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Research Article

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Isolation, identification and screening of cellulolytic activity of some fungi from different sources and localities in Egypt

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KEY WORDS

Cellulolytic fungi, Cellulolytic activity, Congo red, Clear zone, Screening

ABSTRACT

Cellulases are a series of hydrolytic enzymes that can hydrolyze cellulosic biomass into a simpler sugar. In the present study, thirty-one fungal species were isolated from different places in Al-Gharbia and Aswan governorates. These fungal species were identified and found to belong to twelve genera "*Trichoderma, Cladosporium, Aspergillus, Botritrichom, Rhizopus, Cephalosporium, Fusarium, Penicillium, Mucor, Circinella, Alternaria* and *Rhodotorula"*. All identified fungal species were screened for cellulolytic activity based on clear zone diameter using 0.1% congo red. Out of thirty-one fungal species, 27 species showed cellulolytic activity and the highest activity was recorded by *Trichoderma viride* and isolate No. 1 of *Aspergillus niger*. All 27 fungal species were tested for three cellulolytic enzymes as "carboxymethyl cellulase (CMCase), β- glucosidase (βGase), and filter-paper cellulase (FPase)". The highest total cellulolytic activity for three cellulolytic enzymes was recorded by isolate No. 1 of *Aspergillus niger* (79.3 U/ml). Among all tested isolates of *Aspergillus niger* isolate No. 1 isolated from Tanta was the best one.

1. Introduction

Cellulose is the basic structure of the plant cell wall **(Pandey *et al.*, 2020)**. It is unbranched, linear homopolymer of glucose subunits linked by β 1,4 glycosidic linkages **(Rathore *et al.*, 2014)**. Also, it is one of the most important carbon sources all over the world so, the degradation of cellulosic substances is significant for the global carbon source **(Pandey *et al.*, 2020).** Degradation of cellulosic substances may be achieved using chemicals, enzymes, or a combination of them **(Reddy *et al.*, 2014).**

Cellulase is an extracellular enzyme secreted by a variety of microorganisms that causes cellulose hydrolysis by the synergistic action of the enzymes constituent. Three main types of cellulases were described by **(Klysov, 1990).** At the first, Endoglucanase (Endo 1,4 β glucanases) breaks down the internal bond to destroy the cellulose crystalline structure to form individual polysaccharide chains, then cellobiohydrolase (Exo-cellulase) cleaves 2-4 units from the ends of the exposed chains produced by endocellulases, finally cellobiase (β - galactosidase) hydrolyzes the cellobiose into individual glucan. These cellulolytic enzymes work together to complete the hydrolysis of crystalline cellulose **(Sari *et al.*, 2016).**

Cellulases are the most widely utilized commercial enzyme in a variety of industries, including biofuels, drinks, food, feed, textiles, paper, agriculture, and pharmaceuticals. These enzymes are employed alone or in combination with other enzymes in these industries **(Ejaz *et al.*, 2021).** As a result, several studies are being conducted to find new microorganisms capable of producing cellulase with higher activity **(Duarte *et al.*, 2017).** Fungi, bacteria, and actinomycetes are microorganisms that produce cellulases. However, fungi are the primary producers of extracellular cellulase enzyme. High productivity, low cost, rapid production, and ease of recovery from the medium are just a few of the many benefits associated with an enzyme produced from fungus **(Sari *et al.*, 2017).**

This study aimed to isolate, identify and screen fungi that have higher cellulolytic activity.

2. Material and methods

2.1. Samples collection

Eight samples (six samples from Tanta, Al-Gharbia governorate, Egypt, and two samples from Isna, Aswan governorate, Egypt) were investigated.

Samples from Al-Gharbia governorate are two different soil (1, 2), water sample, two agricultural wastes (wheat straw and linen straw), and compost sample from the Faculty of Agriculture, Tanta University. From Aswan Governorate, two soil samples were collected (3, 4). Agricultural wastes and Soil samples were air-dried and stored in sterilized bags at room temperature. Linen straw was soaked for 4 days, and Linen soaked water was stored in a sterilized bottle at 4°C.

2.2. Isolation of Fungi from different collected samples

Fungi were isolated from the above-mentioned collected samples. The isolates were cultured on potato dextrose agar (PDA) medium containing antibacterial streptomycin (100 mg/l). The agricultural wastes were chopped into small pieces (5 mm) and spread on the surface of the plate containing a sterilized PDA media. One ml of liquid sample was distributed on a sterilized plate surface containing sterilized PDA medium. Serial dilutions for soil samples and compost were performed as **Celestino *et al.*, (2014) and Cruz-Lachica *et al.*, (2018)** by mixing 1gm of the sample well with 100 ml sterilized distilled water then, moving 1 ml of this mixture to a tube containing 9 ml sterilized distilled water and shaking thoroughly. After that, dilutions were subsequently made up to 10^{-5}, then 1 ml of each dilution was plated in a sterilized PDA media. The plates were incubated at 28 ± 2°C for 7 days. After incubation, growing fungal colonies were purified and maintained on PDA slants at 4°C.

2.3. Identification of isolated fungi

The pure isolates were identified based on cultural, morphological features as color and growth of the colony, and spore coloration and microscopic features of hyphae and spore's structures according to consult keys given in standard books on mycology **(Booth, 1971; Raper and Fennell, 1977; Moubasher and Abdel-Hafez, 1978; Domsch *et al.*, 1980; Kitch and Pitt, 1992).**

2.4. Screening for the cellulolytic activity of isolated fungi

2.4.1. Screening for cellulolytic activity using solid culture

Fungal isolates were tested for their cellulolytic activity based on the diameter of clear zones around growing fungal colonies using Czapek's agar medium containing carboxy methylcellulose (CMC) as a sole carbon source instead of sucrose **(Hasanin *et al.*, 2018).** Modified Czapek's agar ingredients were (g/l): Carboxymethyl cellulose, 10.0; $NaNO_3$, 2.0; K_2HPO_4, 1.0; KCl, 0.5; $MgSO_4$, 0.5; $FeSO_4$, 0.01; Agar, 20.0. After preparing, the media

were sterilized at 121°C for 15 min and poured into the sterilized Petri dishes. After solidification, separately discs of fungal isolates (5 mm) were inoculated and incubated at 30 ± 2°C for 5 days. After incubation, 5 ml of congo red (0.1% w/v) were added to each dish for 20 min then, the dishes were washed with 1M NaCl for 20 min. After that, dishes were treated with 5% acetic acid for 2 min then washed with distilled water **(Darwesh *et al.*, 2020).** The clear zone is an indicator for cellulose degradation.

2.4.2. Screening for the cellulolytic activity of the isolated fungi using liquid culture

The positive isolates that gave a clear zone around fungal colony in the previous step were tested using Modified Mandel Weber medium: 10.0 carboxymethyl cellulose, 2.0 KH_2PO_4,1.4 $(NH_4)_2SO_4$, 0.3 $MgSO_4.7H_2O$, 0.3 $CaCl_2$, 0.02 tween-80, 0.005 $FeSO_4.7H_2O$, 0.0016 $MnSO_4.7H_2O$, 0.0014 $ZnSO_4.H_2O$, 0.002 $CoCl_2$, 0.75 Peptone, 0.3 Urea, 0.25 yeast extract **(Jasani *et al.*, 2016).** pH was adjusted at 4.8. One disc (5 mm) of each fungal isolates was inoculated in a 100 ml flask containing 25 ml sterilized medium and incubated for 7 days at 30°C. Three replicas were applied for each fungus. After incubation, filtration was carried out using Whatman No. 1 filter paper. All fungal filtrates were centrifuged at 10000 rpm for 10 min, and the supernatants were collected for measuring of activities of carboxymethyl cellulase (CMCase), β- glucosidase (βGase), and filter-paper cellulase (FPase) **(Nathan *et al.*, 2014).**

2.5. Assay of cellulolytic enzymes

Activities of carboxymethyl cellulase (CMCase), β- glucosidase (βGase), and filter-paper cellulase (FPase), were measured as cellulolytic activity according to **Mandels *et al.*, (1976)**. One unit of filter-paper cellulase (FPase), carboxy-methyl cellulase (CMCase), and β- glucosidase (βGase) were defined as µg of reducing sugars released per min per gram of dry weight under standard assay conditions. A standard curve of pure glucose was formed using concentrations from (0.03 to 0.3 mg/ml).

2.5.1. Carboxymethyl cellulase (CMCase) assay

Carboxy-methyl cellulase (CMCase) was measured according to **Mandels *et al.*, (1976)** by adding 0.5 ml of fungal filtrate and 1% of CMC dissolved in 0.05M of Na-citrate buffer (pH 4.8). The reaction mixture was incubated at 45°C for 45 min then, 1ml of DNS reagent was added to each tube and immersed in a boiling water bath for

10 min, after cooling, and the amount of reducing sugar (glucose) was measured at 540 nm. This assay was applied for each one of three replicas of fungal suspension.

2.5.2. β- glucosidase (βGase) assay

β- glucosidase (βGase) was measured according to **Mandels *et al.*, (1976)** by adding 0.5 ml of fungal filtrate and 1% of D-salicin dissolved in 0.05M of Na-citrate buffer (pH 4.8). The reaction mixture was incubated at 45°C for 45 min then, 1ml of DNS reagent was added to each tube and immersed in a boiling water bath for 10 min, after cooling, and the amount of reducing sugar (glucose) was measured at 540 nm. This assay was applied for each one of three replicas of fungal suspension.

2.5.3. Filter-paper cellulase (FPase) assay

Filter-paper cellulase (FPase) was measured according to **Mandels *et al.*, (1976)** by adding 0.5 ml of fungal filtrate and 50 mg of filter paper (Whatman No. 1) dissolved in 1 ml of 0.05M of Na-citrate buffer (pH 4.8). The reaction mixture was incubated at 45°C for 45 min then, 1ml of DNS reagent was added to each tube and immersed in a boiling water bath for 10 min, after cooling, and the amount of reducing sugar (glucose) was measured at 540 nm. This assay was applied for each one of three replicas of fungal suspension. After cooling, and the amount of reducing sugar (glucose) was measured at 540 nm. This assay was applied for each one of three replicas of fungal suspension.

2.6. Statistical analysis

Triplicate was performed for all measurements. Statistical analysis was done using analysis of variance (ANOVA) one-way test, the significance of the mean difference was detected according to **(Duncan, 1955)**. Values are very highly significant when $P \leq 0.001$, highly significant when $P \leq 0.01$, significant when $P \leq 0.05$, and insignificant when $P \geq 0.05$. Results were reported as mean value ± SD. In the figure, SD was displayed as Y-error bars.

3. Results

3.1. Isolation of Fungi from different collected samples

Thirty-one fungal species were isolated from different samples and belonging to twelve genera "*Trichoderma, Cladosporium, Aspergillus, Botritrichom, Rhizopus, Cephalosporium, Fusarium, Penicillium, Mucor, Circinella, Alternaria* and yeast". The fungal isolates were purified and identified according to the morphological and microscopic features of their hyphae and spore's structures as indicated by consult keys in standard mycology books **(Table 1)**.

Table 1. List of fungal species isolated from different sources

Fungal species	Source
Trichoderma viride **(persoon) Harz**	**Soil sample No. 1 from Tanta**
Cladosporium cladosporiodes **Link**	
Aspergillus sediwii **(isolate 1) Thom and Church**	
Botritrichom **spp. Saccardo and Marchal**	
Aspergillus niger **(isolate 1) Van Tieghem**	
Rhizopus **spp. (isolate 1) Ehrenberg**	
Cephalosporium rose-grisium **Saksena**	
Fusarium oxysporium **Schlechtendal**	
Aspergillus flavus **(isolate 1) Link**	
Aspergillus candidus **(isolate 1) Link**	
Rhizopus **spp. (isolate 2) Ehrenberg**	**Wheat straw from Tanta**
Aspergillus flavus **(isolate 2) Link**	**Compost from Tanta**
Aspergillus fumigates **Fresenius**	
Cephalosporium **spp. Corda**	
Rhizopus **spp. (isolate No. 3) Ehrenberg**	**Soil sample No. 2 from Tanta**
Aspergillus sulphorus **(Fresenius) Thom and Church**	
Penicillium digitatum **Saccard**	**Soil sample No. 3 from Aswan**
Aspergillus sediwii **(isolate 2)Thom and Church**	
Aspergillus niger **(isolate 2) Van Tieghem**	
Aspergillus flavus **(isolate 3) Link**	
Aspergillus candidus **(isolate 2) Link**	
Aspergillus terrus **Thom**	
Aspergillus niger **(isolate 3) Van Tieghem**	**Soil sample No. 4 from Aswan**
Aspergillus flavus **(isolate 4) link**	
Aspergillus ochraceus **K. Wilhelm**	
Penicillium notatum **Westling**	
Mucor **spp. (isolate 1) Micheli**	**Linen straw from Tanta**
Mucor **spp. (isolate 2) Micheli**	**water sample from Tanta**
Circinella simplex **Van Tieghem (Plate XII G)**	
Alternaria alternate **Nees**	
Rhodotorula **spp. (Fresen)**	

3.2. Screening for cellulolytic activity from isolated fungi

3.2.1. Screening for cellulolytic activity using solid culture

Among 31 fungi, 27 were found to have cellulolytic activity as indicated by the clear zones, which ranged from 1.5 to 9.0 cm diameter as shown in **Table 2**. The highest clear zone (9 cm) was recorded by isolate 1 of *Aspergillus niger* and *Trichoderma viride*. Moreover, the lowest clear zone diameter (1.5 cm) was recorded by *Cladosporium cladosporiodes*.

Table 2. Screening for cellulolytic activity using solid culture

Fungal species	Clear zone diameter (cm)
Trichoderma viride	**9.0 ± 0.00**
Cladosporium cladosporiodes	**1.5 ± 0.05**
Aspergillus sediwii **(isolate No. 1)**	**3.4 ± 0.10**
Botritrichom **spp.**	**3.3 ± 0.10**
Aspergillus niger **(isolate No. 1)**	**9.0 ± 0.00**
Rhizopus **spp. (isolate No. 1)**	**0.0 ± 0.00**
Cephalosporium rose-grisium	**2.3 ± 0.10**
Fusarium oxysporium	**4.1 ± 0.10**
Aspergillus flavus **(isolate No. 1)**	**5.0 ± 0.00**
Aspergillus candidus **(isolate No. 1)**	**2.9 ± 0.10**
Rhizopus **spp. (isolate No. 2)**	**0.0 ± 0.00**
Aspergillus flavus **(isolate No. 2)**	**3.6 ± 0.20**
Cephalosporium **spp.**	**5.6 ± 0.11**
Rhizopus **spp. (isolate No. 3)**	**0.0 ± 0.00**
Aspergillus sulphorus	**2.2 ± 0.10**
Penicillium digetatum	**2.5 ± 0.00**
Aspergillus sediwii **(isolate No. 2)**	**2.4 ± 0.11**
Aspergillus niger **(isolate No. 2)**	**4.2 ± 0.10**
Aspergillus flavus **(isolate No. 3)**	**4.2 ± 0.05**
Aspergillus candidus **(isolate No. 2)**	**2.3 ± 0.10**
Aspergillus terrus	**2.8 ± 0.15**
Aspergillus niger **(isolate No. 3)**	**6.8 ± 0.20**
Aspergillus flavus **(isolate No. 4)**	**5.2 ± 0.25**
Aspergillus ochraceus	**2.9 ± 0.17**
Penicillium notatum	**2.8 ± 0.11**
Aspergillus fumigates	**6.2 ± 0.10**
Mucor **sp (isolate No. 1)**	**2.6 ± 0.10**
Mucor **sp (isolate No. 2)**	**2.2 ± 0.10**
Circinella simplex	**0.0 ± 0.00**
Alternaria alternate	**3.4 ± 0.10**
Rhodotorula **spp.**	**3.2 ± 0.10**
F-value	**1213.02*** **

All data represented means of 3 replica ± standard Deviation (SD). The P-value is < 0.001. Results non-significant = non-significant difference at $P > 0.05$, *significant at $P \leq 0.05$, **highly significant at $P \leq 0.01$, ***very highly significant at $P \leq 0.001$.

3.2.2. Screening for cellulase activity using liquid culture

Data represented in **Table 3** indicated that the maximum carboxymethyl cellulase (CMCase) activity was recorded by *Aspergillus niger* isolate 1 (28.9 U/ml) followed by *Trichoderma viride* and *Aspergillus fumigates* with (26.1 and 24.7 U/ml) respectively, activities of other remaining isolates were ranged between (24 to 6.9 U/ml). Also, the maximum β-glucosidase (βGase) activity was recorded by *Aspergillus niger* isolate 1 (43.9 U/ml) and the other remaining isolates ranged between (26.0 to 8.1 U/ml). Filter-paper cellulase (FPase) activity was the highest when *Aspergillus fumigates* was applied and recorded (10.2 U/ml) and the other remaining isolates ranged between (10.0 to 2.9 U/ml)**.**

Also, **Table 3** indicated that the total cellulolytic activity was recorded by *Aspergillus niger* isolate 1 (79.3 U/ml), and the remaining isolates were ranged from (61.6 to 19.5 U/ml).

Table 3. Screening for the cellulolytic activity of tested fungi in liquid culture

Fungal species	Enzymatic activity (U/ml)			
	CMCase	βGase	Fpase	Total activity
Trichoderma vir	**26.1 ± 0.3**	**25.4 ± 0.6**	**8.0 ± 0.4**	**59.5 ± 1.3**
Cladosporium Cladosporides	**7.8 ± 0.6**	**8.7 ± 0.4**	**3.4 ± 0.1**	**19.9 ± 1.1**
***Aspergillus sediwii* (isolate 1)**	**16.0 ± 0.5**	**18.6 ± 0.3**	**8.2 ± 0.3**	**42.8 ± 1.1**
***Botritricum* spp.**	**20.5 ± 0.4**	**18.9 ± 0.3**	**6.9 ± 0.4**	**46.3 ± 1.1**
***Aspergillus niger* (isolate 1)**	**28.9 ± 0.3**	**43.9 ± 0.3**	**6.5 ± 0.4**	**79.3 ± 1.0**
Cephalosporium rose-grisium saksena	**8.6 ± 0.5**	**8.1 ± 0.2**	**4.5 ± 0.4**	**21.2 ± 1.1**
Fusarium oxysporium	**18.0 ± 0.7**	**23.1 ± 0.6**	**4.5 ± 0.3**	**45.6 ± 1.6**
***Aspergillus flavus* (isolate 1)**	**21.0 ± 0.3**	**19.9 ± 0.2**	**4.8 ± 0.1**	**45.7 ± 0.6**
***Aspergillus candidus* (isolate 1)**	**10.2 ± 0.3**	**12.0 ± 0.2**	**7.8 ± 0.3**	**30.0 ± 0.8**
***Aspergillus flavus* (isolate 2)**	**20.5 ± 0.6**	**23.3 ± 0.6**	**8.4 ± 0.1**	**52.2 ± 1.3**
Aspergillus fumigates	**24.7 ± 0.6**	**23.5 ± 0.1**	**10.2 ± 0.1**	**58.4 ± 0.8**
***Cephalosporium* spp.**	**16.1 ± 0.5**	**14.9 ± 0.1**	**5.4 ± 0.6**	**36.4 ± 1.2**
Aspergillus sulphorus	**14.0 ± 0.4**	**16.6 ± 0.2**	**7.6 ± 0.1**	**38.2 ± 0.7**
Penicillium digetatum	**17.2 ± 0.4**	**20.5 ± 0.3**	**5.9 ± 0.4**	**43.6 ± 1.1**
***Aspergillus sediwii* (isolate 2)**	**18.8 ± 0.6**	**19.8 ± 0.4**	**5.3 ± 0.4**	**43.9 ± 1.4**
***Aspergillus niger* (isolate 2)**	**20.3 ± 0.5**	**26.0 ± 0.3**	**8.9 ± 0.2**	**55.2 ± 1.0**
***Aspergillus flavus* (isolate 3)**	**17.0 ± 0.4**	**18.0 ± 0.3**	**4.8 ± 0.2**	**39.8 ± 0.9**
***Aspergillus candidus* (isolate 2)**	**13.9 ± 0.1**	**13.9 ± 0.1**	**5.8 ± 0.1**	**33.6 ± 0.3**
Aspergillus terrus	**19.6 ± 0.4**	**17.8 ± 0.9**	**5.4 ± 0.1**	**42.8 ± 1.4**
***Aspergillus niger* (isolate 3)**	**24.0 ± 0.6**	**27.6 ± 0.7**	**10.0 ± 0.1**	**61.6 ± 1.4**
***Aspergillus flavus* (isolate 4)**	**20.9 ± 0.4**	**23.6 ± 0.6**	**5.8 ± 0.1**	**50.3 ± 1.1**
Aspergillus ochracous	**15.7 ± 0.5**	**10.5 ± 0.6**	**6.2 ± 0.1**	**32.4 ± 1.2**
Penicillum notatum	**15.2 ± 0.5**	**14.2 ± 0.4**	**3.7 ± 0.2**	**33.1 ± 1.1**
***Mucor* spp. (isolate 1)**	**6.9 ± 0.6**	**9.7 ± 0.1**	**2.9 ± 0.1**	**19.5 ± 0.8**
***Mucor* spp. (isolate 2)**	**6.9 ± 0.4**	**9.7 ± 0.6**	**2.9 ± 0.1**	**19.5 ± 1.1**
Alternaria alternate	**11.7 ± 0.9**	**13.6 ± 0.3**	**7.0 ± 0.1**	**32.3 ± 1.3**
***Rhodotorula* spp.**	**16.3 ± 0.1**	**15.8 ± 0.1**	**7.6 ± 0.1**	**39.7 ± 0.3**
F-value	**17.65*** **	**53.42*** **	**53.42*** **	**186.42*** **

All data represented means of 3 replica ± standard Deviation (SD). The P-value is < 0.001. Results non-significant = non-significant difference at $P > 0.05$, *significant at $P \leq 0.05$, **highly significant at $P \leq 0.01$, ***very highly significant at $P \leq 0.001$.

4. Discussion

Annually, about 180 billion tons of cellulose were produced by plants, and it's a principal source of organic carbon in the world. Cellulose is the most abundant polymer and renewable resource that available worldwide **(Thottiam *et al.*, 2018),** and it accounts for a major portion of agricultural waste deposited in the soil, therefore its decomposition is crucial to the carbon cycle **(Pandey *et al.*, 2020).** The Fungi are the best decomposition agents for organic matter generally and cellulosic substrate particularly. These fungi can secrete a variety of extracellular enzymes to decompose the lignocellulosic substrate. Among the variety of enzymes, cellulase is one of the most important for the decomposition of lignocellulosic materials **(El-Sheekh *et al.*, 2009; Gahfif *et al.*, 2020; Pandey *et al.*, 2020).**

In this study, isolated fungal species from different sources were identified and screened for cellulase activities. Thirty-one species were identified based on their macro-morphological features, such as colony diameter, colony reverse, texture, colony colour, margin, and exudates. Slide culture technique was used to characterize fungal isolates based on micro-morphological parameters such as hyphal structure, conidiophore structure organization, size and form of conidia, conidia arrangement, phialides, and so on **(Londhe *et al.*, 2019).**

Screening for cellulase activity based on clear zones was done on CMC agar plates that had been flooded with Congo red (0.1% w/v), then de-stained with 1M NaCl and 5% acetic acid. 27 fungal species were identified as Cellulase-producing fungi according to the diameter of the clear zone around the colony. *Aspergillus niger* isolate 1 and *Trichoderma viride* gave the highest clear zone. Generally, cellulases production has been reported in a wide variety of fungi **(Sarao *et al.*, 2010)**, especially filamentous fungi, which can produce high amounts of these enzymes compared to another microbial community **(Mrudula and Murugammal, 2011).** Similar results were obtained by **Zhang *et al.*., (2018)** who reported that members of the genera *Aspergillus* and *Trichoderma* were the most common fungi with cellulolytic activity. Also, **Darwesh *et al.* (2020)** showed that *Aspergillus niger* gave the highest clear zone that showed the largest halo forming zone when all plates were flooding with Congo red.

Results obtained from the screening of cellulolytic tested fungi using liquid medium revealed that the highest total cellulolytic activity was recorded by *Aspergillus niger* isolate 1. The same results were obtained by **Darwesh *et al.*,**

(2020). Also, results showed that isolate No. 1 of *Aspergillus niger* from Al-Gharbia is more cellulolytic than isolates No 2& 3 of *Aspergillus niger* isolated from Aswan. Moreover, the cellulolytic activity of isolate No.1 of *Aspergillus sediwii* from Al-Gharbia and isolate No. 2 of *Aspergillus sediwii* from Aswan were approximately equal but Aswan isolate was a little high on Al-Gharbia isolate. Among four *Aspergillus flavus* isolates (isolate No. 1& 2 from Al-Gharbia) and (isolate 3& 4 from Aswan), *Aspergillus flavus* isolate 2 was more cellulolytic than other *A. flavus* isolates. On the other hand, *Aspergillus candidus* isolate 2 from Aswan recorded cellulolytic activity higher than *A. candidus* isolate 1 that isolated from Al-Gharbia.

Quintanilla *et al.*, (2015); Liu and Kokare, (2017); **Buntić *et al.*, (2019)**reported that fungal cellulases have a different specificity and action mode rather than bacterial cellulases.

5. Conclusion

This study revealed that *Aspergillus niger* and *Trichoderma viride* are promising fungi that have cellulolytic activity. Therefore, we recommend the use of these promising isolates in further study to improve the production of cellulase enzyme for use in saccharification and the use of the resulting reducing sugars in the production of bioethanol.

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عزل و تعريف و فحص الفطريات المحلله للسليلوز من مصادر وأماكن مختلفه فى مصر

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انزيم السليولاز عباره عن سلسله من الانزيمات التى تحلل السليلوز الى سكر بسيط. فى هذه الدراسه تم عزل وتنقيه واحد و ثلاثين نوعا من الفطريات من أماكن مختلفه بمحافظتى الغربيه و أسوان و تم تعريف هذه الفطريات وكانت تنتمى الى أثنى عشر جنسا وهى ترايكوديرما، كلادوسبوريم، اسبرجيللس، بوتريتريكم، ريزوبس، سيفالوسبوريم، فيوزاريم، بنسليوم، ميوكر، سيرسينيلا، الترناريا و الخميره. و تم فحص جميع الفطريات التى تم عزلها وتعريفها لمعرفه اي الفطريات لديها القدره على انتاج السليلولاز وتكسير السليلوز و ذلك بناءْ على قياس قطر منطقه التحلل الواضحه حول نمو الفطر باستخدام ٠.١ % من صبغ احمر الكونجو. و او ضحت النتائج ان ٢٧ نوعا لديهم القدره على تكسير السليلوز، و تم تسجيل اكبر منطقه تحلل بواسطه الفطر ترايكوديرما فيريدى واسبرجيللس نيجر عزله ١. بعد ذلك تم اختبار نشاط هذه الفطريات لانزيم كربوكسي ميثيل سليلولاز، بيتا جلوكوسيداز، و سليلولاز المحلل لورق الترشيح واسبرجيللس نيجر عزله ١ اعلى نشاط اجمالى لثلاثه انزيمات السليلوزيه حيث سجل ٧٩.٣ وحده/ مللى.