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CORRELATION BETWEEN QUALITATIVE AND QUANTITATIVE CELLULASE ENZYMES ACTIVITIES IN SOME *TRICHODERMA SPP.*

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ABSTRACT: Twenty-six isolates used in qualitative screening of Cellulase done in two media CMC-Congo red dye plate and 1% PASC (phosphoric acid swollen cellulose) plate. Quantitative Enzymes activities were tested in two fermentation methods SSF (solid state fermentation) and SmF (Submerged fermentation). SmF was detected at 2,3,4,6 and 7 days in Mandel's Media with CMC 0.5%. Moreover, SSF was carried out by using straws (Rice and wheat) for all isolates. The cellulase activities measured as (FPase, CMCase and β-glucosidase). In this study positive correlation coefficient in enzymes activity between SSF and qualitative screening in (CMC & PASC) plate. Higher correlation have been detected between PACS and SSF rice straw was about (0.595**) for β-Glucosidase. After all these tests we can compare enzyme production by SmF and SSF. Data showed SmF production for high yield and high quality. The cellulase genes, *Chb* gene encoding cellobiohydrolase 2 (CBH2) and *Bgl* gene that encoding endo-β-gluconase (BGL1) were present in all *Trichoderma* isolates.

Key words: *Trichoderma Spp*. -Cellulase genes- SSF -SmF-CMCase-FPase- *β*-glucosidase-*CBH2*-*BGL1*.

INTRODUCTION

Bioethanol from agricultural waste is an attractive way to turn waste into added value that will solve the problem of feed competition and waste management. Napier grass is a highly productive and effective lignocellulosic biomass **(**Mueansichai *et al.,* 2022**).** The cellulolytic activity of fungal strains determined by their ability to grow and form clear zones around colonies on Mandel's agar medium (Mandel's mineral solution (MS) supplemented with trace elements, 2% agar and 0.5% w/v low viscosity CMC (Teather & Wood, 1982). For cellulase activity assays, there is always a gap between initial cellulase activity assays and final hydrolysis measurement. To be most meaningful, individual cellulase component assays must be based on a reliable estimation of the amount of individual enzyme component present in the assay. This information permits the calculation of specific activity, i.e., bonds broken per milligram enzyme per unit time. It was showed that the total cellulase system consists of endoglucanases, exoglucanases, and β-D-glucosidases, all of which hydrolyze crystalline cellulose synergically (Liu *et al.,* 2011) .

Solid-state fermentation is one of the easiest and cheapest methods for producing microbial bioactive compounds. *Trichoderma harzianum* has long been recognized as one of the potential fungi for this purpose. *Trichoderma spp*. was isolated from banana rhizosphere using the soil dilution method and later screened for their ability to produce cellulases using filter paper activity (FPase) and the (CMCase) test. *Trichoderma sp.* was also subjected to one factor change at a time to determine the effects of different parameters on cellulase production. It was observed that *T. harzianum* showed the ability to produce higher cellulase activity when wheat straw was used as the substrate. The results showed that 38.5 U/g of cellulase was produced with the use of wheat straw coupled with an incubation temperature of 28 °C and moisture content of 60%. *T. harzianum* showed solid-state fermentation, with the possibility of its application to industry (Heng & Hamzah, 2022).

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CMCase is an amorphous cellulose is used to study CMCase (endoglucanase), while Aciel, an example of crystalline cellulose is used to study FPase (exoglucanases). Isolates produced higher CMCase than FPase even when CMC was used as the only carbon source. Therefore, it could be inferred that the cellulase secreted by methods is an endoglucanase with lesser exoglucanases activities(Kazeem *et al.,* 2021). Lower CMCase activity compared to FPase on the same inducer substrate is a common occurrence in numerous studies. This is because CMC is an amorphous form of cellulose that is easier to digest than filter paper (Oni *et al.,* 2020). The filamentous fungi can grow on solid substrate under solid-state fermentation (SSF) and submerged fermentation (SmF). SSF is regarded as the growth of fungi on solid substrates in the absence of free water. The solid substrates not only supply as nutrients to the fungi but also serve as anchorage for the fungi (Gupta, 2017). Additionally, it offers environments for growth that are comparable to those in which the fungi naturally grow. Moreover, better contact between microorganisms and their substrates can be achieved using SSF, which results in better growth and higher bio production. Industrially important enzymes have traditionally been obtained from submerged fermentation (SmF) because of the ease of handling and greater control of environmental factors such as temperature and pH. However, solid state fermentation (SSF) technique can improve the yield and reduces the cost of enzyme production. There are several reports describing use of agro-industrial residues for the production of cellulose such as wheat straw, wheat bran and rice straw as substrates (Alegre *et al.,* 2009; Kshirsagar *et al.,* 2020; Patel *et al.,* 2019; Yadav *et al.,* 2018) The other advantages of SSF include superior productivity, simple technique; low capital investment, low energy requirement and less water output (Zeng & Chen, 2009; Shad *et al.,* 2023).

The main objectives of this study is to detect the correlation between cellulase activities quantitatively and qualitatively, and screening of (CBH2, BGL1) genes for 26 *Trichoderma* isolates.

MATERIAL AND METHODS

Strains

Twenty-six Trichoderma *spp.* strains obtained from Department of Genetics, Faculty of Agriculture, Menoufia University, Shibeen EL-Kom, Egypt.(El-Sobky *et al.,* 2019)

Qualitative screening (Total cellulases)

Dye staining of carboxy methyl cellulose agar (CMC agar) was autoclaved, dispensed into Petri dishes, allowed to solidify, and inoculated with 5×10^5 spores of *Trichoderma* strains and incubated at 28◦C. After 5 days, plates were flooded with 1% aqueous Congo red and allowed to be stained for 15 minutes. The stain was washed off from the agar surface with distilled water and the plates were flooded with 1 M NaCl to destining for 15 minutes. The diameter of the clear zone was measured and recorded **(**Pointing, 1999)**.**

- **Walseth cellulose plate-clearing assay** this method of (Bose, 1963) with some modifications was carried out to screen for high cellulase-producing isolates. The basic medium consisted of Mandel's mineral solution MS supplemented with 2% agar. Phosphoric acid-swollen cellulose (PASC) (Walseth cellulose) **(**Rautela & Cowling, 1966) **,**was used as the sole source of carbon at a concentration of 1%. The plates were seeded with 5×10^5 spores in 20µl and incubated at 28◦C for 48 hours. Then the diameter of the radial growth was measured and recorded.

Quantitative determination

(CMC) was used as a sole carbon source in submerged fermentation SmF. The measurements were performed after 2,3,4,6 and 7 days of incubation from the filtrate of the inoculated flasks for in Mandel's Media with CMC 0.5% as a substrate of Cellulase. Filter paper Assay (FPase) Total cellulase activity was measured by the filter paper (FPase) method, with Whatman No. 1 filter paper strips (1 cm×6 cm) used as substrate (Ghose, 1987). Endoglucanase (CMCase) CMC (2% w/v) in 50 mM citrate buffer pH.4.8 (freshly made) was used as substrate solutions for Endoglucanase assays. 0.5 mL of the culture filtrate was transferred into test tube with a volume of at least 10 mL. The culture filtrate

solution and substrate solution were equilibrated at 50°. (Miller, 1959). ß-glucosidase activity assay was determined according to (Ghose, 1987)as follows: Cellobiose (15 mM) in 50 mM citrate buffer pH.4.8 (freshly made) was used as substrate solutions for β-Glucosidase assays .

Solid state fermentation

The solid-state culture was kept in 250-mL flasks; the flasks were closed with a cotton covered plug. Each flask contained 3 g of straw and 12 mL Mandel's basal salt solution (MS). The flasks were autoclaved at 121°C for 30 min. Each flask was then inoculated with 1 mL conidial suspension to produce a final concentration of 1×10^7 conidia. The flasks were incubated at 30 ◦C with a relative humidity of 70% for 10 days.

Detection of Cellulase genes of *Trichoderma* species

Trichoderma isolates were cultured on PDA broth at 28 °C for 5 days, after which total genomic DNA for each strain was extracted, using method reported by (Elsobky *et al.,* 2019). Amplification of Chb gene was achieved according to **(**Wang & Gao, 1999) and the Bgl gene was achieved according to **(**González *et al.,* 1992) primer sequences, amplification conditions, and amplicon sizes listed in

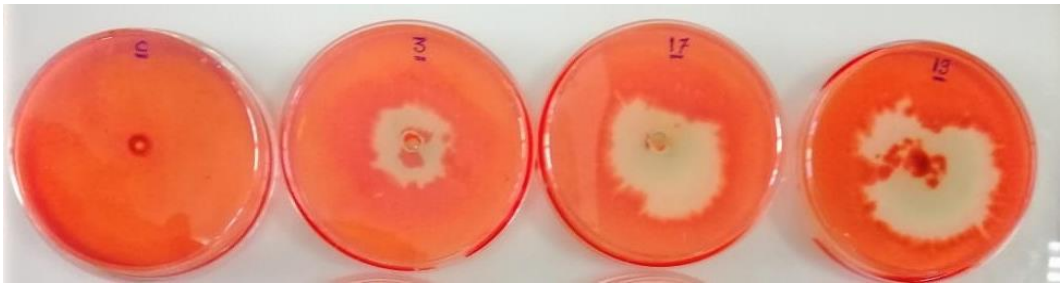
Data analysis

All experiments that were conducted in this study were implemented in accordance with the Completely Randomized Design (CRD). The determination of significant differences was calculated (Duncan Test). Statistical tests were performed using a statistical analysis by computer program system developed by IBM Corp. in 2017, specifically IBM SPSS Statistics for Windows, Version 25.0, denoting their significance at $P < 0.05$.

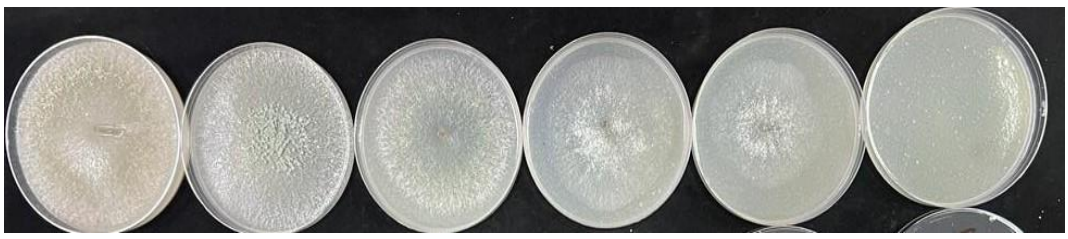
RESULTS AND DISCUSSION

Qualitative measurement of cellulase production

Trichoderma isolates were identified for their endoglucanase activities on plate by using assay that appeared when using (CMC) and Congo red dye. Based on measurements of clear zone diameter, six isolates gave clear zones of cellulase activity having diameter significantly larger than other isolates namely, MNF-MAS-*Tricho*1, MNF-MAS-*Tricho*6, MNF-MAS-*Tricho*23, and ASI-MAS-*Tricho*26 Figure (1).



a)



b)

Figure (1): a) Endoglucanase activities on plate by using (CMC) staining by Congo red dye.
b) Radial growth of Trichoderma Isolates in Mandel's Media containing PASC Phosphoric acid-swollen cellulose 1%.

Data presented in Table (1) showed that (CMC) was a substrate for endoglucanase and so can be used as a test for endoglucanase activity and the 1% PASC media full plate radial growth showed in isolates MNF-MAS-*Tricho*2, 3, 5, 6, 12, 15, 18, 21, and MNF-MAS-*Tricho*23 after 48 hrs from incubation and the detection of the sporulation type showed green spores in 7 isolates Table (1). Various studies based on method of Congo red to screen the different *Trichoderma* cellulolytic isolates (Castrillo *et al.,* 2017; Suirta *et al.,* 2021) Avicel as a" PASC "has been used in our study to be measuring exo-glucanase activities that cleave the accessible ends of cellulose modules to liberate glucose and cellobiose (Fahmi *et al.,* 2016; Sharrock, 1988).

Table (1): Qualitative screening of *Trichoderma spp.* isolates using (CMC), and micro-crystalline cellulose as substrates.

***Trichoderma* isolate**	**Endo-glucanase (Clear zone diameter in cm) ***		**Sporulation type in 1% PASC Media**
	PASC	**CMC**	
MNF-MAS-*Tricho*1	0.30 ^{a}	5.83 ^{jk}	Green spores*
MNF-MAS- *Tricho* 2	9.00^{h}	2.97 ^{de}	No spores
MNF-MAS- *Tricho* 3	9.00 ^{h}	2.83 ^{d}	No spores
MNF-MAS- *Tricho* 4	7.00^{g}	4.20^{h}	Green spores*
MNF-MAS- *Tricho* 5	9.00 ^{h}	0.17^{a}	Green spores*
MNF-MAS- *Tricho* 6	9.00 ^{h}	5.57 ^{jk}	Light green spores *
MNF-MAS- *Tricho* 7	5.00^{e}	3.73^{fg}	No spores
GIZ-MAS- *Tricho* 8	3.00^{d}	4.20 ^{h}	No spores
MNF-MAS- *Tricho* 9	6.20 ^{f}	4.00^{gh}	No spores
MNF-MAS- *Tricho* 10	7.10 ^{g}	3.40 ^{ef}	No spores
MNF-MAS- *Tricho* 11	5.20 ^{e}	4.00 ^{gh}	Green spores**
MNF-MAS- *Tricho* 12	9.00 ^{h}	4.93^{i}	Green spores**
MNF-MAS- *Tricho* 13	7.13 ^{g}	1.17^{b}	No spores
MNF-MAS- *Tricho* 14	5.00 ^{e}	1.97 ^{c}	No spores
MNF-MAS- *Tricho* 15	9.00 ^{h}	1.43 ^{b}	Green spores**
MNF-MAS- *Tricho* 16	0.10 ^{a}	0.17 ^{a}	No spores
MNF-MAS- *Tricho* 17	1.10^{b}	0.27 ^{a}	No spores
MNF-MAS- *Tricho* 18	9.00 ^{h}	4.43 ^{h}	No spores
MNF-MAS- *Tricho* 19	6.13 ^{f}	5.00^{i}	No spores
MNF-MAS- *Tricho* 20	0.27 ^{a}	3.00^{de}	No spores
MNF-MAS- *Tricho* 21	9.00 ^{h}	5.43^{j}	No spores
MNF-MAS- *Tricho* 22	0.17 ^{a}	2.10 ^{c}	No spores
MNF-MAS- *Tricho* 23	9.00 ^{h}	5.93 ^{k}	Light green spores **
GIZ-MAS-2 *Tricho* 24	2.00^{c}	4.33 ^{h}	No spores
AST-MAS- *Tricho* 25	0.00^{a}	4.43 ^{h}	No spores
AST-MAS- *Tricho* 26	6.10^{f}	5.93 ^{k}	Green spores **

*Within columns, values with a common letter do not differ significantly ($P<0.05$), according to $Duncan_{a,b,c}$

Table (2): Filter paper enzyme activity FPase in Mandel's Media with CMC 0.5% .

Isolate Code		Enzymes activities (IU mL $^{-1}$)				
		2 days	3 days	4 days	6 days	7 days
1	MNF-MAS-*Tricho*1	0.28 ^{f}	0.30 ^{e}	0.31^{ef}	0.34 ^{gh}	0.43 ^{efg}
2	MNF-MAS- *Tricho* 2	0.18 ^{a}	0.31 ^{ef}	0.37 ^{i}	0.45 ^{j}	0.49 ^{g}
3	MNF-MAS- *Tricho* 3	0.26 ^{ef}	0.28 ^{c}	0.32^{fg}	0.27 ^{cd}	0.47 ^{g}
4	MNF-MAS- *Tricho* 4	0.25 ^{def}	0.27 ^{bc}	0.32 ^{fg}	0.27 ^{cd}	0.43 ^{efg}
5	MNF-MAS- *Tricho* 5	0.24^{cde}	0.26 ^{ab}	0.28 ^{bc}	0.24 ^{ab}	0.43 ^{efg}
6	MNF-MAS- *Tricho* 6	0.24^{cdef}	0.38 ^{h}	0.40 ^{j}	0.27 ^{cd}	0.40 ^{def}
7	MNF-MAS- *Tricho* 7	0.22 ^{bcd}	0.27 ^{bc}	0.41 ^{j}	0.30 ^{e}	0.44 ^{efg}
8	GIZ-MAS- *Tricho* 8	0.21 ^{abc}	0.25^{a}	0.25 ^{a}	0.26 ^{cd}	0.26 ^{a}
9	MNF-MAS- *Tricho* 9	0.25 ^{def}	0.27 ^{bc}	0.34 ^{h}	0.25 ^{bc}	0.38 ^{cde}
10	MNF-MAS- *Tricho* 10	0.24^{cde}	0.29 ^{de}	0.28 ^{b}	0.35 ^{h}	0.38 ^{cde}
11	MNF-MAS- *Tricho* 11	0.19 ^{ab}	0.24 ^{a}	0.25 ^{a}	0.26 ^{cd}	0.26 ^{a}
12	MNF-MAS- *Tricho* 12	0.25^{def}	0.27 ^{bc}	0.28 ^{b}	0.33 ^{fg}	0.35 ^{cd}
13	MNF-MAS- *Tricho* 13	0.23 ^{bcde}	0.27 ^{c}	0.30 ^{def}	0.23 ^{a}	0.45 ^{fg}
14	MNF-MAS- *Tricho* 14	0.19 ^{ab}	0.24^{a}	0.25 a	0.26 ^{cd}	0.26 ^{a}
15	MNF-MAS- *Tricho* 15	0.31 ^{f}	0.31 ^{ef}	0.32 ^{fg}	0.27 ^{cd}	0.47 ^{g}
16	MNF-MAS- *Tricho* 16	0.21 ^{abc}	0.24^{a}	0.25 ^{a}	0.26 ^{cd}	0.26 ^{a}
17	MNF-MAS- *Tricho* 17	0.35 ^{g}	0.25 ^{ab}	0.30 ^{cde}	0.28 ^{d}	0.45 ^{fg}
18	MNF-MAS- *Tricho* 18	0.27 ^{ef}	0.33 ^{g}	0.33 ^{gh}	0.42 ^{i}	0.34 ^{cd}
19	MNF-MAS- *Tricho* 19	0.25 ^{def}	0.31 ^{ef}	0.32 ^{fg}	0.27 ^{cd}	0.33 ^{abc}
20	MNF-MAS- *Tricho* 20	0.25 ^{def}	0.28 ^{cd}	0.33 ^{gh}	0.30 ^{e}	0.35 ^{cd}
21	MNF-MAS- *Tricho* 21	0.26 ^{ef}	0.28 ^{cd}	0.29 ^{bcd}	0.33 ^{gh}	0.28 ^{ab}
22	MNF-MAS- *Tricho* 22	0.24^{cdef}	0.27 ^{bc}	0.27 ^{b}	0.26 ^{cd}	0.34 ^{cd}
23	MNF-MAS- *Tricho* 23	0.25 ^{def}	0.36 ^{h}	0.36 ^{i}	0.31 ^{e}	0.34 ^{bcd}
24	GIZ-MAS-2 *Tricho* 24	0.25 ^{def}	0.32 ^{fg}	0.30 ^{cde}	0.31 ^{ef}	0.32 ^{abc}
25	AST-MAS- *Tricho* 25	0.21 ^{abc}	0.24 ^{a}	0.25 ^{a}	0.25 ^{bc}	0.34 ^{bcd}
26	AST-MAS- *Tricho* 26	0.20 ^{ab}	0.24 ^{a}	0.25 ^{a}	0.26 ^{cd}	0.40 ^{def}

*Within columns, values with a common letter do not differ significantly (P<0.05), according to Duncan$_{a,b,c}$

Quantitative measurement of cellulase from *Trichoderma*.

All isolates were screened for enzyme activity (filter paper activity (FPase) assay, CMCase for endo- β - 1,4-gluconase and cellobiase assay for β-glucosidase). Therefore, submerged fermentation experiment was carried out by 26 *Trichoderma* isolates.

Cellulase production in submerged fermentation (SmF) cultures.

The measurements were taken after 2,3,4,6 and 7 days of incubation from the filtrate of the inoculated flasks for in Mandel's Media with CMC 0.5% as a substrate of Cellulase. Results represented in the Table (2,3 and 4) showed every enzyme separated and the kinetic study of the best isolates in enzyme production.

Moreover, data presented in Table (2) showed cellulases enzyme activity (measured as FPase after different incubation periods), free sugar produced in Submerged fermentation (SmF) cultures for all isolates. Generally, all isolates indicated outstanding FPase activities. Four isolates demonstrated highly significant FPase activities after 7 days of incubation namely, MNF-MAS-*Tricho*2, MNF-MAS-*Tricho*3 MNF-MAS-*Tricho*15 and MNF-MAS-*Tricho*17(0.49, 0.47, 0.47 and 0.45 IU mL^{-1}, respectively). Whereas

isolates GIZ-MAS-*Tricho*8, MNF-MAS-*Tricho*11, MNF-MAS-*Tricho*14, MNF-MAS-*Tricho*16 and MNF-MAS-*Tricho*21 showed the lowest FPase activity (0.26 IU mL^{-1}) were in average values. MNF-MAS-*Tricho*2 showed the highest level of FPase after 4, 6, 7 days of incubation (0.37,0.45 and 0.49 IU mL^{-1}, respectively). MNF-MAS-*Tricho*15 showed the high level of FPase in two, seven days of incubation (0.31and 0.47 IU mL^{-1}, respectively). Moreover, MNF-MAS-*Tricho*17 showed the high level of FPase in 2, 7days of incubation they came to be (0.35 and 0.45 IU mL^{-1}, respectively). MNF-MAS-*Tricho*7 showed a high level of FPase activity after four days of incubation (0.41 IU $mL^{-1)}$ but in the remaining days of incubation didn't show any high levels of FPase. This Table (2) showed that the high significant values of FPase enzyme activity came after 6 days of incubation.

Table (3) CMCase activity in Mandel's Media with CMC 0.5%.

Isolate Code		Enzymes activities (IU mL^{-1})				
		2 days	3 days	4 days	6 days	7 days
1	MNF-MAS-*Tricho*1	0.43^{cdef}	0.43^{abc}	0.48^{ab}	0.52^{ab}	0.59^{cdef}
2	MNF-MAS- *Tricho* 2	0.37^{ab}	0.43^{abc}	0.53^{bcde}	0.53^{abc}	0.61^{efgh}
3	MNF-MAS- *Tricho* 3	0.51^{hi}	0.53^{e}	0.51^{bcd}	0.54^{bc}	0.65^{ghij}
4	MNF-MAS- *Tricho* 4	0.45^{defg}	0.43^{abc}	0.51^{bcd}	0.64^{f}	0.69^{j}
5	MNF-MAS- *Tricho* 5	0.38^{abc}	0.44^{abcd}	0.56^{defgh}	0.60^{ef}	0.75^{k}
6	MNF-MAS- *Tricho* 6	0.38^{abc}	0.45^{abcd}	0.59^{fghi}	0.64^{f}	0.80^{l}
7	MNF-MAS- *Tricho* 7	0.51^{hi}	0.46^{bcd}	0.62^{i}	0.62^{ef}	0.67^{ij}
8	GIZ-MAS- *Tricho* 8	0.41^{abcd}	0.44^{abcd}	0.45^{a}	0.49^{a}	0.51^{a}
9	MNF-MAS- *Tricho* 9	0.47^{fghi}	0.44^{abcd}	0.60^{hi}	0.60^{ef}	0.64^{ghi}
10	MNF-MAS- *Tricho* 10	0.51^{hi}	0.44^{abcd}	0.58^{fghi}	0.54^{bc}	0.60^{defg}
11	MNF-MAS- *Tricho* 11	0.41^{abcd}	0.44^{abcd}	0.45^{a}	0.49^{a}	0.51^{a}
12	MNF-MAS- *Tricho* 12	0.49^{hi}	0.43^{abc}	0.57^{efghi}	0.55^{bcd}	0.55^{abcd}
13	MNF-MAS- *Tricho* 13	0.36^{a}	0.41^{a}	0.58^{efghi}	0.55^{bcd}	0.62^{efgh}
14	MNF-MAS- *Tricho* 14	0.41^{abcd}	0.44^{abcd}	0.45^{a}	0.49^{a}	0.51^{a}
15	MNF-MAS- *Tricho* 15	0.43^{cdef}	0.45^{abcd}	0.58^{efghi}	0.59^{de}	0.63^{fghi}
16	MNF-MAS- *Tricho* 16	0.41^{abcd}	0.44^{abcd}	0.45^{a}	0.49^{a}	0.51^{a}
17	MNF-MAS- *Tricho* 17	0.52^{i}	0.42^{ab}	0.53^{bcde}	0.53^{abc}	0.58^{bcde}
18	MNF-MAS- *Tricho* 18	0.47^{fghi}	0.46^{bcd}	0.50^{abc}	0.53^{ab}	0.59^{cdef}
19	MNF-MAS- *Tricho* 19	0.46^{efgh}	0.48^{d}	0.51^{bcd}	0.52^{ab}	0.60^{defg}
20	MNF-MAS- *Tricho* 20	0.47^{fghi}	0.46^{cd}	0.54^{cdef}	0.53^{abc}	0.55^{abcd}
21	MNF-MAS- *Tricho* 21	0.51^{hi}	0.61^{f}	0.57^{efghi}	0.55^{bcd}	0.55^{abc}
22	MNF-MAS- *Tricho* 22	0.41^{bcde}	0.68^{g}	0.70^{j}	0.74^{g}	0.90^{m}
23	MNF-MAS- *Tricho* 23	0.41^{bcde}	0.44^{abcd}	0.54^{cdefg}	0.53^{abc}	0.53^{a}
24	GIZ-MAS-2 *Tricho* 24	0.44^{def}	0.48^{d}	0.50^{abc}	0.55^{bcd}	0.59^{cdef}
25	AST-MAS- *Tricho* 25	0.49^{ghi}	0.44^{abcd}	0.59^{ghi}	0.58^{de}	0.61^{efgh}
26	AST-MAS- *Tricho* 26	0.44^{def}	0.44^{abcd}	0.49^{abc}	0.52^{ab}	0.54^{ab}

*Within columns, values with a common letter do not differ significantly (P<0.05), according to $Duncan_{a,b,c}$

Table (4): β-Glucosidase in Mandel's Media with CMC 0.5%.

Isolate Code		Enzymes activities (IU mL $^{-1}$)				
		2 days	3 days	4 days	6 days	7 days
1	MNF-MAS-*Tricho*1	0.34 ^{a}	0.30 ^{a}	0.65 ^{hi}	0.61^{efg}	0.59 ^{abcd}
2	MNF-MAS- *Tricho* 2	0.61 ^{i}	0.62 ^{d}	0.65 ^{hi}	0.66^{hi}	0.75^{hi}
3	MNF-MAS- *Tricho* 3	0.53 ^{fg}	0.45 ^{b}	0.51 ^{ab}	0.59^{cdef}	0.70^{fgh}
4	MNF-MAS- *Tricho* 4	0.58 ^{hi}	0.46 ^{b}	0.52 ^{abc}	0.53 ^{a}	0.73 ^{ghi}
5	MNF-MAS- *Tricho* 5	0.48 ^{cde}	0.47 ^{b}	0.55 ^{bcd}	0.62 ^{fgh}	0.72 ^{ghi}
6	MNF-MAS- *Tricho* 6	0.55 ^{gh}	0.60 ^{d}	0.59 ^{efg}	0.59^{bcdef}	0.67^{efg}
7	MNF-MAS- *Tricho* 7	0.45 ^{cd}	0.49 ^{b}	0.51 ^{ab}	0.53 ^{a}	0.53 ^{a}
8	GIZ-MAS- *Tricho* 8	0.47 ^{cde}	0.48 ^{b}	0.59 ^{efg}	0.60^{def}	0.65^{def}
9	MNF-MAS- *Tricho* 9	0.48 ^{cde}	0.47 ^{b}	0.55 ^{cde}	0.62^{fg}	0.76^{i}
10	MNF-MAS- *Tricho* 10	0.45 ^{cd}	0.49 ^{b}	0.51 ^{ab}	0.53 ^{a}	0.53 ^{a}
11	MNF-MAS- *Tricho* 11	0.48^{de}	0.47 ^{b}	0.53 ^{abc}	0.55 ^{abc}	0.63^{cde}
12	MNF-MAS- *Tricho* 12	0.46 ^{cde}	0.47 ^{b}	0.55 ^{bcd}	0.57 ^{abcde}	0.61 ^{bcde}
13	MNF-MAS- *Tricho* 13	0.45 ^{cd}	0.49 ^{b}	0.51 ^{ab}	0.53 ^{a}	0.53 ^{a}
14	MNF-MAS- *Tricho* 14	0.54 ^{gh}	0.54 ^{c}	0.53 ^{abc}	0.60^{def}	0.72 ^{ghi}
15	MNF-MAS- *Tricho* 15	0.45 ^{cd}	0.49 ^{b}	0.51 ^{ab}	0.53 ^{a}	0.53 ^{a}
16	MNF-MAS- *Tricho* 16	0.54 ^{gh}	0.47 ^{b}	0.62 ^{gh}	0.64 ^{ghi}	0.62^{cde}
17	MNF-MAS- *Tricho* 17	0.58 ^{hi}	0.62 ^{d}	0.73 ^{j}	0.77 ^{ij}	0.75^{hi}
18	MNF-MAS- *Tricho* 18	0.56 ^{gh}	0.60 ^{d}	0.60 ^{fg}	0.62 ^{fgh}	0.70 ^{fghi}
19	MNF-MAS- *Tricho* 19	0.50 ^{ef}	0.48 ^{b}	0.58 ^{def}	0.60^{defg}	0.90 ^{j}
20	MNF-MAS- *Tricho* 20	0.40 ^{b}	0.49 ^{b}	0.80 k	0.67^{i}	0.58 ^{abc}
21	MNF-MAS- *Tricho* 21	0.34 ^{a}	0.54 ^{c}	0.71 ^{j}	0.62 ^{fgh}	0.55 ^{ab}
22	MNF-MAS- *Tricho* 22	0.45 ^{cd}	0.62 ^{d}	0.62 ^{gh}	0.62 ^{fgh}	0.61 ^{bcde}
23	MNF-MAS- *Tricho* 23	0.31 ^{a}	0.48 ^{b}	0.66^{i}	0.62 ^{fgh}	0.58 ^{abc}
24	GIZ-MAS-2 *Tricho* 24	0.44 ^{bc}	0.48 ^{b}	0.50 ^{a}	0.55 ^{ab}	0.59 ^{abc}
25	AST-MAS- *Tricho* 25	0.46 ^{cde}	0.48 ^{b}	0.52 ^{abc}	0.56 ^{abcd}	0.61 ^{bcde}
26	AST-MAS- *Tricho* 26	0.47 ^{cde}	0.47 ^{b}	0.53 ^{abc}	0.55 ^{ab}	0.60 ^{abcd}

*Within columns, values with a common letter do not differ significantly (P<0.05), according to Duncan$_{a,b,c}$

Data presented in Table (3) showed cellulases enzyme activity (measured as CMCase after different incubation periods), Generally, all isolates indicated outstanding endoglucanase activity. While MNF-MAS-Tricho22 isolate showed the highest significant CMCase activity (0.90 IU mL^{-1}) after seven days of incubation. Furthermore, this isolate showed high level of CMCase after 3,4 and 6 days of incubation (0.68, 0.70 and 0.74 IU mL ^{1}, respectively). Those results clearly showed that isolates MNF-MAS-*Tricho*6 and MNF-MAS-*Tricho*5. The lowest level of CMCase appeared after incubation seven isolates GIZ-MAS-*Tricho*8, MNF-MAS-*Tricho*11, MNF-MAS-*Tricho*14, MNF-MAS-*Tricho*16 MNF-MAS-*Tricho*23 and AST-MAS-*Tricho*26 all of them come to be lower than the above tested isolates (0.55IU mL^{-1}). MNF-MAS-*Tricho*17 showed higher enzyme activity after two days of growth as it came to be 0.52 IUmL^{-1}. In fact, this isolate showed steady of enzyme activity as they came to be 0.52, 0.42, 0.53, 0.53 and 0.58 IU mL^{-1} respectively in different days of the measurement.

Table (4) showed the cellulases enzyme activities (β-glucosidase after four different incubation periods), MNF-MAS-*Tricho*19 was the highest (0.90 IU mL^{-1}) while MNF-MAS-*Tricho*7, MNF-MAS-*Tricho10,* MNF-MAS-*Tricho13,* MNF-MAS-*Tricho15,* MNF-MAS-*Tricho23* and GIZ-MAS-*Tricho24* were the

lowest (0.5 IU mL^{-1}) after 7 days of incubation. MNF-MSH-*Trich*2 showed the highest value for β-glucosidase enzyme activity in the culture filtrate at the beginning of the experiment after two days and three days their values come to be (0.61 and 0.62 IU mL^{-1}, respectively), While most isolates showed low level of the enzyme activities, at the first sample after two days and the end of experiment ,whereas it showed high significant values of β-glucosidase enzyme activity after 7 days of growth .

Successful enzymatic hydrolysis of cellulose necessitates the combined action of exoglucanases, endoglucanases, and beta-glucosidase (Zhang *et al.,* 2018) **.** To evaluate the activities of FPase, CMCase, and β-glucosidase; Qualitative and quantitative assays were conducted on minimal media supplemented with sugar cane bagasse and rice straw as the sole carbon sources. The results obtained from both types of assays were highly consistent with those reported by (Sazci *et al.,* 1986; Castrillo *et al.,* 2021) . They affirmed the congruence between qualitative and quantitative methods and have recommended the use of the clearing zone assay as a fast, cost-effective, and sensitive test for screening many isolates. In a study conducted by (Marecik *et al.,* 2018), 123 strains of *Trichoderma* were tested for their ability to degrade cellulose. Approximately 30 strains exhibited significantly higher levels of cellulase activities compared to the reference strain. The variation in cellulolytic activities among *Trichoderma* isolates may be attributed to differences isolates in genetic content, origin, and the number of cellulase enzymes that present in tested fungus.

Our obtained data agreed nicely with what reported by Nathan *et al.,* (2014) they demonstrated that the optimum enzyme recovery period was identified between 5th to 9th days of incubation.

Zahra *et al.,* (2020) demonstrated that the Cellulase Production by *Trichoderma viride* in Submerged Fermentation SmF represents as cheaper process and the good alternative for industrial applications. These findings were consistent with the result that most of the isolates belonged to *T. longibarchiatum* and *T. harzianum*. These species have been adopted in various industries because of their ability to secrete large amounts of protein and metabolites (Gupta *et al.,* 2014) Nutrient supplies have been reported to be key elements in the synthesis of cellulase and xylanase carbon source is an essential nutrient affecting enzyme production. Moreover, the pure CMC using as sole carbon source represents a suitable substrate for quantitative cellulase activity measurements in SmF (Dewiyanti *et al.,* 2022).

Avicel has been used for measuring exoglucanase activities that cleave the accessible ends of cellulose modules to liberate glucose and cellobiose (Sharrock, 1988).

Figure (2) Showed mean cellulase produce from tested *Trichoderma* isolates in Solid state fermentation (SSF) with the wheat and rice straw after different incubation days. The soft mycelium of *Trichoderma* showed for the first time after three days and the sporulation detected with the green color observed on the straw after five days. The hydrolysis of the rice and wheat straw (weight loss and glucose free sugar) is shown in Table (5) and the enzyme production (FPase, CMCase and β-glucosidase) showed in Table (6).

Table (5) showed the total weight loss and free glucose sugar produced in (SSF) rice and wheat straw by *Trichoderma* isolates. There were high variations in losing weight from (4% to 40%) in rice straw after 10 days of incubation and showed differences in glucose concentration levels are ranging from (1.3: 3.14 mg/ml). Isolate MNF-MAS-*Tricho*18 shows a high level on both substrate (34.56%and 3.14mg/ml). Moreover, there were high variations weight loss in wheat straw from (6% to 20%) after 10 days of incubation that showed lower than weight loss in rice straw (4% to 39%) in the same incubation condition. Glucose concentrations are ranging from (0.24 to 5mg/ml). Isolate MNF-MAS-*Tricho*3 showed high level in both (4.32mg/ml and 19.6%).

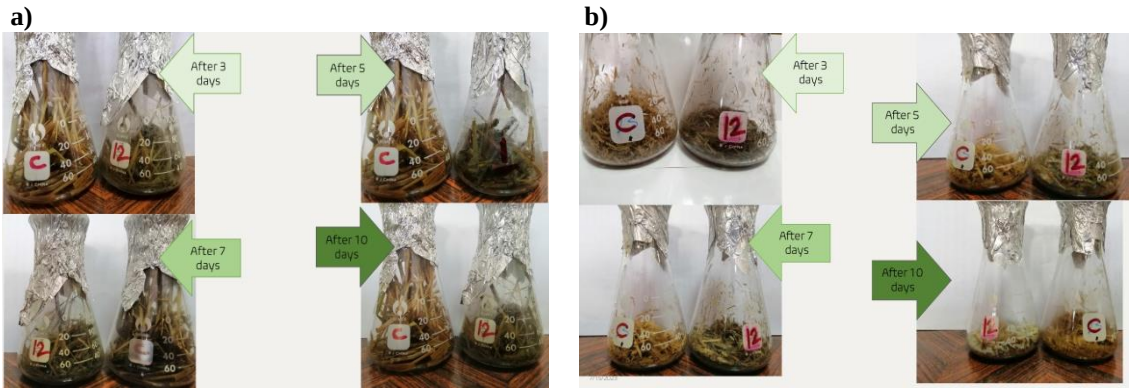


Figure (2): Solid state fermentation (SSF) of some tested *Trichoderma* strain MNF-MAS- *Tricho* 12 a) Rice straw b) Wheat straw.

Table (5): Total weight loss and glucose free sugar produced in (SSF) rice and wheat straw by *Trichoderma* strains after 10 days.

Isolates		Rice straw		Wheat straw	
		Weight loss %	Glucose mg/ml	Weight loss %	Glucose mg/ml
1	MNF-MAS-*Tricho*1	31.72% ^{jk}	2.01 ^{ghi}	16.62%^{ij}	7.47 ^{k}
2	MNF-MAS- *Tricho* 2	29.29% ^{hi}	2.13^{i}	19.88%^{l}	0.90^{d}
3	MNF-MAS- *Tricho* 3	24.56%^{g}	1.45 ^{abcd}	19.60% ^{kl}	4.32 ^{i}
4	MNF-MAS- *Tricho* 4	33.79% ^{kl}	1.86 ^{fg}	13.53% ^{fg}	1.45^{e}
5	MNF-MAS- *Tricho* 5	21.83%^{f}	1.76^{f}	18.02% ^{jk}	0.44 ^{ab}
6	MNF-MAS- *Tricho* 6	4.38% ^{a}	2.07^{hi}	14.09% ^{fgh}	4.30 ^{i}
7	MNF-MAS- *Tricho* 7	11.18% ^{c}	1.94 ^{gh}	10.72%^{cd}	0.24 ^{a}
8	GIZ-MAS- *Tricho* 8	8.28% ^{b}	1.36 ^{ab}	10.16% ^{bc}	0.24 ^{a}
9	MNF-MAS- *Tricho* 9	12.90% ^{c}	1.41 ^{abcd}	8.70% ^{b}	3.63 ^{h}
10	MNF-MAS- *Tricho* 10	39.59%^{m}	1.56 ^{cd}	12.97%^{ef}	0.77^{cd}
11	MNF-MAS- *Tricho* 11	15.38% ^{d}	1.39 ^{abc}	10.16% ^{bc}	0.30 ^{a}
12	MNF-MAS- *Tricho* 12	30.77% ^{ij}	2.54^{ik}	9.38% ^{bc}	1.81 ^{f}
13	MNF-MAS- *Tricho* 13	15.27% ^{d}	1.44 ^{abcd}	5.67% ^{a}	0.26 ^{a}
14	MNF-MAS- *Tricho* 14	7.69% ^{b}	1.37 ^{ab}	10.16% ^{bc}	0.30 ^{a}
15	MNF-MAS- *Tricho* 15	17.75% ^{e}	2.05^{hi}	10.56% ^{bc}	4.90 ^{j}
16	MNF-MAS- *Tricho* 16	34.32%^{l}	1.30 ^{a}	9.60% ^{bc}	0.27 ^{a}
17	MNF-MAS- *Tricho* 17	6.69% ^{b}	2.55^{ik}	13.25%^{f}	0.68 ^{bcd}
18	MNF-MAS- *Tricho* 18	34.56%^{l}	3.14^{l}	17.91%^{jk}	0.62 ^{bc}
19	MNF-MAS- *Tricho* 19	12.60% ^{c}	1.51 ^{bcd}	10.50% ^{bc}	1.41 ^{e}
20	MNF-MAS- *Tricho* 20	26.98%^{h}	1.59^{d}	15.78%^{hi}	1.86 ^{f}
21	MNF-MAS- *Tricho* 21	27.75% ^{h}	2.71^{k}	18.59% ^{kl}	2.17 ^{g}
22	MNF-MAS- *Tricho* 22	18.34% ^{e}	2.15^{i}	18.02% ^{jk}	2.35 ^{g}
23	MNF-MAS- *Tricho* 23	34.32%^{l}	2.16^{i}	13.25%^{f}	4.25 ^{i}
24	GIZ-MAS-2 *Tricho* 24	28.93% ^{hi}	2.40^{j}	11.29% ^{cde}	2.22 ^{g}
25	AST-MAS- *Tricho* 25	26.98% ^{h}	1.36 ^{ab}	11.29% ^{cde}	0.24 ^{a}
26	AST-MAS- *Tricho* 26	7.69% ^{b}	2.71^{k}	12.41%^{def}	2.17 ^{g}

*Within columns, values with a common letter do not differ significantly (P<0.05), according to Duncan$_{a,b,c}$

Table (6): Total Cellulase enzymes activity (IU g-1) (FPase, CMCase and β-glucosidase), in (SSF) Rice and wheat straw by *Trichoderma* strains after 10 days.

Isolates	Cellulase enzymes activity activities (IU g^{-1})					
	Rice straw			Wheat straw		
	FPase	CMCase	β-Glucosidase	FPase	CMCase	β-Glucosidase
MNF-MAS-*Tricho*1	0.085^{gh}	0.120 ^{ab}	0.076^{a}	0.063^{ef}	0.080 ^{ab}	0.078 ^{abc}
MNF-MAS- *Tricho* 2	0.114^{j}	0.156^{hij}	0.154^{e}	0.076^{i}	0.104^{hij}	0.103 ^{d}
MNF-MAS- *Tricho* 3	0.068 ^{abcde}	0.127 ^{abcd}	0.112 ^{bcd}	0.065^{fgh}	0.084 ^{abcd}	0.075 ^{abc}
MNF-MAS- *Tricho* 4	0.069^{bcde}	0.127 ^{abcd}	0.114 ^{bcd}	0.046 ^{bc}	0.084 ^{abcd}	0.076 ^{abc}
MNF-MAS- *Tricho* 5	0.062^{ab}	0.139^{cdefg}	0.116 ^{bcd}	0.041^{ab}	0.093^{cdefg}	0.078 ^{abc}
MNF-MAS- *Tricho* 6	0.102 ^{i}	0.149^{fghi}	0.149^{e}	0.068 ^{fgh}	0.099 ^{fghi}	0.099 ^{d}
MNF-MAS- *Tricho* 7	0.075^{ef}	0.154^{ghi}	0.106^{bc}	0.050^{cd}	0.103^{ghi}	0.071 ^{ab}
GIZ-MAS- *Tricho* 8	0.067 ^{abcd}	0.112^{a}	0.120^{cd}	0.044 ^{abc}	0.075^{a}	0.080 ^{bc}
MNF-MAS- *Tricho* 9	0.064^{abc}	0.149^{fghi}	0.117 ^{bcd}	0.043 ^{ab}	0.099 ^{fghi}	0.078 ^{abc}
MNF-MAS- *Tricho* 10	0.088^{h}	0.146 ^{efghi}	0.114 ^{bcd}	0.059^{e}	0.097^{efgh}	0.076 ^{abc}
MNF-MAS- *Tricho* 11	0.067 ^{abcd}	0.112 ^{a}	0.117 ^{bcd}	0.044 ^{abc}	0.075 ^{a}	0.078 ^{abc}
MNF-MAS- *Tricho* 12	0.103 ^{i}	0.159^{ij}	0.147^{e}	0.069^{gh}	0.106 ^{hij}	0.098^{d}
MNF-MAS- *Tricho* 13	0.060^{a}	0.143^{efgh}	0.106 ^{bc}	0.040^{a}	0.096^{efgh}	0.071 ^{ab}
MNF-MAS- *Tricho* 14	0.067 ^{abcd}	0.112 ^{a}	0.110 ^{bcd}	0.044 ^{abc}	0.075 ^{a}	0.073 ^{abc}
MNF-MAS- *Tricho* 15	0.067 ^{abcd}	0.143 ^{efgh}	0.123^{d}	0.045 ^{abc}	0.096^{efgh}	0.082^{c}
MNF-MAS- *Tricho* 16	0.067 ^{abcd}	0.112 ^{a}	0.108 ^{bcd}	0.044 ^{abc}	0.075 ^{a}	0.072 ^{abc}
MNF-MAS- *Tricho* 17	0.070^{cde}	0.131^{bcde}	0.108 ^{bcd}	0.047 ^{bc}	0.087^{bcde}	0.072 ^{abc}
MNF-MAS- *Tricho* 18	0.105 ^{i}	0.140^{cdefg}	0.151^{e}	0.070^{h}	0.094 ^{cdefg}	0.101 ^{d}
MNF-MAS- *Tricho* 19	0.068^{bcde}	0.126 ^{abc}	0.111 ^{bcd}	0.045 ^{abc}	0.084 ^{abc}	0.074 ^{abc}
MNF-MAS- *Tricho* 20	0.074^{def}	0.134^{bcdef}	0.105^{b}	0.050^{cd}	0.089^{bcdef}	0.070^{a}
MNF-MAS- *Tricho* 21	0.084^{gh}	0.141^{defgh}	0.109 ^{bcd}	0.066 ^{fgh}	0.094^{defgh}	0.073 ^{abc}
MNF-MAS- *Tricho* 22	0.066 ^{abc}	0.176 ^{k}	0.105^{b}	0.064^{fg}	0.117^{k}	0.070^{a}
MNF-MAS- *Tricho* 23	0.103 ^{i}	0.168^{jk}	0.153^{e}	0.069^{gh}	0.112^{jk}	0.103 ^{d}
GIZ-MAS-2 *Tricho* 24	0.079 ^{fg}	0.124 ^{ab}	0.110 ^{bcd}	0.052^{d}	0.082 ^{ab}	0.073 ^{abc}
AST-MAS- *Tricho* 25	0.064 ^{abc}	0.147^{fghi}	0.116 ^{bcd}	0.043 ^{ab}	0.098^{fghi}	0.077 ^{abc}
AST-MAS- *Tricho* 26	0.067 ^{abcd}	0.123 ^{ab}	0.119 ^{bcd}	0.044 ^{abc}	0.082 ^{ab}	0.079 ^{abc}

*Within columns, values with a common letter do not differ significantly (P<0.05), according to Duncan$_{a,b,c}$

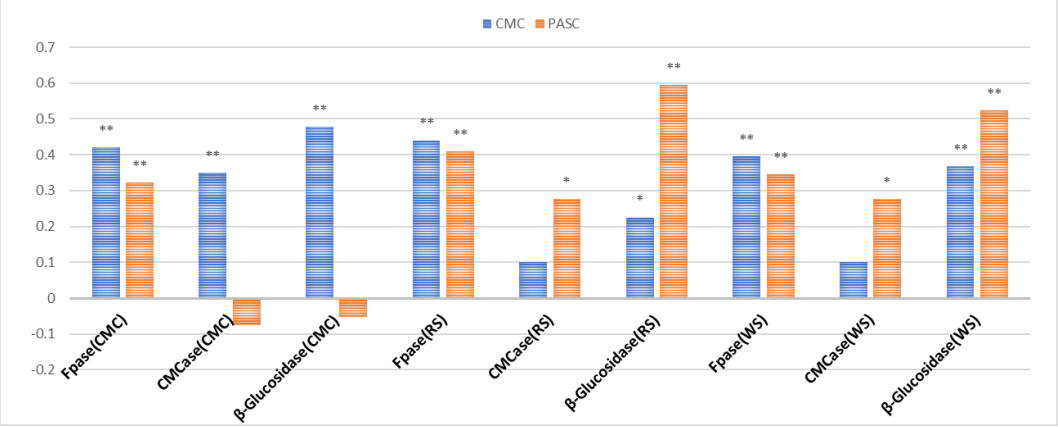
Data presented in Table (6) clearly showed the cellulases enzyme activities (measured as FPase, CMCase and β-glucosidase) in SSF (Rice straw) for all isolates. Generally, all isolates indicated outstanding endoglucanase activity, when compared to FPase and β-glucosidase. Five isolates demonstrated highly significant FPase activities namely, MNF-MAS-*Tricho*2, 6,12,18 and 23 (0.114 ,0.102, 0.103, 0.105 and 0.103 IU g^{-1}, respectively). MNF-MAS-Tricho 13 showed the lowest FPase activity (0.06 IU g^{-1}). While MNF-MAS-*Tricho*2,12,22 and 23 showed the highest significant CMCase activity (0.156, 0.159, 0.176 and 0.168 IU g^{-1}, respectively) and the lowest were GIZ-MAS-*Tricho* 8,11,14, and 16 (0.112 IU g^{-1}). As for β-glucosidase MNF-MAS-*Tricho* 2,6,12,18 and 23 were the highest in the same level without significant (0.15 IU g^{-1}) while MNF-MAS-*Tricho*1 were the lowest (0.076 IU g^{-1}).

In SSF of wheat straw two isolates demonstrated highly significant FPase activities namely, MNF-MAS-*Tricho*2, and18 (0.076 and 0.070 IU g^{-1}, respectively). MNF-MAS-*Tricho* 13 showed the lowest FPase activity (0.04 IU g^{-1}).

While MNF-MAS-*Tricho* 22 and 23 showed the highest significant CMCase activity (0.112 and 0.117 IU g^{-1}, respectively) and the lowest were GIZ-MAS-*Tricho* 8, MNF-MAS- *Tricho* 11,14, and 16 (0.075 IU g^{-1}). As for β-glucosidase MNF-MAS-*Tricho* 2 and 23 was the highest in the same level without significant (0.103 IU g^{-1}) while MNF-MSH-*Tricho*20 are the lowest (0.070 IU g^{-1}).

It has been revealed that most filamentous fungi are able to grow well under SSF and produce higher levels of extracellular enzymes and also other important metabolites than those under submerged fermentation (SmF). These findings were consistent with the results that most of the isolates belonged to *T. longibarchiatum* and *T. harzianum*. These species have been adopted in various industries because of their ability to secrete large amounts of protein and metabolites (Gupta *et al.,* 2014). Moreover, the utilization of rice straw for cellulase production has the potential to mitigate national rice straw waste, while also offering cost-effective advantages to the enzyme industry (Naher *et al.,* 2021). Different methods and different substrates have been used to compare between SSF and SmF for enzyme production; Firstly, Using SmF for enzyme production gave high yield and high quality. Different substrate showed higher enzymatic activity when compared on CMC substrate than the microcrystalline cellulose (Pirzadah *et al.,* 2014). The results showed that the activities of Filter paper enzyme (FPase), Endoglucanase (CMCase) and β-glucosidase were significantly affected by substrate mixture (Oni *et al.,* 2020)*T. reesei* shows higher enzymatic activity when compared with *T. viride* on CMC substrate than the microcrystalline cellulose. (Pirzadah *et al.,* 2014).

Data in Figure (3) showed the correlation between the qualitative and quantitative of cellulase activity. Data showed high positive correlation coefficient in enzyme activity in SSF and qualitative screening with (CMC & PASC) and the higher correlation showed with PACS and SSF rice straw was about (0.595**) in β-Glucosidase. Our obtained results are in coherence with (Florencio *et al.,* 2012) that studied the screening procedure using plates were compared with cellulase production under SSF. A correlation coefficient was correlation between the Congo red test and SSF, demonstrating that the two methodologies were in good agreement. Results showed that there are a negative correlation between SmF CMC substrate and PASC Qualitative screening in levels of β-Glucosidase and CMCase. Moreover, there were negative correlation in SmF (Avicel) substrate and CMC Qualitative screening in levels of FPase, CMCase and β-Glucosidase.



**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Figure (3): Correlation coefficient between qualitative screening with (CMC & PASC) substrate and quantitative in SmF (Avicel &CMC) substrate and SSF (RS&WS) substrate.

Firstly, Using SmF production for high yield and high quality, but it can't be used in second generation biofuel. Moreover, SmF was very expensive (Figure 4). Secondly, SSF are producing low yield of enzymes that can be used in second generation biofuel. Therefore, we need to improve the isolates to obtain high yield of enzymes and releasing high amount of glucose in SSF. Different substrate showed higher enzymatic activity when compared on CMC substrate than the microcrystalline cellulose (Pirzadah *et al.,* 2014).

Detection of Cellulase genes of *Trichoderma* species

The existence of cellulase genes was shown in Figure (5). The cellulase genes, *Chb* gene encoding cellobiohydrolase 2 (CBH2) recorded in all *Trichoderma* isolates. Moreover, the *Bgl* gene which was encoding endo-β-glucanase (BGL1) in all *Trichoderma* isolates except two isolates MNF-MAS-*Tricho* 5 and 6.

We carried out PCR for detection of important cellulase genes (Cbh and *Bgl*) in the genomes of the twenty-six *Trichoderma* isolates as shown in Figure (5). The primers utilized for amplification of *Cbh* gene yielded one band (230:270 bp) in all tested isolates (Figure 5a). Moreover, for Bgl, the PCR produced one band (420 bp) that appeared in all isolates except two isolate Named MNF-MAS-*Tricho*6 and 7 (Figure 5b) in our result we found more DNA band (750bp) appeared in 9 isolate MNF-MAS-*Tricho* 2, MNF-MAS-*Tricho* 4, MNF-MAS-*Tricho* 7, MNF-MAS-*Tricho* 11, MNF-MAS-*Tricho* 13, MNF-MAS-*Tricho* 19, MNF-MAS-*Tricho* 20, MNF-MAS-*Tricho* 22, MNF-MAS-*Tricho* 23.

Genes encoding the cello-bio-hydrolase enzyme (CBHI), designated as *cbhI*, The CBH enzymes are known as the key component of cellulase system that display an exo-type of digesting biopolymers, and the major product of their action on cellulose was cellobiose (Teeri, 1997) Based on amino acid sequence similarity, the CBH enzymes can be grouped into glycoside hydrolase families: GH6, GH7, and GH48(.β-glucosidase I (BGLI) gene plays a major role in the conversion of randomly cleaved cello oligosaccharides into glucose.

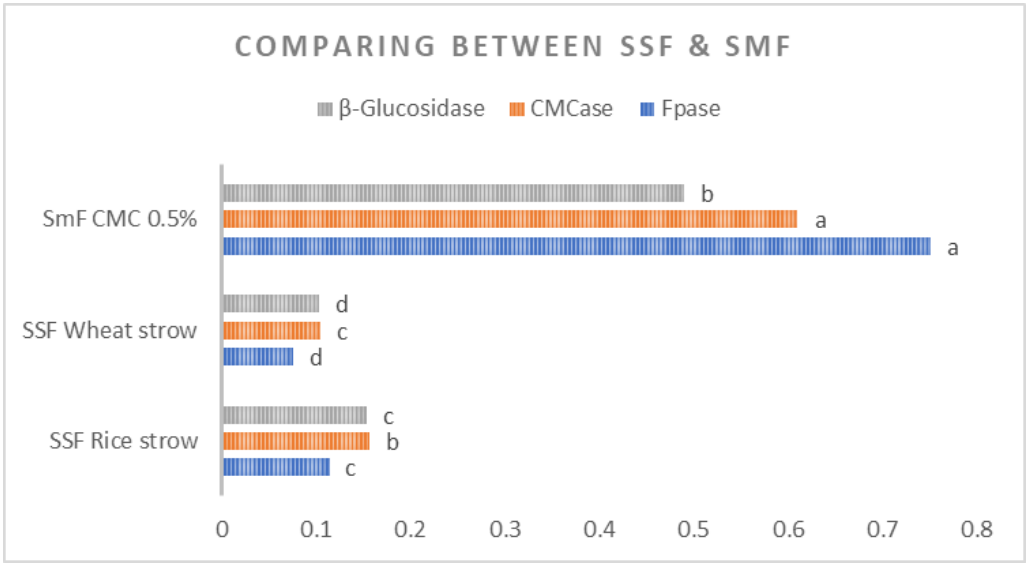


Figure (4): Comparison between different methods of enzyme production SSF (IU g-1) and SmF (IU ml-1)

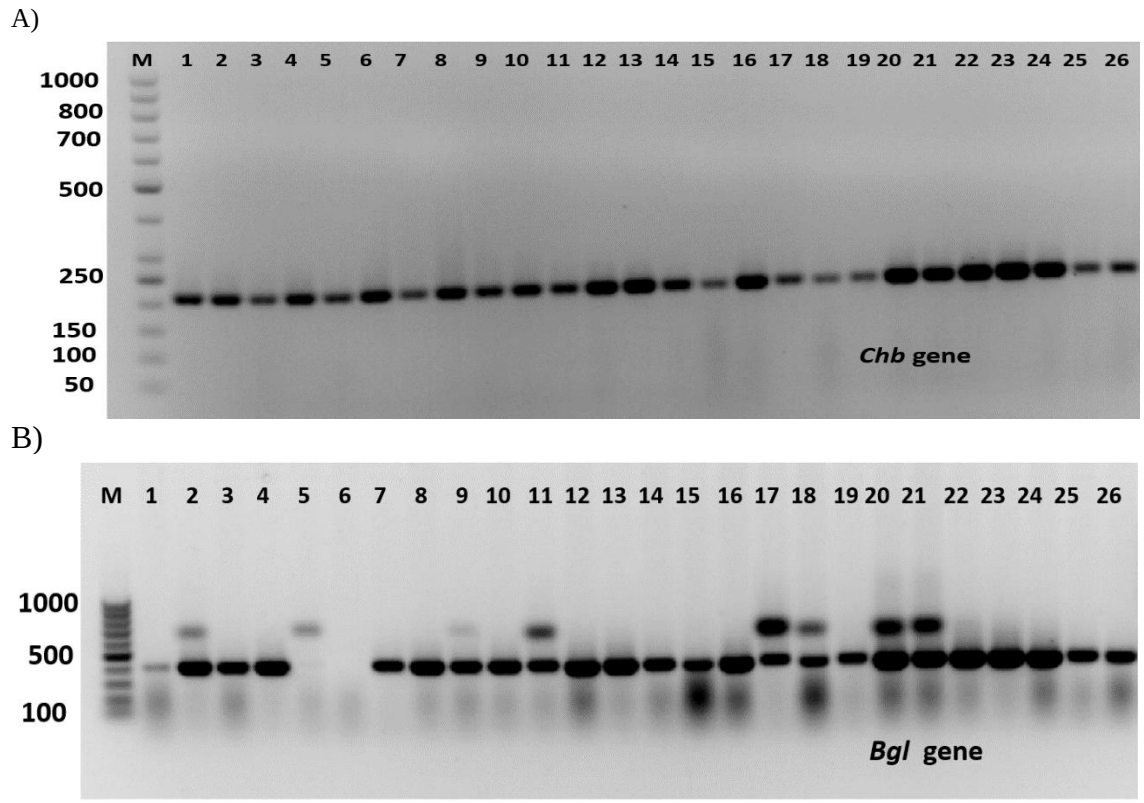


Figure (5): Agarose gel analysis of specific-PCR products from amplification a:*Cbh* gene with about 230 bp of *Trichoderma* isolates. Lane 1: 50 bp DNA Ladder, B: *Bgl* gene with about 420 bp of *Trichoderma* isolates(Lane 1: 100 bp DNA Ladder).

Conclusion

In our study we were able to detect the correlation between Qualitative and quantitative characters of cellulase enzymes. Theise was a significant relationship between tested Trichoderma isolates. Those isolates were collected from various locations. In fact, we recommended usage this approach screening various Trichoderma isolates using PASC and CMC plate. This will facilitate identification of most promising high cellulases isolates.

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دراسات مقارنة لنشاط إنزيمات السليوليز في الاختبارات الوصفية والكمية في فطر الترايكوديرما

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تم دراسة ستة و عشرون عزلة من فطر الترايكوديرما من خلال الاختبارات الوصفية لأنزيم السيليوليز من خلال اطباق CMC مع صبغة الكونجو الأحمر و كذلك اطباق PASC. بالإضافة الي الاختبارات الكمية من خلال التخمرات الصلبة والسائلة. كما تم عمل التخمرات السائلة علي بيئة ماندل مع CMC ٠,٥% تم قياس الانزيمات β- (FPase, CMCase , glucosidase) علي عدة أيام من اليوم ٢ و ٣و٤و ٦و ٧ . و تم قياس كفاءة هذه الانزيمات علي البيئات الصلبة علي قش الأرز و القمح. و تم قياس الارتباط بين القياسات الوصفية و الكمية في التخمرات السائلة و الصلبة واتضح ان الارتباط بين الوصفي و الكمي موجب المعنوية الي درجة كبير عند ٠,٥٩٥ بين β-Glucosidase وبين التخمرات الصلبة في قش الأرز. و بالمقارنة بين طرق إنتاج الانزيمات علي البيئة الصلبة و السائلة اتضح ان انتاج الانزيم من التخمرات السائلة تعطى كمية اكثر كما إنها ذات جودة مرتفعة. وبدراسة الجينات Chb وكذلك Bgl اتضح ان كل السلالات المدروسة يوجد بها هذه الجينات .