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# Production of Cellulases Via Solid State Fermentation by Different Fungal Isolates using Various Agricultural Wastes as a Substrate

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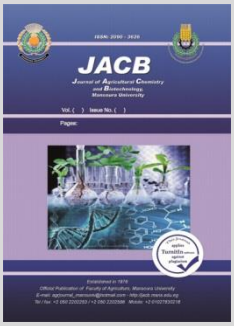


## ABSTRACT

Four local cellulolytic fungal isolates were chosen among 30 isolates according to CMC-ase production via SSF using clover straw as agricultural waste. Isolates were identified as *Trichoderma asperellum*, *Aspergillus flavus*, *Aspergillus flavipes*, *Aspergillus terreus*. Different agricultural wastes were used for CMC-ase production by the isolates and the combination of clover straw and wheat brane (1:1) proved to be the best for CMC-ase production by the four isolates, while low values of CMC-ase were noticed by all fungal isolates by using the other agricultural wastes. Molasses proved to be the best additional carbon source for CMC-ase production by all tested fungi, also cellulose and carboxymethyl cellulose enhanced CMC-ase production. All addition nitrogen sources improved CMC-ase production, yeast extract and ammonium sulphate were the best nitrogen sources for CMC-ase production by *Aspergillus flavus* and *Trichoderma asperellum*, while ammonium chloride proved to be the best nitrogen source for CMC-ase production by *Aspergillus flavipes* and *Aspergillus terreus*. *Trichoderma asperellum* is in the first order in CMC-ase production followed by *Aspergillus flavus*, *Aspergillus flavipes* and *Aspergillus terreus*. The Three isolates of *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus flavipes* proved to be good producers for FP-ase, while few amount of FP-ase is produced by *Trichoderma asperellum*.

***Keywords***: Agricultural wastes, Cellulases, CMC-ase, Solid state fermentation.

## INTRODUCTION

Over 150 years ago, French chemist Anselme Payen discovered and isolated cellulose from plants, the most abundant renewable bio-resource on Earth, primarily found in plant cell walls with production of 100 billion dry tons annually (Zhang and Lynd, 2004). Structurally, cellulose is a homo-polysaccharide composed of β-D-glucopyranose units, linked by β-(1-4)-glycosidic bonds (Read and Bacic, 2002). Cellobiose is the smallest repeating unit of cellulose and can be enzymatically converted into glucose units. Therefore, one of the applications of cellulases enzymes is the conversion of cellulose to glucose as a first step in the second-generation of ethanol production process (Zhang *et al*., 2024).

Consequently, numerous studies focus on reducing the production cost of these enzymes by identifying highly efficient microbial isolates and utilizing agricultural wastes as a primary substrate for enzymes production (Zhang *et al*., 2025).

Aerobic fungal cellulases play a crucial role in various industries Sajith *et al*., (2016); Zhang *et al*., (2024). (de França Passos *et al*., 2018) mentioned that three genera *Penicillium*, *Trichoderma* and *Aspergillus*, among all filamentous fungi are considered as model for cellulases production from bench to industrial scale. According to the International Union of Biochemistry and Molecular Biology (IUBMB) cellulases categorized into three categories i.e. cellobiohydrolases or exocellulases, endocellulases and β-glucosidases or cellobiases Schülein, (1988). These enzymes convert cellulose into glucose by targeting different parts of cellulose molecule during hydrolysis process by synergistic action. The main function of endoglucanase (EG or CMC-ase) (EC 3.2.1.4) is the hydrolysis of carboxymethyl cellulose (CMC). Based on their enzymatic action mechanisms and substrate specificities, three classes of cellulases are recognized: β-glucosidases (EC 3.2.1.21), exoglucanases (EC 3.2.1.74 and EC 3.2.1.91) and endoglucanases (EC 3.2.1.4), Teeri, (1997). Endo-β-glucanase breaks down cellulose chains randomly, producing cello-oligosaccharides, while exo-β-glucanase splits off cellobiose from exposed chain ends Raghuwanshi *et al.*, (2014). Cellobiose is then hydrolyzed by cellobiase to form glucose Lee *et al.*, (2000). This hypothesis is more widely applicable and accepted for the degradation of cellulosic biomass Lynd *et al*., (2002).

Solid State Fermentation mimics the natural environment of filamentous fungi, promoting growth similar to their native habitat. SSF requires adequate moisture for microbial growth, allowing fungal strains to colonize and form mycelial mats on solid substrates Singhania *et al*., (2009).

Nowadays, by using SSF, many enzymes, such as cellulases, is produced by utilizing lignocellulosic biomass as a substrate, which provides both surface as well as essential nutrients for fungal growth. Prévot *et al*., (2013) reported that the product yield is more in SSF than SmF process when comparing the same strain for both processes.

So, the use of SSF enables the utilization of cellulosic wastes as a primary substrate for cellulases enzyme production, significantly reducing production costs.

This study aims to isolate and identify high potential fungal strains capable of utilizing some available cellulosic agricultural wastes for cellulases production and to determine the most effective waste materials, either individually or in combinations, for optimal production.

## MATERIALS AND METHODS

### Microorganisms and culture conditions

The fungal isolates were obtained from soil samples collected from the Faculty of Agriculture farm, Mansoura

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University, during a screening study for fungal cellulase producers using modified Czapek Dox agar medium, where sucrose was replaced with carboxymethyl cellulose (CMC) as the sole carbon source. Isolates were identified to genus and species level whenever possible based on morphological characteristics on Czapek yeast extract agar (CYA) medium and microscopic investigation according to the following universally keys for identification of fungi: Domsch *et al*., (1993), for compendium of soil fungi; Raper and Fennell (1965), for *Aspergillus*. Samuels, *et al.,* (1999) for *Trichoderma* sp.

For maintenance, fungal isolates were grown on potato dextrose agar (PDA) at 30°C for 7 days, then stored at 4°C. Stock cultures were subculture onto fresh PDA every two months under the same incubation conditions.

**Inoculum preparation:** Fungal spore suspension was prepared by adding 10 mL of sterile 0.9% sodium chloride solution to a 7-day old PDA slant culture. The surface of the culture was gently scraped with a sterile wire loop to release spores. The resulting suspension was then counted using a Neubauer chamber and adjusted to a final concentration of 1 × $10^6$ spores/mL.

**Cellulases/CMC-ase production**

For SSF, the basal medium consisted of 4 g of dry substrate in a 250 mL Erlenmeyer flask, moistened with 6 mL of tap water. The flasks containing medium were autoclaved at 121 °C for 20 min. Each sterilized medium was inoculated with 1 mL of a spore suspension (1 × $10^6$ spores/mL) and incubated at 30 ± 2 °C for 7 days.

**Extracellular Cellulases extraction**

Following solid-state fermentation, the crude enzyme was extracted by shaking the fermented substrate with 40 mL of distilled water at 150 rpm (30 ± 2 °C) for 1 hour. The resulting slurry was filtered through double-layered gauze. The pooled extracts were then centrifuged at 5,000 rpm for 20 minutes to remove cells, spores, and residual substrate particles. The clear supernatant obtained after centrifugation served as the crude enzyme preparation for subsequent cellulases assays.

**Extracellular Cellulases assay**

Carboxymethyl cellulase and Filter paper-ase (FP-ase) activity were determined using the dinitrosalicylic acid (DNS) method which measures the reducing sugars released from the hydrolysis of the substrate (Miller, 1959), while β-glucosidase (cellobiase) was estimated using the glucose oxidase/peroxidase reagent (Wood and Bhat, 1988).

To determine CMC-ase activity, 0.9 mL of 1% carboxymethyl cellulose (CMC) solution (prepared in 0.1 mM citrate buffer, pH 5.5) was mixed with 0.1 mL of crude enzyme. The reaction mixture was incubated at 50 ºC for 10 min. The released reducing sugars were determined using 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The reaction was stopped by adding 1.5 mL (DNS) reagent and the content was boiled for 5 min. to develop the color, the reaction mixture was cooled down and diluted by distilled water. Absorbance of samples was measured at 540 nm against a blank containing all the reagents except crude enzyme. glucose is used as standard for cellulases activity. One unit of CMC-ase is defined as the amount of enzyme that liberates 1 µmol of glucose equivalents per minute.

FP-ase activity was determined according to the International Union of Pure and Applied Chemistry (IUPAC) standard method (Ghose, 1987). Briefly, a 1 × 6 cm strip of Whatman No. 1 filter paper (50 ± 0.1 mg), cut into small pieces in 1 mL of citrate buffer, was incubated with 0.5 mL of crude enzyme at 50°C for 1 hour. The released reducing sugars were quantified using 3 mL of DNS reagent, as described previously. One International Unit (IU) or Filter Paper Unit (FPU) of FP-ase activity is defined as the amount of enzyme that releases 2 mg of glucose per hour under the specified assay conditions.

Modified method of Wood and Bhat, (1988) was used to determine β-glucosidase (cellobiase) activity, in brief, 0.9 mL of 1% cellobiose solution (prepared in 0.1 mM citrate buffer, pH 5.5) was mixed with 0.1 mL of crude enzyme. The reaction mixture was incubated at 50 ºC for 10 min and stopped by adding 1 ml HCL (2 N), then 2 ml of glucose oxidase reagent was added and the reaction allowed to proceed at 37°c for 30 min. Finally, the reaction mixture was mixed well and the absorbance was read at 520 nm against the blank.

**Effect of different agricultural wastes on CMC-ase production:**

To identify a cost-effective substrate for solid-state fermentation (SSF) that enhances cellulase production, various agricultural wastes, including wheat bran (WB), rice husk (RH), corn cobs (CC), clover straw (CS), and wheat straw (WS), were collected from local farms and markets. These substrates were tested both individually and in 1:1 (w/w) combinations.

**Effect of additional carbon sources**

CMC-ase production was investigated using basal medium supplemented with various carbon sources (1% w/w), including starch, maltose, sucrose, glucose, cellulose, molasses, and CMC. A control flask without any additional carbon source was used for comparison. CMC-ase activity was assessed after 7 days of incubation at 30 ± 2°C.

**Effect of additional nitrogen sources:**

The impact of nitrogen sources on CMC-ase production was assessed by supplementing the fermentation medium with 0.5% (w/w) of various organic and inorganic nitrogen sources. Seven different sources were tested, and flasks without additional nitrogen source served as control. After inoculation by 1 mL spore suspension, the flasks were incubated at 30 ± 2°C for 7 days.

## RESULTS AND DISCUSSION

**Screening of fungal isolates exhibiting potent cellulolytic activity:**

Although 30 isolates of cellulolytic fungi were isolated and purified, only four of them –morphologically different- have been chosen according to its capacity of CMC-ase production. Data in (Table 1) show that the isolates No 4, 8, 20 and 22 were the most active CMC-ase producers after 7 days of incubation at 30°C±2 using clover straw under solid state fermentation. CMC-ase production by isolates No 4, 8, 20 and 22 were 25.62, 26.60, 22.63 and 20.88 IU/gds, respectively. On the other hand, a few amounts of CMC-ase 4.99, 4.31, 3.91 and 3.28 IU/gds were produced by isolates No 9, 18, 21 and 30, respectively. previous studies on cellulase showed that high level of extracellulases capable of solubilizing cellulose are produced by fungi. Most of the cellulases enzymes exploited for industrial applications are produced by filamentous fungi such as *Trichoderma, Fusarium* , *Penicillium* and

*Humicola*. Picart *et al,* (2007) and Prasanna *et al*.(2016) reported that considerable number of cellulases has been produced from fungal species such as *penicillium* sp. and Santos *et al*. (2016) used *Aspergillus niger* and *Rhizopus sp.* for cellulases production. Toor and Ilyas (2014) produced cellulases from *Aspergillus ornatus* Also. Qurat-ul-Ain *et al*., (2012) produced cellulases from *Aspergillus niger* and *Trichoderma longibrachiatum.*

**Identification of the most active cellulolytic fungal isolates**

Four fungal isolates were selected based on their CMC-ase productivity, considering their morphological diversity. The identification of the selected fungal isolates was carried out based on morphological characteristics and microscopic investigation as observed under light microscope (Figure 1).

Smears of the selected fungi were prepared in Lactophenol cotton blue and examined with a microscope and results are mentioned in Table (2).

**Table 1. Screening for cellulase (CMC-ase) production by different fungal isolates under SSF using clover straw as a substrate after 7 days of incubation at 30±2° C:**

| Isolate No. | CMC-ase production IU/gds | Isolate No. | CMC-ase production IU/gds |
|---|---|---|---|
| 1 | 20.44 ± 0.64$^{e}$ | 16 | 8.48 ± 0.13 |
| 2 | 24.53 ± 0.01$^{b}$ | 17 | 19.62 ± 0.20$^{f}$ |
| 3 | 21.39 ± 0.25$^{d}$ | 18 | 4.31 ± 0.60 |
| 4 | 25.62 ± 0.65$^{a}$ | 19 | 12.35 ± 0.42 |
| 5 | 14.16 ± 0.51 | 20 | 22.63 ± 0.34$^{c}$ |
| 6 | 10.48 ± 0.20 | 21 | 3.91 ± 0.18 |
| 7 | 17.76 ± 0.62 | 22 | 20.88 ± 0.25$^{e}$ |
| 8 | 26.60 ± 0.16$^{a}$ | 23 | 11.28 ± 0.09 |
| 9 | 4.99 ± 0.18 | 24 | 9.10 ± 0.13 |
| 10 | 7.56 ± 0.07 | 25 | 6.30 ± 0.26 |
| 11 | 18.98 ± 0.44$^{g}$ | 26 | 15.77 ± 0.20 |
| 12 | 12.18 ± 0.34 | 27 | 5.98 ± 0.54 |
| 13 | 8.79 ± 0.56 | 28 | 7.91 ± 0.39 |
| 14 | 7.23 ± 0.33 | 29 | 7.61 ± 0.51 |
| 15 | 12.15 ± 0.22 | 30 | 3.28 ± 0.33 |
| LSD at 0.05 = 1.05 | | | |

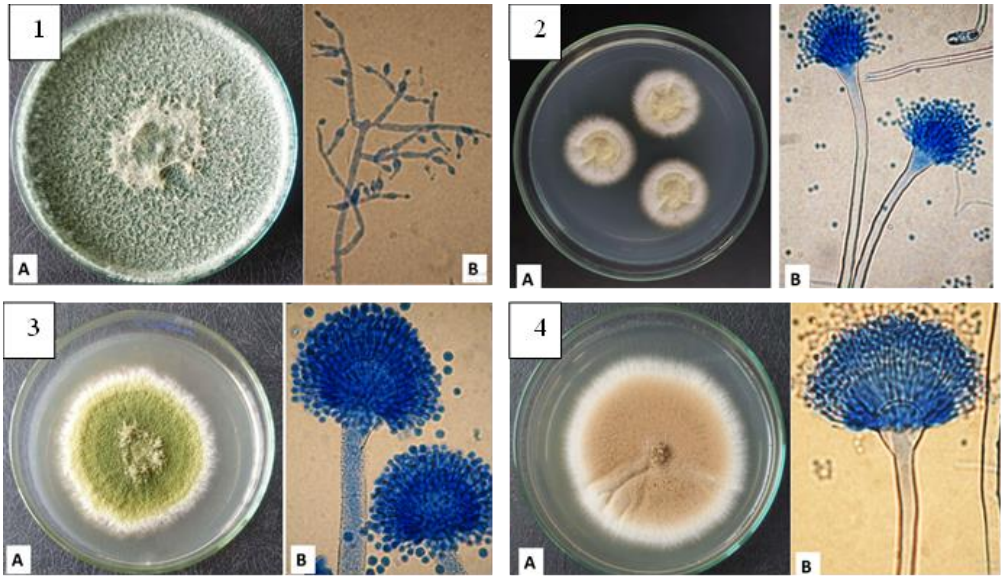


**Figure 1. A) Colonies characterization on CYA medium and (B) microscopic investigation of (1) Trichoderma asperellum, (2) Aspergillus flavipes, (3) Aspergillus flavus and (4) Aspergillus terreus.**

**Table 2. Morphological characteristics of the selected local fungal CMC-ase producers:**

| Isolate No. | Colony and microscopic examination | Fungal strain |
|---|---|---|
| 2 | Dull-green colony on CYA at 28° C.<br>Conidiophores are branched.<br>Verticillae ampulliform phialides with slightly ovoidal conidia. | *Trichoderma asperellum* |
| 4 | Yellow-green colony on CYA at 28° C.<br>Conidial heads are small (about 220 µm in diameter.<br>Conidiophores are rough-walled, hyaline.<br>Vesicles appear as globose in shape, and 25 µm in diameter.<br>Biseriate conidiogenous cells and chains of spherical conidia. | *Aspergillus flavus* |
| 20 | Pale yellow colony on CYA at 28° C.<br>Conidial heads sparse, loosely columnar to radiate.<br>Conidiophores are smooth-walled, hyaline.<br>Vesicles appear subspherical<br>Biseriate conidiogenous cells and chains of spherical conidia. | *Aspergillus flavipes* |
| 22 | Cinnamon-brown colony on CYA at 28° C.<br>Conidial heads are columnar.<br>Conidiophores are smooth-walled, hyaline.<br>Vesicles appear as globose in shape.<br>Biseriate conidiogenous cells and chains of small spherical conidia. | *Aspergillus terreus* |

**Effect of different agricultural wastes on CMC-ase production:**

The results on the effect of different agricultural wastes on CMC-ase production by the four selected fungal isolates (Table 3) show that the kind of agricultural wastes either individual or in combination and organism were greatly affected CMC-ase production by all fungal isolates. (CS+ WB) proved to be the best agricultural waste for CMC-ase

production by the fungal isolates. Enzyme production after 7 days at 30±2°C was 26.31, 27.34, 27.93 and 29.62 IU/gds by *Aspergillus flavus*, *Trichoderma asperellum*, *Aspergillus flavipes* and *Aspergillus terreus*, respectively. Also, the four fungi produced good amount of CMC-ase with WB and CS, the amounts of enzymes produced were 21.92, 26.15, 22.62 and 24.15 IU/gds by *Aspergillus flavus*, *Trichoderma asperellum*, *Aspergillus flavipes* and *Aspergillus terreus*, respectively, when WB was used as agric. waste, while by using CS as substrate the amounts were 25.62, 24.56, 24.35 and 21.32, respectively. On the other hand, low values of CMC-ase were noticed by all fungal isolates when CS, CC, WS, WS+CC, RH+WS, RS+CC was used as agricultural wastes. Many investigators produced cellulases from fungi using agricultural wastes. Toor and Ilyas (2014) used *Cicer arietinum* for cellulases production from *Aspergillus ornatus*. corn stover was used by Gao *et al*. (2008) for cellulases production from *Aspergillus terreus* M1, while Santos *et al*. (2016) used prickly pear for cellulases production by *Aspergillus niger* and *Rhizopus* sp. Qurat-ul-Ain *et al*., (2012) produced cellulases from *Aspergillus niger* , *Trichoderma longibrachiatum* using rice husk and saw dust By using wheat bran, Dos Reis *et al*., (2013) produced cellulases from *Penicillium decumbens*. Hideno *et al*., (2011) used rice straw for cellulases production from *Acremonium cellulolyticus*. Also wheat straw was used by Lechner and Papinutti, (2006) for cellulases production from *Lentinus tigrinus* and Membrillo *et al*., (2008) produced cellulases from *Pleurotus ostreatus* using sugarcane bagasse.It is obvious from data presented in Table (3) that CMC-ase was not of the same order as the used agricultural wastes.

**Table 3. Effect of using different agricultural wastes (individual and in combinations) on CMC-ase production by 4 selected fungal isolates after 7 days of incubation at 30±2° c:**

| Isolate | *Aspergillus flavus* | *Trichoderma asperellum* | *Aspergillus flavipes* | *Aspergillus terreus* |
|---|---|---|---|---|
| Waste | CMC-ase production (IU/gds) | | | |
| WB | 21.92$^{b}$±1.55 | 26.15$^{a}$±1.55 | 22.62$^{c}$±1.06 | 24.15$^{b}$±0.75 |
| CS | 25.62$^{a}$±1.17 | 24.56$^{b}$±0.84 | 24.35$^{b}$±1.12 | 21.32$^{c}$±0.76 |
| RH | 1.48±0.87 | 4.56±0.49 | 0.46±0.35 | 2.52±0.43 |
| CC | 2.34±0.92 | 2.54±0.51 | 1.32±0.56 | 3.18±0.35 |
| WS | 4.22±0.41 | 7.95±0.51 | 1.77±0.32 | 3.42±0.60 |
| CS+WS | 12.68±0.84 | 12.62±0.99 | 12.37±1.19 | 13.09±0.65 |
| CS+RB | 8.59±0.42 | 11.38±0.91 | 9.88±0.78 | 16.51±0.99 |
| CS+CC | 9.31±1.04 | 13.58±0.71 | 8.32±1.17 | 11.62±0.64 |
| CS+WB | 26.31$^{a}$±1.22 | 27.34$^{a}$±0.45 | 27.93$^{a}$±0.62 | 29.62$^{a}$±1.12 |
| WS+CC | 4.46±0.76 | 7.51±0.56 | 0.44±0.11 | 5.51±1.14 |
| WB+RH | 14.83±0.82 | 18.28±1.44 | 13.29±0.94 | 14.03±1.44 |
| WB+WS | 18.36$^{c}$±1.42 | 23.70±1.88 | 18.89±0.72 | 12.55±0.91 |
| RH+WS | 2.23±0.28 | 1.27±0.24 | 1.73±0.46 | 3.66±1.30 |
| RH+CC | 1.56±0.33 | 5.94±0.33 | 0.35±0.11 | 3.46±1.06 |
| WB+CC | 9.25±0.43 | 15.73±0.65 | 10.97±1.35 | 11.92±0.76 |
| LSD at 0.05 | 1.53 | 1.55 | 1.37 | 1.52 |

**Effect of additional carbon sources:**

Carbon source significantly impacts cellulases production, because the fact that cellulases are inducible enzymes that are expressed in response to different carbon sources present in the fermentation media Saini *et al*., (2017) and Zhang Y. *et al*., (2017).

Data in Table (4) show the effect of additional carbon sources on CMC-ase production by the four selected fungal isolates. Molasses proved to be the best additional carbon source for CMC-ase production by all fungal isolates. By molasses addition, CMC-ase production after 7 days of incubation at 30±2°C by *Aspergillus flavus*, *Trichoderma asperellum*, *Aspergillus flavipes* and *Aspergillus terreus* were 30.83, 30.56, 28.56 and 25.65 IU/gds, respectively. Also, CMC seemed to be an active additional carbon source for CMC-ase production especially with *Trichoderma asperellum* (31.75 IU/gds) and *Aspergillus flavus* (29.92 IU/gds) but it slightly enhanced CMC-ase production by *A. flavipes* (24.75 IU/gds) and *A. terreus* (23.52 IU/gd).

Additionally, cellulose induced CMC-ase secretion in all tested fungal isolates. By addition cellulose CMC-ase production reached 28.39, 31.94, 25.94 and 25.00 IU/gds by *A. flavus*, *T. asperellum*, *A. flavipes* and *A. terreus* respectively. On the other hand, some carbon sources such as starch, maltose, sucrose and glucose did not enhance CMC-ase secretion by *A. flavus* and *T. asperellum*. With regard to *Aspergillus flavipes* and *Aspergillus terreus*, all additive carbon sources enhanced CMC-ase production.

**Table 4. Effect of adding different additional carbon sources on CMC-ase production by 4 selected fungal isolates after 7 days of incubation at 30±2° c:**

| Isolate | *A. flavus* | *T. asperellum* | *A. flavipes* | *A. terreus* |
|---|---|---|---|---|
| Carbon source | CMC-ase production (IU/gds) | | | |
| Control | 25.87$^{c}$±1.22 | 26.30±1.35 | 22.30±1.17 | 21.66±1.08 |
| Starch | 24.03±1.08 | 26.21±1.11 | 26.21$^{b}$±1.44 | 24.08bc±0.47 |
| Maltose | 22.88±0.38 | 25.46±0.78 | 24.46±0.66 | 22.96±0.58 |
| Sucrose | 25.40±0.95 | 24.82±0.59 | 24.82±0.53 | 23.75±1.09 |
| Glucose | 25.38±1.16 | 27.03$^{b}$±1.34 | 24.03±0.38 | 22.54±1.00 |
| Cellulose | 28.39$^{b}$±0.55 | 31.94$^{a}$±0.47 | 25.94$^{bc}$±0.90 | 25.00ab±0.64 |
| Molasses | 30.83$^{a}$±0.88 | 30.56$^{a}$±0.72 | 28.56$^{a}$±0.58 | 25.65a±0.44 |
| CMC | 29.92ab±0.40 | 31.75$^{a}$±1.03 | 24.75±0.62 | 23.52±1.17 |
| LSD at 0.05 | 1.54 | 1.69 | 1.48 | 1.48 |

Data show that cellulose proved to be a good inducer for CMC-ase production by all fungal isolates, similar results were obtained by Dashtban *et al*., (2011) who reported that the optimal cellulase production from *Hypocrea jecorina* QM6a, QM9414, and RUTC-30 was achieved in medium microcrystalline cellulose was the sole carbon source. Also similar results on the effect of CMC on cellulases production by the saprophytic fungus *Phlebia gigantean* were reported by Niranjane *et al*., (2007) who found that CMC proved to be the best carbon source for cellulases production. In the following experiments molasses is chosen among the active additional carbon sources because of its inexpensive price.

**Effect of additional nitrogen sources:**

Nitrogen is an important factor affecting protein secretion in fungi, and so different nitrogen sources can be used in fermentation medium for production of cellulases .

Data in (Table 5) show the effect of additional nitrogen sources on CMC-ase production by the fungal isolates after 7 days of incubation at 30±2°C. Data show that yeast extract and Ammonium sulphate were the best nitrogen sources for CMC-ase production by *Aspergillus flavus* and *Trichoderma asperellum*, by using yeast extract, the enzyme production attained 43.46 IU/gds and 42.56 IU/gds, respectively. While ammonium chloride proved to be the best nitrogen source for CMC-ase production by both *Aspergillus flavipes* and *Aspergillus terreus*, their CMC-ase production were 39.67 and 34.47 IU/gds respectively. It is interesting to note that all nitrogen additive sources improved CMC-ase production with all fungal isolates compared to control without nitrogen addition. Also Kachlishvili *et al*., (2006) found that ammonium sulfate, ammonium hydrogen phosphate and ammonium chloride can be used as inorganic nitrogen sources for cellulases production. On the other hand, peptone, beef extract or yeast extract, tryptone or soyabean meal can be used as organic nitrogen source.

The data obtained concerning the effect of yeast extract on cellulases production by the tested fungi are in accordance with the results obtained by Prasanna *et al.* (2016) who found that optimum cellulase activity was obtained from *penicillium sp.* cultivated on yeast extract containing medium. Also, Saini *et al*., (2017) mentioned that the optimum production of cellulases from *Trichoderma reesei* was obtained when cultivated on parthenium biomass containing peptone, yeast extract or ammonium molybdate, as nitrogen source.

**Table 5. Effect of adding different additional nitrogen sources on CMC-ase production by 4 selected fungal isolates after 7 days of incubation at 30±2° c:**

| Isolate | *A. flavus* | *T. asperellum* | *A. flavipes* | *A. terreus* |
|---|---|---|---|---|
| Nitrogen source | CMC-ase production (IU/gds) | | | |
| Control | 31.14$^{e}$±0.25 | 28.64$^{e}$±0.13 | 29.86$^{f}$±2.02 | 24.35$^{d}$±0.70 |
| Ammonium chloride | 41.98$^{ab}$±0.74 | 35.48$^{b}$±2.80 | 39.67$^{a}$±1.98 | 34.47$^{a}$±2.40 |
| Ammonium phosphate | 40.93a$^{b}$±1.40 | 31.75±1.72 | 37.25$^{abc}$±1.22 | 32.65$^{ab}$±0.89 |
| Ammonium sulfate | 38.80±2.08 | 42.56$^{a}$±0.19 | 38.65$^{ab}$±1.56 | 33.65$^{ab}$±2.20 |
| Peptone | 39.99±0.55 | 34.60$^{bc}$±0.76 | 35.24$^{cde}$±2.71 | 32.65$^{ab}$±1.00 |
| Yeast extract | 43.46$^{a}$±0.51 | 36.39$^{b}$±2.48 | 36.55$^{bcd}$±0.91 | 33.20$^{ab}$±1.77 |
| Beef extract | 42.39$^{ab}$±0.18 | 32.05±1.12 | 33.54±2.00 | 31.24$^{bc}$±2.30 |
| Ammonium nitrate | 41.85$^{ab}$±0.38 | 27.03$^{e}$±0.79 | 32.54±1.11 | 28.65$^{c}$±2.22 |
| LSD at 0.05 | 1.70 | 2.70 | 3.07 | 3.13 |

Data in (Table 6) show production of CMC-ase, FP-ase and β-glucosidase by the four fungal isolates after 7 days of incubation at 30±2°C using the optimum agricultural waste, additional carbon and nitrogen source, Data revealed that, for CMC-ase production, all the fungal isolates produced the enzyme. *Trichoderma asperellum* is in the first order of production (41.68 IU/gds) followed by *Aspergillus flavus* (40.65 IU/gds), *Aspergillus flavipes* (38.75 IU/gds) and *Aspergillus terreus* (35.47 IU/gds). As for FP-ase, only three fungal isolates proved to be good producers for enzyme production, the production was 56.97, 47.98 and 43.84 IU/gds for *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus flavipes*, respectively. On the other hand few amount of FP-ase (4.59 IU/gds) is produced by *Trichoderma asperellum.* Data indicated that none of the fungal isolates produced considerable amounts of β-glucosidase, the units were 1.00, 0.92, 5.80 and 4.28 IU/gds for *Aspergillus flavus*, *Trichoderma asperellum*, *Aspergillus flavipes* and *Aspergillus terreus*, respectively.

**Table 6. Production of CMC-ase, FP-ase and β-glucosidase by 4 selected fungal isolates after 7 days of incubation at 30±2° c:**

| Enzyme | CMC-ase | FP-ase | β-glucosidase |
|---|---|---|---|
| Fungal isolate | IU/gds | FPU/gds | U/gds |
| *Aspergillus flavus* | 40.65±0.59 | 56.97±2.7 | 1.00±0.51 |
| *Trichoderma asperellum* | 41.68±1.94 | 4.59±0.9 | 0.92±0.21 |
| *Aspergillus flavipes* | 38.75±0.9 | 43.84±2.6 | 5.80±0.98 |
| *Aspergillus terreus* | 35.47±0.93 | 47.98±3.7 | 4.28±0.74 |

Data clearly show that none of the fungal isolates secreted the three enzymes of cellulases. *Aspergillus flavus*, *Aspergillus flavipes*, *Aspergillus terreus* are good producers for CMC-ase and FP-ase but failed to produce considerable amount of β-glucosidase. On the other hand, *Trichoderma asperellum* produced the highest amount of CMC-ase and produced the lowest amount of FP-ase and β-glucosidase. Reese *et al*., (1950) reported that few of fungi and bacteria produced the three cellulases capable of degradation of crystalline cellulose.

## CONCLUSION

a wide variety of local agro-cellulosic residues can be utilized for fungal cellulase production. In this study, four cellulolytic fungal isolates, identified as *Aspergillus flavus*, *Trichoderma asperellum*, *Aspergillus flavipes* and *Aspergillus terreus*, were used to produce cellulases using agroresidues in an attempt to reduce production cost.

The effect of agricultural wastes on cellulase production revealed that both the type of waste and fungus significantly impacted enzyme production, consistent with findings by Kamande *et al*. (2024) that cellulase production via SSF is greatly influenced by lignocellulosic substrates.

The current study's results revealed that using molasses as additional carbon source induced CMC-ase production in all fungal isolates.

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# إنتاج إنزيمات السليوليز بطريقة التخمير الصلب بواسطة عزلات فطرية مختلفة باستخدام مخلفات زراعية متنوعة كمادة أساسية

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## الملخص

تم اختيار أربع عزلات فطرية محلية ذات قدرة على تحليل السليلوز من بين ٣٠ عزلة بناءً على إنتاج إنزيم CMC-ase من خلال التخمير الصلب باستخدام تبن البرسيم كمخلفات زراعية. تم تعريف العزلات على أنها *Trichoderma asperellum* ، *Aspergillus flavus*، *Aspergillus flavipes*، *Aspergillus terreus* . تم استخدام مخلفات زراعية مختلفة لإنتاج CMC-ase من قبل العزلات، وأثبتت تركيبة قش البرسيم وردة القمح (١:١) أنها الأفضل لإنتاج CMC-ase من قبل العزلات الأربع، بينما لوحظت قيم منخفضة من CMC-ase قبل جميع العزلات الفطرية عند استخدام المخلفات الزراعية الأخرى. أثبت المولاس أنه أفضل مصدر كربوني إضافي لإنتاج CMC-ase من قبل جميع الفطريات المختبرة، كما عزز السليلوز إنتاج CMC-ase. حسنت جميع مصادر النيتروجين الإضافية إنتاج CMC-ase ، وكان مستخلص الخميرة وكبريتات الأمونيوم أفضل مصدري نيتروجين لإنتاج CMC-ase من قبل *Aspergillus flavus* و*Aspergillus flavipes* بواسطة CMC-ase بينما أثبت كلوريد الأمونيوم أنه أفضل مصدر نيتروجين لإنتاج *Trichoderma asperellum* و*Aspergillus terreus*. يأتي فطر *Trichoderma asperellum* في المرتبة الأولى في إنتاج CMC-ase من العزلات المختبرة يليه *Aspergillus flavus* ، *Aspergillus flavipes* و *Aspergillus terreus*. أثبتت ثلاث عزلات وهي *Aspergillus flavus* ، *Aspergillus flavipes* و*Aspergillus terreus* أنها تنتج إنزيم FP-ase بصورة جيدة ، بينما يتم إنتاج كمية قليلة من FP-ase بواسطة *Trichoderma asperellum* . ولقد أوضحت النتائج المتحصل عليها أن جميع العزلات لم تستطع أن تنتج كميات كبيرة من إنزيم β-glucosidase .