"Diversity assessment of Cellulase production fungus from different soil type of Chhindwara district"

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Abstract:

Modern molecular biology technique is leading researches towards developing microorganisms which can produce a greater number of and more efficient cellulases, the tradition microbiology technique of isolation still plays an important role. New cellulase-degrading strain may represent a good host or framework to further improve or add additional enzyme genes for further improvement. Similarly, a cellulase produced by an isolate may be more efficient and may be worth cloning and introducing to an already good industrial cellulase producer to further improve its cellulose-degrading repertoire. Therefore, there has been much research aimed at obtaining new microorganisms producing cellulase enzyme with higher specific activities and greater efficiency. Our study carried out in 9 places with different soil types of Chhindwara district. Screening performed by using CMC agar plate assey method 33 fungal isolated that out of 28 fungal isolates were cellulase producer. However the isolated percentage Showed that *Aspergillus* sp.(51.51%),*Penicillium* sp.(12.12%), *Fusarium s*p.(12.12%), *Mucor* sp.(9.09) and *Pythium* sp.(6.06) and lowest percentage showed *Geotricum* sp.(3.03),*Rhizopus sp*.(3.03**).** The screenings of cellulase producing fungi were performed on CMC agar plates flooded with Congo red and washed with NaCl. depending on the diameter of clear zone around the colony. The 28 Cellulolytic fungal isolated from different soil and different area. Mostly fungus are isolated from Shikarpus and parasia. The appearance of the clear zone around the colony after the addition of Congo red solution was strong evidence that the fungi produced cellulase in order to degrade cellulose . Among the 28 fungus isolates, 7 isolate have showed the maximum zone of clearance range (1.03-3.66mm)**.**

In this study, efficient cellulose producing microorganisms were isolated from different natural sources like compost soil, decayed wood and lignocellulosic waste. The purpose was to identify and characterize those isolates displaying the greatest cellulase activity for the possible use in large scale bio refining.

Keywords: Cellulase, Aspergillus, lignocellulosic.

Introduction:

Cellulose, hemicelluloses and lignin are the major structural component of woody plants and non-woody plants such as grass and represent a major source of renewable organic matter. The total amount of cellulose on earth has been estimated at 7×10^{11} tons (Nayebyazdi *et al.* 2012). Cellulose is considered as one of the most important sources of carbon in this planet and its annual biosynthesis by both land plants and marine algae occurs at a rate of 0.85×10^{11} tone per annum (Kumari *et al.*, 2011). Cellulose is polysaccharide with the formula $(C_6H_5O_{10})$ n, where ranges from 500 to 5000, depending on the source of the polymer, consisting of a linear chain of several hundred to over ten thousand β (1→4) linked D-glucose unit (Nayebyazdi *et al.* 2012). Cellulose is a natural substance that forms the cell walls of all plants and trees. It makes up 45% of palm trunk, 47% of fronds, and 41% of on dry basis (Abdullah *et al.*, 2011). Cellulase is a hydrolytic enzyme which breaks down cellulose into smaller oligosaccharides and glucose. Cellulose constitutes the largest supply of biomass material and 20-45% of cellulose is present in plant tissue in dry weight.

Fungal cellulases are inducible enzymes that are usually excreted into the environment and this depends on the type of cellulose (amorphous or crystalline) being acted upon. The role of the fungi such as *Acremonium* sp., *Chaetomium s*p., *Trichoderma reesei, Trichoderma viride, Penicillium pinophilum, Phanerochaete chrysosporium (Sporotrichum pulvenlentum), Fusarium solani, Talaromyces emersonii, Trichoderma koningi, Fusarium oxysporium, Aspregillus niger and Rhizopus oryzae* in the cellulose

degradation process in various environment has been well documented (Mukut and Godiya 2010). Many fungi capable of degrading cellulose synthesize large quantities of extracellular cellulases that are more efficient in depolymerising the cellulose substrate. Most commonly studied cellulolytic organisms include fungal sp*: Trichoderma, Humicola, Penicillium* and *Aspergillus*. Among *Trichoderma* spp., *T. harzianum* and *T. koningii* have been studied. Already an impressive collection of more than 14,000 fungi which were active against cellulose and other insoluble fibers were collected (Gautam *et al*. 2011).Cellulose degrading fungal pathogens play an important role in the biosphere by recycling cellulose mediated by cellulose enzyme and common in field such as forest soils, in manure and on decaying plant tissues.

Material and Method:

Table 1- Collection of the soil sample from chhindwara districts.

S.No.	District	Places	Soil sample collection
1.	Chhindwara	Khajeri	
2.		Pertala	
3.		Shikarpur	
4.		Rajpalchock	
5.		Umaria	
6.		Ghatparasia	
7.		Khirsadoh	
8.		Parasia	
9.		Navegao	

Result and Discussion:

Table-2 Physico -chemical properties of soil samples.

Location	**Soil types**	**pH**	**EC**	**C-organic**	**N**	**P**	**K**
Khajeri	Forest	6.3	.14	1.0	325	22.95	340
Pertala	Forest	7.4	.25	0.5	400	9.6	210
Shikarpur	Forest	7.7	.16	1.2	290	17.44	370
Rajpalchock	Normal	7.7	.31	0.8	445	20.44	250
Umaria	Forest	7.8	.41	1.09	325	14.78	210
Ghatparasia	Forest	6.4	.18	1.5	275	9.6	350
Khirsadoh	Forest	6.8	.46	0.8	350	4.7	445
Parasia	Wheat	7.5	.24	0.8	350	9.6	300
Navegao	Sugar	6.7	.17	1.3	275	2.33	250

Table-3 Enumeration of fungi in cfu/gm in different places of soil.

S. No.	Place	Dilution	Concentration of fungi cfu/gm
1.	Khajeri	10^{-1}	13×10^{1}
		10^{-2}	9×10^{2}
2.	partala	10^{-1}	23×10^{1}
		10^{-2}	10×10^{2}
3.	shikarpur	10^{-1}	10×10^{1}
		10^{-2}	6×10^{2}
4.	Rajpalchock	10^{-1}	15×10^{1}
		10^{-2}	13×10^{2}
5.	Umaria	10^{-1}	17×10^{1}
		10^{-2}	9×10^{2}
6.	Ghatparasia	10^{-1}	10×10^{1}
		10^{-2}	10×10^{2}
7.	khirsadoh	10^{-1}	10×10^{1}
		10^{-2}	7×10^{2}
8.	parasia	10^{-1}	25×10^{1}
		10^{-2}	13×10^{2}
9.	Navegao	10^{-1}	3×10^{1}
		10^{-2}	3×10^{2}

Table-4 Total percent occurrence (frequency %) of mycoflora from different Places of soil

S.No.	place	Dilution	No.of colonies	Frequency (%)
1.	Khajri	10^{-1}	6.5	6.28
		10^{-2}	4.5	4.34
2.	partala	10^{-1}	11.5	11.11
		10^{-2}	5	4.83
3.	Shikarpur	10^{-1}	5	4.83
		10^{-2}	3	2.89
4.	Rajpalchock	10^{-1}	7.5	7.24
		10^{-2}	6.5	6.28
5.	umaria	10^{-1}	8.5	8.21
		10^{-2}	4.5	4.34
6.	Ghatparasia	10^{-1}	5	4.83
		10^{-2}	4.5	4.34
7.	khirsadoh	10^{-1}	6	5.79
		10^{-2}	3.5	3.38
8.	parasia	10^{-1}	12.5	12.07
		10^{-2}	6.5	6.28
9.	Nawegao	10^{-1}	1.5	1.44
		10^{-2}	1.5	1.44

Table- 5 Percentage of Isolated Fungi

S.No	Number of fungus	Name of isolates	Percentage (%)

1.	*Aspergillus sp.*	17	51.51
2.	*Mucor sp.*	3	9.09
3.	*Penicillium sp.*	4	12.12
4.	*Epicoccum sp.*	1	3.03
5.	*Fusarium sp.*	4	12.12
6.	*Geotricum sp.*	1	3.03
7.	*Rhizopus sp.*	1	3.03
8.	*Pythium sp.*	2	6.06

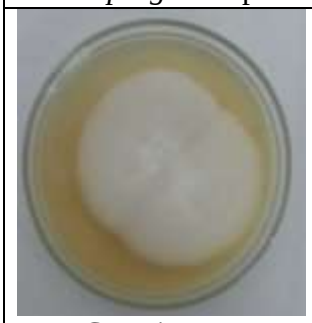
Images of Isolated Fungi


Aspergillus sp.


Penicillium sp.


Fusarium sp.


Pythium sp.


Geotricum sp.


Rhizopus sp.


Mucor sp.


Epicoccum sp.

Table-6 isolates the cellulolytic fungi from different types of soil.

S.No.	Type of soil	No. of cellulolytic fungal isolate
1.	Forest soil	18
2.	Normal soil	3
3.	Wheat soil	6
4.	Sugarcane	1

Table-7 Cellulolytic fungus isolated from different area of chhindwara.

S. No.	Isolated genera	Soil sample								
		Khajeri	Pertala	Shikarpur	Ghatparasia	Umaria	Khirshadoh	Parasia	Navegao	Rajpalchock
1.	*Aspegillus* sp.	+	-	+	+	-	+	-	+	+
2.	*Geotricum* sp.	-	-	-	-	-	-	+	-	-

3.	*Penicilliium* sp.	-	-	+	+	-	-	+	-	-
4.	*Epicoccum* sp.	-	-	+	-	-	-	-	-	-
5.	*Mucor* sp.	-	+	-	-	-	-	-	-	-
6.	*Rhizopus* sp.	-	-	-	-	+	-	-	-	-
7.	*Fusarium* sp.	-	-	-	-	-	-	+	-	-
8.	*Pythium* sp.	-	-	+	-	-	-	+	-	-

(-):negative,(+):Positive

Table-8. Biometric feature of cellulolutic fungal culture and enzyme activity.

S. No.	**Place**	**Name of fungus**	**Colony color**	**Colony diameter (range in mm)**	**Zone of hydrolysis(range in mm)**	**Relative zone of cellulolytic hydrolysis (range in mm)**
1.	Khajeri	*Aspergillus* sp.	Blackish white	27-55	37-72	1.15-1.37
2.	Pertala	*Mucor* sp.	white	47	58	1.23
3.	Shikarpur	*Aspergillus* sp.	Greenish white and powdery	8-10	15-30	1.5-3
		Penicillium sp.	Greenish powdery	9-14	14-28	1.55-2
		Epicoccum sp.	Orange white	12	16	1.33
		Pythium sp.	Light brown	9	17	1.88
4.	Rajpalchock	*Aspergillus* sp.	Blackish white & greenish	6-31	22-40	1.29-3.66
5.	Ghatparasia	*Aspergillus* sp.	Light yellowish	42	49	1.16
		Penicillium sp	Greenish white	11	16	1.14
6.	Umaria	*Rhizopus* sp.	white	47	58	1.23
7.	Khirshadoh	*Aspergillus* sp.	Orange white,black & greenish	9-52	24-54	1.03-3
8.	Parasia	*Penicillium* sp.	Yellowish white	15	25	1.6
		Pythium sp.	Yellowish	33	38	1.15
		Fusarium sp.	Purple with white	20-41	29-54	1.16-1.45
		Geotricum sp.	white	30	39	1.3
9.	Navegao	*Aspergillus* sp.	Blackish white	29	37	1.27

Table- 9 Effect of pH 4, 5, 6 and 7on the Biomass (g/l) of isolated fungus.

S.No.	Isolated fungus	Days	Biomass				
			pH-4	pH-5	pH-6	pH-7	pH-8
1.	*Aspergillus* sp.	7	10.3	9.43	17.43	17.3	13.96
2.	*Penicillium* sp.		10.3	8.73	14.2	12.63	14.4
3.	*Fusarium* sp.		10.2	6.2	11.93	12.33	15.06
4.	Control	7	No growth				

Aspergillus sp.:- pH- 7 ≠ 6 > 8 > 4 > 5
Penicillium sp.:- pH- 8 = 6 > 7 > 4 > 5

Fusarium sp.:- pH -8 > 7 > 6 > 4 > 5

Physico-chemical properties of soil: -The result of the physicochemical properties of the soil of the 9 different sites. The result of physical and chemical paremeters (pH, conductivity, organic carbon, nitrogen, phosphorus, potassium) obtained from the analysis of soil sample are shown in table -1. The table 1 shows the different values of the parameters determined in the research along with the recommended standards.

pH:-An examination of the soil sample **(Table-2)** shows that the values for pH in the different ranged from 6.3-7.8. The pH of all the soil samples was in agreement with pH assigned by P.K. Basu 2011, as the standard pH of soil which ranges varied from 4.6-8.5.

Wagh et al.(2013) reported that the values for pH ranged from 7.32 to 8.52 indicating that the soils are alkaline and under such conditions the solubility of minerals decreases creating nutrient deficiencies in the soils. Plant growth is therefore limited by deficiencies in iron, manganese, zinc, copper and boron. Chaudhari et al. 2012 reoperted the pH (6.88 - 8.34) values indicated that four soil samples were moderately alkaline and two were neutral. Patil et al. 2013 reported the pH value (5.30 -8.2) indicate that the two samples were alkaline two samples were acidic and remaining samples were neutral.

Conductivity:-The Electrical Conductivity value ranged from 0.14 mS/cm to 46 mS/cm. Electrical conductivity is used to estimate the soluble salt concentrations in soil and is as a measure of salinity. Soil with EC below 0.4mS/cm are considered marginally or non-saline. (P.K. Basu, 2011), as the standard conductivity of soil range from 0.8-2.5 (P.K. Basu, 2011). Wagh et al.(2013) reported Electrical Conductivity value ranged from 0.20 mS/cm to 3.02 mS/cm, however sample shows excess content of soluble salts which may due to excess use of fertilizer like P and K. Electrical conductivity is used to estimate the soluble salt concentrations in soil and is commonly used as a measure of salinity. Soil with EC below 0.4mS/cm are considered marginally or non-saline, while soils above 0.8 mS/cm are considered severely saline. Patil et al. 2013 reported the electrical conductivity (0.03 - 0.51 ds/m) values showed that all red soil samples were non saline in nature. Chaudhari et al. 2012 reoperted the electrical conductivity (0.1 - 0.26 ds/m) values showed that all soil samples were non saline in nature.

Organic carbon:-The organic carbon (%) ranged from 0.5 to 1.3%. The organic soil matter includes all the dead plant materials and live or dead animals. Most living things in soils, including plants, insects, bacteria and fungi, are dependent on organic matter for nutrients and energy. Soils have varying organic compounds in varying degrees of decomposition. According to the P.K. Basu 2011, as the standard organic carbon of soil ranged from 0.5-0.75. Wagh et al.(2013) reported the organic carbon (%) ranges from 0.38 to 1.5 %. The organic soil matter includes all the dead plant materials and live or dead animals. Most living things in soils, including plants, insects, bacteria and fungi, are dependent on organic matter for nutrients and energy.

Nitrogen:-The organic nitrogen (kg/ha) ranged from 275 kg/ha to 445 kg/ha. Seven samples were found to be medium while 2 samples found to be high limit. Nitrogen of soil range was varied from 280-560 (P.K. Basu 2011).

With this consideration the available Nitrogen content (150 - 313 kg/ha) of two samples was found to be low while for remaining samples contains high limit. The Phosphorus content (3.05 – 22.73 kg/ha) of soils indicated that the three samples were contain high, one sample contain normal and the remaining three samples were contain very low amount of Phosphorus (wage at al.2013). According to Methods Manual of Soil Testing in India13 the critical limits of Nitrogen, Phosphorus and Potassium for normal growth of plant were 280 kg/ha, 10 kg/ha and 108 kg/ha respectively. With this consideration the available Nitrogen content (151 - 188 kg/ha) of almost all samples was found to be low (Chaudhari et al. 2012).

Phosphorus:- Phosphorus is one of the key macronutrient required for plant growth and metabolism. Phosphorous in the present soils vary from 2.33 kg/ha to 22.95 kg/ha. Two soil samples were containing very low, three samples contain low, two samples contain medium, and two samples contain high.

Standard phosphorus of soil ranged was 10-24.6kg/ha . Soluble P converted into insoluble phosphate involves microorganisms. Phosphorous in the present soils vary from 10 Kg/hectare to 172.9

Kg/hectare the highest value in sample No. 6 may be due to use of excessive phosphorous fertilizers. Application of phosphorus (P) is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crop Application of phosphorus (P) is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crop(wagh et.al 2013). According to Methods Manual of Soil Testing in India13 the critical limits of Nitrogen, Phosphorus and Potassium for normal growth of plant were 280 kg/ha, 10 kg/ha and 108 kg/ha respectively. The Phosphorus content (2.49 - 63.76 kg/ha) of soils indicated that the two samples were contain very low, one sample contain low and the remaining three samples were contain very high amount of Phosphorus (Chaudhari et al. 2012).

Potassium:-The analyzed samples potassium ranged from 210 kg/ha to 445 kg/ha. Two samples were containing low, six samples contain medium and one sample contain high. Standard potassium of soil range varied from 108-280 .

Potassium fixation occurs when soils dry and the potassium is bonded between layers of clay. Under certain conditions, dependent on the soil texture, intensity of drying, and initial amount of exchangeable potassium. From the analyzed samples potassium ranged from 112 Kg/hectare to 840 Kg/hectare indicating sufficient K in most of the sample. Patil et al. 2013 reported According to Methods Manual of Soil Testing in India20 the critical limits of Nitrogen, Phosphorus and Potassium for normal growth of plant were 280 kg/ha, 10 kg/ha and 108 kg/ha respectively.(wage et al.2013). According to Methods Manual of Soil Testing in India13 the critical limits of Nitrogen, Phosphorus and Potassium for normal growth of plant were 280 kg/ha, 10 kg/ha and 108 kg/ha respectively. The available Potassium (115.4 -290 kg/ha) showed that four samples were contain medium amount and remaining two samples contain high amount of Potassium (Chaudhari et al. 2012).

Isolation and Enumeration of fungi:-In the present study Nine soil samples from chhindwara, were used to isolate cellulase producing fungi by using to a screening medium containing CMC and CMC as carbon source and inducers. The results of total fungal count showed **(Table-3).**

All soil samples showed different value of fungal propgules able to use the carbon sources. The total fungal count in dilution of 10^{-1} showed that Pertala and Parasia had the highest count of 23×10^{1} and 25×10^{1} cfu/ml followed by Khajeri, (13×10^{1} cfu/ml) Rajpalchock(15×10^{1} cfu/ml) and Umaria(17×10^{1} cfu/ml) respectively. However Shikarpur, Ghatparasia and Khirsadoh had 10×10^{1}cfu/ml while Navegao had the lowest fungal count of 3×10^{1} cfu/ml. However the total fungal count in dilution of 10^{-2} showed rajpalchock and parasia had the highest count of 13×10^{2} cfu/ml followed by Partala(10×10^{2} cfu/ml), Khajeri(9×10^{2} cfu/ml), Umaria(10×10^{2} cfu/ml), Ghatparasia(7×10^{2} cfu/ml) and Khirsadoh (7×10^{2}) respectively while Shikarpur and Navegao had the lowest fungal count (7×10^{2} and 3×10^{2} cfu/ml) **(Table-3).**Mukut et al. 2010 reported the results of total fungal count showed that Angwan Mada and New Keffi hotel had the highest count of 6.3 x 10^{3} TFC/ml, followed by Main Campus, High Court,Angwan Lambu, Tudun Amama, Low Cost, BCG and Angwan Rimi, which had 5.0 x 10^{3}, 4.9 x 10^{3}, 4 x 10^{3}, 4.0 x 10^{3}, 3.8 x 10^{3} and 3.7 x10^{3} TFC/ml, respectively, while Tsho Kasuwa had the lowest fungal count of 2.8 x 10^{3} TFC/ml.

Vega et al. (2012) reported the soil dilution plate method was used for the enumeration and isolation of fast growing cellulolytic fungi on an enriched selective medium. Numbers of colony forming units per gram of dry soil as presented in are higher than those reported elsewhere for saprophytic fungi in different types of soils, indicating high degrading activity of plant biomass. Due to the selection procedure final selected strains producing high alkaline cellulase levels were members of the Penicillin and Aspergillis groups since they are fast growing and highly sporulating fungi. Representatives of these fungal groups with alkalophilic or thermophilic enzymes are currently isolated from soils. All soil samples show similar values of fungal propagules able to use the carbon sources ($4.5–7 \times 10^{4}$ UFC g^{-1} dry soil). The percentage occurrence (frequency %) of mycoflora isolates from the different location indicated in dilution of 10^{-1} showed that Partala and Parasia had the highest frequency of 11.11 and 12.07% followed by Khajeri, Rajpalchock and Umaria, which had 6.28, 7.24 and 8.21% respectively. However Shikarpur, Ghatparasia

and Khirsadoh, had the moderate frequency 4.83, and 5.79% respectively while Navegao had the lowest frequency of 1.44 % **(Table -4).**

However the total percent occurrence of mycoflora isolates from dilution of 10^{-2} showed that Rajpalchock and Parasia had the highest frequency of 6.28% followed by Khajeri, Partala, Umaria and Ghatparasia which had 4.34, 4.83 and 4.34% while Shikarpur, Khirhadoh and Navegao, had lowest frequency of 2.89, 3.38 and 1.44 %. respectively. Mukut at al. 2010 reported the percentage occurrence(frequencies) of fungal isolates from the different locations indicated that *A. niger* and *Penicillium* species had the highest percentage (60%) followed by *A. flavus* and *Rhizopus stolonifer* which had 50%, respectively. *A. fumigatus* and *Absidia corymbifera* had 40%, *F. solani* had 30%, *Mucor* sp. had 20%, while *R. oryzae* had the lowest percentage occurrence frequencies of 10% respectively.

Screening of Cellulolytic fungi: -Screening performed by using CMC agar plate assey method 33 fungal isolated that out of 28 fungal isolates were cellulase producer. However the isolated percentage Showed that *Aspergillus* sp.(51.51%),*Penicillium* sp.(12.12%), *Fusarium s*p.(12.12%), *Mucor* sp.(9.09) and *Pythium* sp.(6.06) and lowest percentage showed *Geotricum* sp.(3.03),*Rhizopus sp*.(3.03),**(Table-5).**

The cellulolytic 28 fungal isolates from different type of soil was semi quantitative tested using the plate clearing assay with CMC as substrate in **(Table-6).** The isolated 28 cellulolytic fungus reveled that *Aspergillus* and *Penicillium* sp. mostly dominant in that area, followed by *Aspergillus* sp. *Penocillium* sp. *Pythium* sp., *Fusarium* sp., *Mucor* sp. , *Rhizopus* sp. , *Geotricum* sp. and *Epicoccum* sp.respectively.Mostly fungus isolated from forest soil.

The screenings of cellulase producing fungi were performed on CMC agar plates flooded with Congo red and washed with NaCl. depending on the diameter of clear zone around the colony. The cellulolytic activity of the 50 fungal isolates was semiquantitative tested using the plate clearing assay with CMC as substrate at different pH values(vega et al.2012). The 28 Cellulolytic fungal isolated from different soil and different area. Mostly fungus are isolated from Shikarpus and parasia**(Table -7).**

The screening of the cellulolytic fungal isolate was based on the diameter of clearing zone surrounding the colony on CMC medium. All the fungal culture produced zone of hydrolysis in CMC agar plate within 3 days. Congo red intercalates between the cellulose. The diameters of clearing zone for each isolate are shown in **(Table -8).** Screening performed by using test tube assay showed that out of 115 fungal isolates, 78 (67.83%) isolates were cellulase producer(Jahangeer et al 2005).The appearance of the clear zone around the colony after the addition of Congo red solution was strong evidence that the fungi produced cellulase in order to degrade cellulose . Among the 28 fungus isolates, 7 isolate have showed the maximum zone of clearance range (1.03-3.66mm) **(Figure-2).** Among the isolated cultures of fungal strains, *Aspergillus* sp. showed bigger zone of cellulose hydrolysis (1.03-3.66mm) **(Table-8).**

Strains were screened out for cellulolytic potential on the basis of diameter of clear zone produced due to cellulose hydrolysis by different cultures of the same species. These results indicated that among the cultures of *A. fumigatus*, TAS-10; among *C. thermophile*, TAS-02; among *H. grisea*, TAS-81; among *H. insolens* TAS-13; among *S. thermophile*, TAS-73; among *T. duponti*, TAS- 76 and among *T. thermophila*, TAS-82 were the best cellulase producers giving 1.70, 1.40, 1.66, 1.90, 1.34, 1.45 and 1.25 mm zone, respectively. Among the 87 isolated cultures of fungal strains, *H. insolens* TAS-13 showed bigger zone of cellulose hydrolysis.

Identification of fungi based on morphologically and microscopically:-The isolated fungi were purified by repeated sub-culturing on the Potato Dextrose agar medium at regular interval and incubating 28^0c.Twenty nine fungal stains were identified as cellulase producing fungi and its initial identification was done by fungal staining and colony morphology. Twenty Eight sp. From 8 genera were isolated from the different type of soil. These were *Aspergillus* sp. *penicillium* sp. *Mucor* sp. *Rhizopus* sp., *Epicoccum* sp., *Geotricum* sp., and *Fsarium* sp. The isolate fungus identified up to genus level based on the morphological and microscopic features are given below.

Effect of pH on biomass (cellulase producing fungi):- Biomass production was observed on biomass of 3different sp. at pH value of 4,5,6,7 and 8. Maximum number of isolates showed high production at pH- 6 followed by pH-7 and pH-8 and minimum at pH 4, followed by pH-5 (Table- 8).Significant variation in biomass production was observed among all isolates of *Trichoderma* species as well as different isolates of same species at all test pH values of 4.5, 5.5, 6.5, and 7.5. Maximum number of isolates showed high biomass production at pH 7.5 followed by 5.5 and 6.5 and minimum at pH 4.5. (kolli *et al.*2012).

Conclusion: -In nature, a variety of bacteria and fungi produce cellulases to hydrolyze these insoluble polysaccharide to soluble oligomers, and subsequently to monomer. However, this conversion is quite difficult owing to the complex structure of plant cell wall designed to resist microbial various cellulolytic microorganisms .'Actinomycetes' one of the known cellulase-producer, has attracted considerable research interest due to its potential application in recovery of fermentation sugar from cellulose. That can be of benefit for human consumption and to the ease of their growth. They are capable of producing an array of different extracellular enzyme including cellulase, chitinase and xylanase.

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