases are the group of extracellular enzymes; Klysove in 1990 described 3 major types of cellulases first is endoglucanase (Endo 1, 4 β glucanases), which break down the internal bond to disrupt the crystalline structure of cellulase polysaccharides chain, second is cellobiohydrolase (Exo-cellulase) which cleave 2-4 units from the ends of the exposed chains produce by endocellulases and third is cellobiase (β-galactosidase) hydrolyse the cellobiose in to individual monosaccharides such as glucose. A complete hydrolysis of crystalline cellulose involves synergistic action of these cellulolytic enzymes (Lynd *et al.,* 2002 and Sari *et al.,* 2016).

Cellulases are the most important commercial enzyme used in many industries such as biofuel, food, feed, beverages, paper, textile, pharmacutical, agriculture and so on. These industries it used alone or in the combination with other enzymes (Kuhad *et al.,* 2011) so the isolation and identification of cellulolytic microbes provide a great starting point for the discovery of such beneficial enzymes. There for a lot of researches are going on to discover new microorganism which can produce cellulase with higher specific activity (Rathnam *et al.,* 2012).

Cellulases are produced by number of microorganisms including bacteria, fungi, actinomycetes etc. But among all fungi are main producer of extracellular cellulase enzyme. Some of the great advantages related to the enzyme production from the fungi are low cost material, with high productivity, faster production and easily recoverable from the culture media (Vishwanatha *et al.,* 2010, de Souza *et al.,* 2015 and Sari *et al.,* 2017).

So the main objective of the present study was isolation, identification and screening of cellulolytic fungi

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from the degrading leaf litter of *Saccharum officinarum* L. which is rich source of lignocellulosic material because these lignocellulolytic materials are good source for the discovery of cellulolytic fungi producing novel cellulases enzymes.

Material and method

Collection of material

Sonagir area of Datia district selected for the collection of litter sample because it is a major Sugarcane growing area. Collected sample was filled in nylon mesh bags. These bags placed into the pits for degradation process and from these degraded litter, isolation of fungi have been carried out.

Isolation of fungi

For the isolation of fungi from the litter, serial dilution agar plate (Apinis, 1963) and direct inoculation method were used (Warcup, 1950). For this purpose the suspension of litter was prepared with double distilled water and diluted up to $1:10^3$ solutions. 1.0 ml of each suspension was streaked over PDA (Potato Agar Media), supplemented with 0.1mg antibiotic (streptomycin) to avoid bacterial contamination. Inoculated Petri plates were incubated at 30ºC for fungal growth. Then each Petri plate was used for identification.

Identification of fungi

For the identification of isolated fungi Lacto phenol cotton blue stain was used. The identification process was based on analysis of morphological and microscopic characters of fungi by using taxonomic guide and tools. The morphological characteristics included growth and colour of colony, presence and absence of mycelia growth, spore pigmentations. For this purpose standard textbooks and photographs were also used (Nagmani, 2006, Gilman

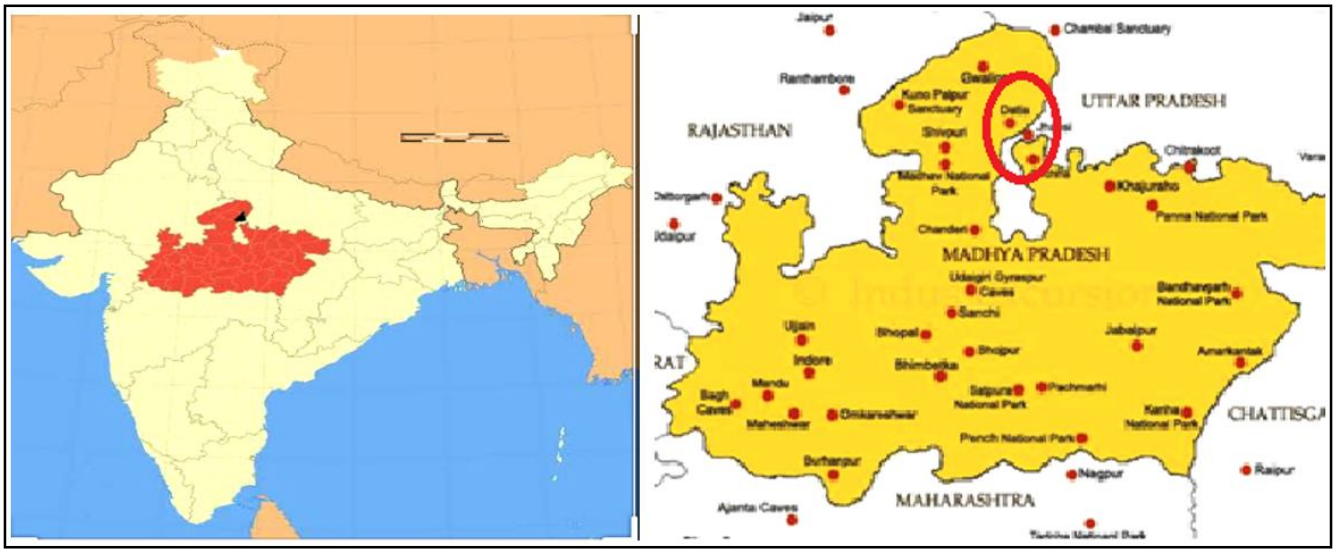


Fig. 1: Map showing study site *i.e.* Datia district comes under Madhya Pradesh state of India.

A **B**

Fig. 2: (A) Agriculture field of sugarcane crop. (B) Litter bags placed in the pits.

1957, Barnett and Hunter 1972).

Screening of Cellulolytic fungi

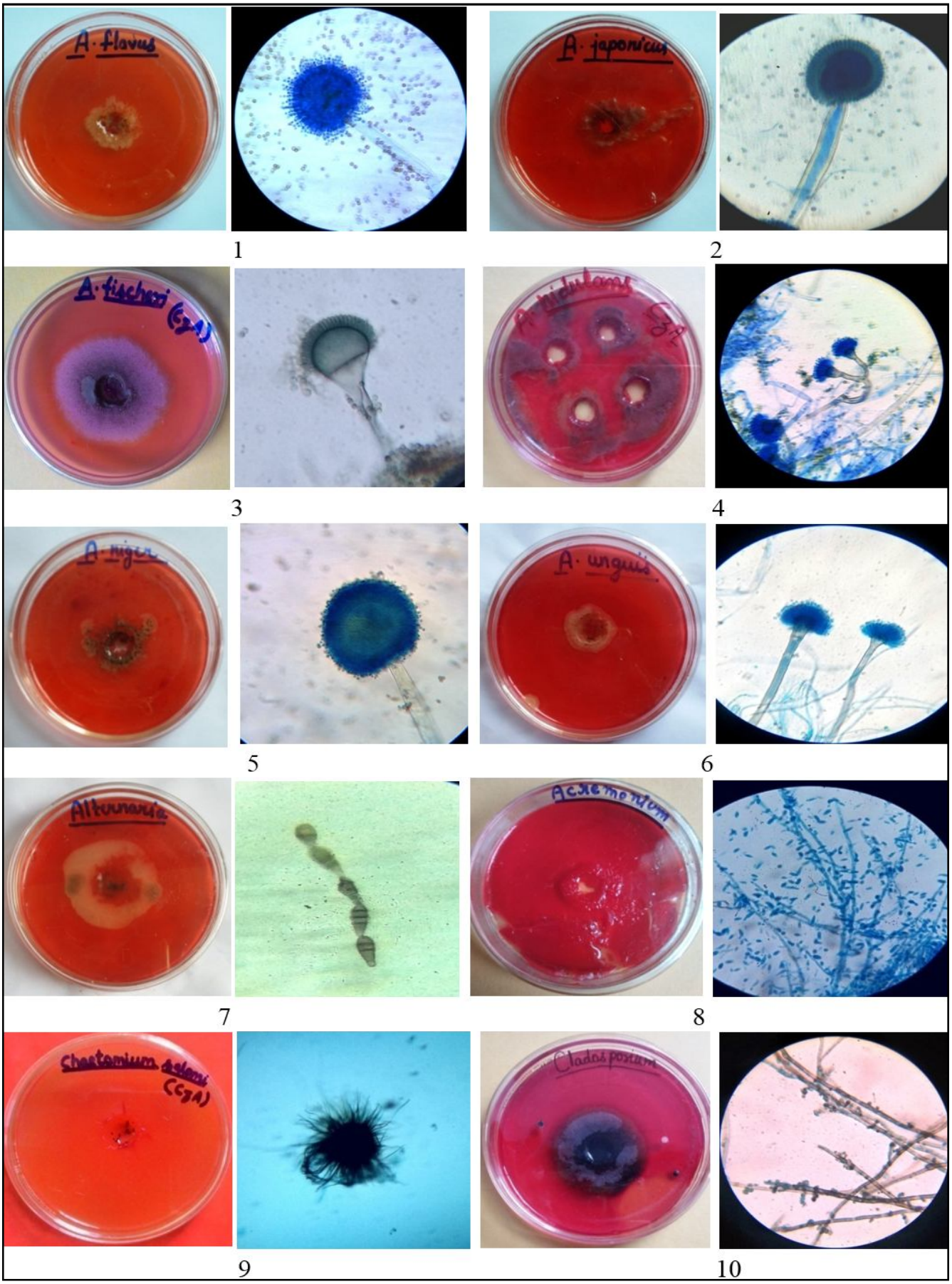
Isolated and identified fungal strains were used for the screening of cellulolytic fungi. This process was based on clear zone formation by identified fungal strains on carboxymethyl cellulose (CMC) media contains (g/l): $NaNO_3$ – 2, K_2HPO_4 – 1 $MgSO_4$ – 0.05, KCl– 0.5, $FeSO_4$ – 0.01, CMC – 1% Agar-agar – 2.0, pH of medium was adjusted to 5 (Lekhram *et al.,* 2014). Isolated fungal strains were inoculated in to the wells on CMC media and incubated at 30°C for 48 hours. To visualize the clear zone the plates were treated with 1% Congo red staining solution for 15 minutes and washed with 1M NaCl (Teather and wood 1982).

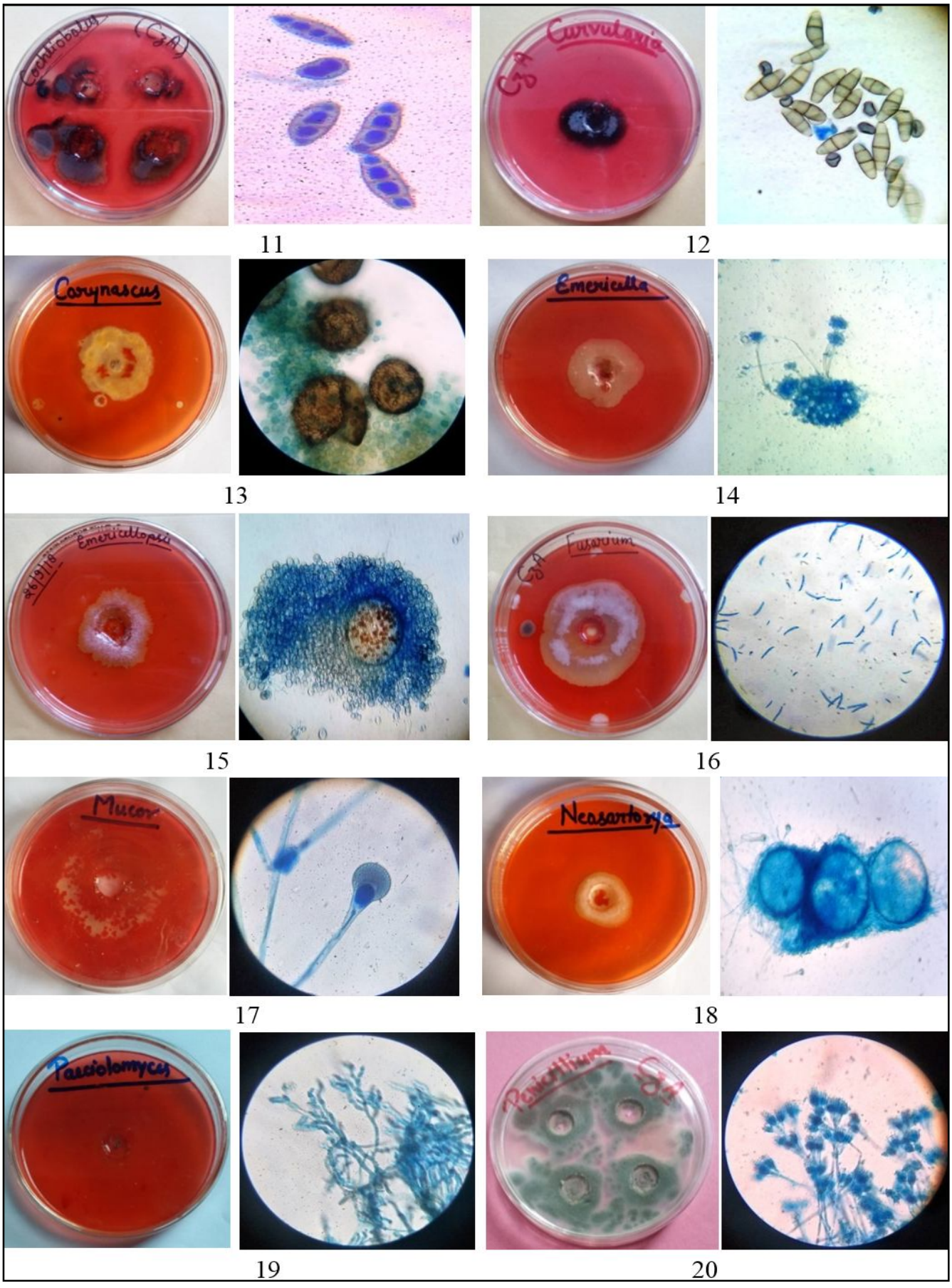
Results

During the decomposition of leaf litter of *Saccharum officinarum* total 47 species belonging to 26 genera of fungi have been successfully isolated and identified. The isolated fungal strains were identified on the basis of morphological as well as microscopic characteristics. In which 11 species of genus *Aspergillus*, 7 species of *Chaetomium,* 4 species of *Penicillium,* 2 species of *Cochliobolus* and *Fusarium* and single species of each genera of *Acremonium, candida, Ceratocystis, Cladosporium, Curvularia, Colletotrichum, Emericella, Emericellopsis, Eurotium, Mucor, Neosatorya, Pecilomyces, Phoma, Rhizopus, Rhodotorula, Trichoderma, Trichosporon* and *Sordaria* reported (Pandey *et al.,* 2020). The cellulolytic activities of these identified fungal strains were tested by clear zone formation around the colony growing on CMC media. The appearance of clear zone around the well is indicative of cellulolytic activity of test fungus. The zone appears due to hydrolysis of CMC i.e. source of cellulose. The results of which are present in table 1. Out of 46 fungal strains only 28 produce zone of hydrolysis on CMC agar media plate their name as *Aspergillus flavus, A. nidulans, A. Japonicas, A. Niger, A. fischeri, A. unguis, Alternaria* sp., *Acremonium* sp., *Chaetomium salami, Cladosporium* sp., *Cochliobolus* sp., *Corynascus* sp., *Curvularia* sp., *Emericella* sp., *Emericellopsis* sp., *Fusarium* sp., *Mucor* sp., *Neosartorya* spp., *Penicillium chrysogenum, P. degetatum, P.decumbens, P. herquei, Pecilomyces* sp., *Phoma sp, Rhizopus* sp., *Rhodotorula* sp., *Trichoderma* sp., *Trichosporon* sp., besides this out of 28 cellulolytic fungi 6 belongs to ascomycetes class, 18 belongs to deuteromycetes class and 2 belongs to zygomycetes and 2 belongs basidiomycetes class. Results shows that fungi belonging to class ascomycetes and deuteromycetes have greater cellulolytic activity as

Table 1: Name of fungal strains which shows the Cellulolytic activity. Here + sign indicate the presence of cellulolytic activity while – sign indicate the absence of cellulolytic activity.

Name of the fungal strains	Presence of cellulolytic activity
Aspergillus flavus	+
A. flavipes	-
A. candidus	-
A. nidulans	+
A. niger	+
A. japonicus	+
A. stellatus	-
A. fumigatus	-
A. tamarii	-
A. fishcherei	+
A. unguis	+
Acremonium implicatum	+
Alternaria alternata	+
Alternaria longipes	-
Candida albicans	-
Chaetomium spirale	-
C. osmanae	-
C. fumicola	-
C. salami	+
C. aureum	-
C. molisanum	-
Colletotrichum capsici	-
Corynacus	-
Curvularia lunata	+
Cladosporium	+
Ceratocystis	-
Cochliobolus	+
Cochliobolus tuberculata	-
Emericella nidulans	+
Emericellopsis	+
Eurotium	-
Fusarium	+
Fusarium endophthalamitis	-
Mucor	+
Myrothecium roridum	-
Neosartorya	+
Penicillium chrysogenum	+
P. digitatum	+
P. decumbens	+
P. herquii	+
Paecilomyces	+
Phoma	+
Rhizopus	+
Rhodotorula	+
Sordaria	-
Trichoderma	+
Trichosporon	+


A. flavus
A. japonicus
A. fischeri (CCFA)
A. nidulans
A. niger
A. unguis
Alternaria
Acremonium
Chaetomium globosum (CCFA)
Cladosporium
1
2
3
4
5
6
7
8
9
10


Cochliobolus (CzA)
11
Curvularia
12
Corynascus
13
Emericella
14
Emericellopsis
15
Fusarium
16
Mucor
17
Neosartorya
18
Paeciolomyces
19
Penicillium
20

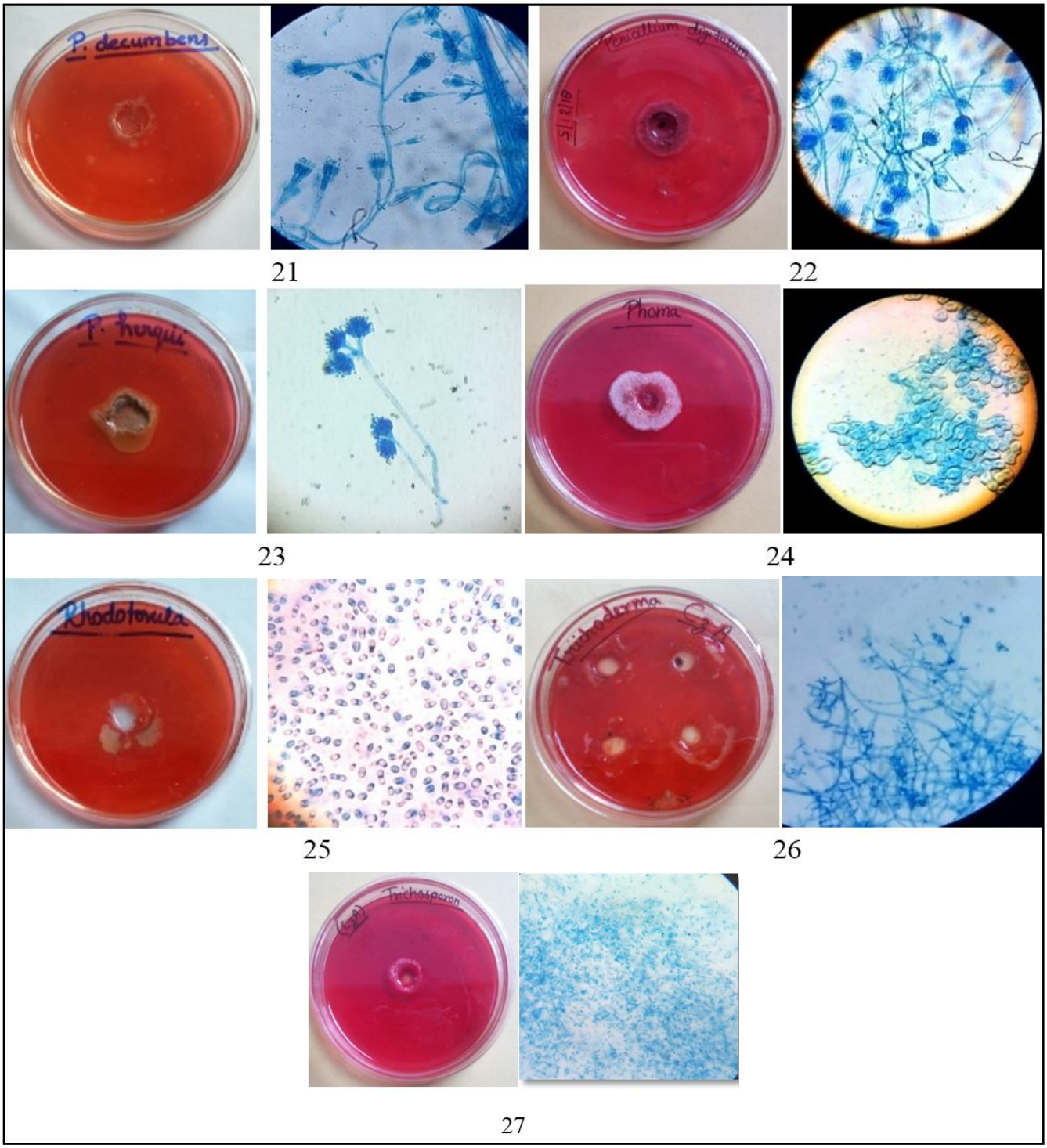


Fig. 1: Isolated and identified fungal colonies growing on carboxymethyl cellulose (CMC) media. Showing cellulase activity, microscopic view of the fungus has also been given just after Petri plates. **1**. *Aspergillus flavus* **2**. *A. niger* **3.** *A. nidulens* **4.** *A. fishcherei* **5.** *A. japunicus* **6** *A. unguis*. **7.** *Alternaria* **8.** *Acremonium* **9.** *C. salami* **10**. *Corynascus* **11.** *Cladosporium* **12.***Curvularia* **13.** *Cochliobolus* **14.** *Emericella* **15.** *Emericellopsis* **16.** *Fusarium* **17.** *Mucor* **18.** *Neosartorya* **19.** *Paecilomyces* **20.** *Penicillium chrysogenum* **21.** *P. decumbens* **22.** *P. digitatum* **23.** *P. herquii* **24.** *Phoma* **25.***Rhizopus* **26.** *Rhodotorula* **27.** *Trichoderma* **28.** *Trichosporon.*

compare to bsidiomycetes and zygomycetes.

Discussion

Cellulose is the world's most abundant organic substance (Rutloff, 1987) and a large proportion of agricultural waste added to the soil is cellulose therefore its decomposition has a special significance in the biological cycle of carbon (Lederberg, 1992). Fungi are

well known agent of decomposition of organic matter in general and of cellulosic substrate in particular (Lynd *et al.,* 2002). These fungi produce a wide range of extra cellular enzyme to degrade the lignocellulosic material. Among the various category of enzyme cellulose is one of the key enzymes for the degradation of lignocellulosic material.

The result of this study shows that the leaf litter of *Saccharum officinarum* is a good source of cellulosic fungi for cellulase production because it contains high sugar as well as lignocelluloses percent as important food substrate for their growth. Many other studies also mentioned that lignocellulosic materials are good source for the growth of high variety of cellulolytic fungi. These cellulolytic fungi produce cellulase enzyme to hydrolyze cellulose which is the main component of plant cell wall (Sari *et al.,* 2017). Besides this bacteria are also known to produce cellulase but only fungi can produce cellulase complex which can completely degrades the lignocellulosic materials without pre-treatment (Amore and Faraco 2012).

During the study total 46 fungal strains were isolate and identified but only 28 showed cellulolytic activities. Besides this various species of *Aspergillus* and *Penicillium,* single species of *Trichoderma* and *Fusarium* were notice to show maximum zone of hydrolysis on CMC. So on the basis of clear zone dimension form by fungi on CMC media, it is suggested that *Aspergillus*, *Penicillium, Trichoderma* and *Fusarium* genera were found to have relatively high cellulolytic activity and *Alternaria, Cladosporium, Curvularia, Emericella, Emericellopsis, Mucor, Neosartorya, Phoma, Rhizopus,* in moderate range while *Candida,, Chaetomium, Cochliobolus, Corynascus, Rhodotorula, Trichosporon* showed low cellulase activity. Same results that *Aspergillus, Penicillium, Fusarium, Trichoderma* are the most potential cellulase producer were also reported by Yapani in 1987.

Various species of *Aspergillus* fungi produce wide range of enzyme capable of degrading lignocellulosic material. Even de Vries and Visser in 2001 concluded from their work that a wide range of *Aspergillus* species have possess all the component of cellulase enzyme complex which is also a agreement with the present study.

Similarly *Penicillium* sp. and *Trichoderma* sp. are also known to have most effective cellulase producer for the lignocellulosic material. Andersen *et al.,* 2016 reported in their work that *Penicillium* spp. are good candidates for over production of enzyme in order to supply industrial enzyme or also help in bioconversion of lignocellulosic rich biomass. But in the case of *Trichoderma* it is most expensively studied because their growth was suppressed by other rapidly growing fungi (Jahangeer, 2005).

A part from this some other fungal strains such as *Fusarium, Cladosporium, Chaetomium a*nd *Alternaria* are also reported as efficient cellulolytic fungi. Similar finding were also reported by Sajith in 2016 and Lal & Mishra in 1978.

While *Candida*, *Cochliobolus*, *Corynascus*, *Trichosporon* are the group of fungi which can grow on lignocellulosic substrate but the ability to produce lignocelluloses degrading enzymes has not been much reported. So this research showed that these isolates can further be explored as a new cellulase producer.

Conclusion

This research succeeded in screening of cellulolytic fungi from the leaf litter of *Saccharum officinarum* during degradation process. *Saccharum officinarum i.e.* Sugarcane is widely cultivated sugar rich crop in India. This plant produce huge amount of lignocelluloses biomass as agricultural waste material during its cultivation. Isolated cellulolytic fungal strains from the degrading litter of *Saccharum officinarum* have potential application as a source of inoculums for degradation process so that it can be utilized to increase the rate of decomposition or in many other lignocellulosic conversion processes for biotechnological process in various industries.

Acknowledgement

Authors are thankful to Principal, Bipin Bihari College, Jhansi, U.P. for providing required laboratory facilities and also for his kind support for this work. We are grateful to owner of sugarcane fields in Sonagir, without whose support, this work was impossible. A humble and heart full thanks to Almighty.

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