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Research Article **MICROBIOLOGY**

Screening of cellulolytic activity of some rhizospheric soil fungi isolated from different sources

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KEY WORDS

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ABSTRACT

Cellulases are enzymes that catalyze the bioconversion of cellulose molecule into monosaccharides (simple sugers) such as beta glucose or shorter polysaccharides and oligosaccharides. Most fungal cellulase enzymes degrade the cellulose molecule and several other polysaccharides into monosaccharides. In this study, twenty fungal species were isolated from several locations in Al-Gharbia governorate, Egypt. The soil samples are collected from various plant rhizospheres (rice, wheat, bean, sugar beet, and pea). These fungi were identified and referred to seven different genera "*Trichoderma, Aspergillus, Rhizopus, Cephalosporium, Fusarium, Penicillium, Mucor"*. All identified fungal species were tested for cellulolytic activity in the solid medium. Out of twenty fungal species, seventeen species showed cellulolytic activity and the highest activity was recorded in *Trichoderma viride* (Rs-4) (67.2 U/ml) and *Aspergillus sydowii* (Rs-2) (52.1 U/ml). The selected cellulolytic fungal enzymes were carboxymethyl cellulase (CMCase), β-glucosidase (βGase) and filter paper cellulase (FPase) in the liquid medium. All tested fungi showed significant activity for the three enzymes. *Trichoderma viride* (Rs-4) had the highest total cellulolytic activity of the three cellulolytic enzymes.

Introduction

Lignocellulosic material is primarily composed of three different polymers: lignin, hemicellulose, and cellulose that are linked together. Cellulose is the most common organic compound in the world and the primary component of plant cell walls. It is composed of nearly 8,000 to 12,000 D-glucose residues linked together by β-1,4 bonds **(Aro *et al*., 2005).** Cellulose chains have a flat structure that is stabilized by internal hydrogen bonds. **(Festucci-Buselli *et al*., 2007).**

Hemicellulose is a complex carbohydrate structure composed of various polymers such as pentoses (arabinose and xylose), hexoses (glucose, mannose, and galactose), and sugar acids. In hardwood and agricultural plants such as grasses and straw, xylan is the most abundant component of hemicellulose, whereas glucomannan is the most abundant component in softwood **(Fengel and Wegener, 1984; Saha, 2003).** Lignin is an amorphous heteropolymer composed of three different phenylpropane units (coniferyl, p-coumaryl and sinapyl alcohol) held together by various linkages. Lignin's major function is to provide impermeability, structural support, and resistance to microbial attack and oxidative stress to plants. The amorphous heteropolymer is also non-water soluble and optically inactive, making lignin breakdown extremely difficult **(Fengel and Wegener, 1984).**

Lignocellulosic biomass can be transformed into fuels and other compounds by using a multienzyme system that hydrolyzes biomass to glucose. Hydrolytic efficiency is well established to be the result of the synergistic actions of a multi-component system containing at least three major groups of enzymes: Endoglucanases hydrolyze the cellulose polymer internally, while exoglucanases or cellobiohydrolases act on the reducing and non-reducing ends, releasing cellobiose and cellooligosaccharides; and β-glucosidases which cleaves cellobiose and releases two molecules of glucose **(Gottschalk *et al*., 2010; Maeda *et al*., 2011; Delabona *et al*., 2013).** Several enzymes are required for completing hydrolysis of lignocellulosic biomass, including xylanase, cellulase, glucanase, ligninase, hemicellulases, pectinase, etc., Cellulase is the most important of these enzymes **(El-Sheekh *et al*., 2009; Liu *et al*., 2013; Awadalla *et al*., 2017).**

Cellulase is a multienzyme complex composed of three different enzymes, carboxymethyl cellulase, filter paper cellulase, and β- glucosidase, that work together to complete the hydrolysis of cellulose to cellobiose (an intermediate product of cellulose hydrolysis) and then to glucose **(Rawat *et al*., 2014; Awadalla *et al*., 2017).** Cellulase enzyme is used in various industrial applications, including textile, paper, food, and biofuel. In these industries, these enzymes are used alone or in combination with other enzymes **(Ejaz *et al*., 2021).**

Many soil fungi and bacteria secrete these enzymes **(Onsori *et al*., 2004).** On the other hand, Fungi are more efficient in degrading cellulosic material because they can grow on the surface or penetrate the cellulosic

material **(Boer *et al.*, 2004).** It was also proved that using cellulolytic fungi improves cellulose waste's decomposition potential **(Metwally *et al.*, 2021).**

The present work aimed to isolate, identify, and screen the cellulolytic activity of some rhizospheric soil fungi.

Material and methods

Sample collection

Six samples were collected from Al-Gharbia governorate, Egypt. The soil samples were from various plant rhizospheres (rice, wheat, bean, sugar beet, and pea).

Soil samples were collected from Al-Gharbia governorate at a depth of 5 cm from the top and sieved using a two mm sieve. The samples were collected in bags and transported to the lab. The soil sample was air-dried before being used in vitro to isolate the fungi **(Johnson *et al.*, 1959).**

Isolation of fungi from the collected soil samples

The dilution plate method was used to isolate soil fungi from the collected soil samples **(Johnson *et al.*, 1959).** One gram of each soil sample was dissolved in 9 ml of sterilized distilled water in a sterile 100 ml conical flask. The flask containing the soil suspension was shaken vigorously in a shaker for 30 min at 160 rpm. One ml of the suspension was pipetted into a sterilized 100 ml Erlenmeyer flask containing 9 ml of sterile distilled water and shaked for a few minutes. Consecutive dilutions were made in the same way until a 10^{-4} dilution was found to be suitable for plating, 0.1ml of 10^{-4} dilution was transferred to a plate containing Czapek's -Dox agar medium with rose Bengal 25 µg/ml and spread over the surface. Three replicas of agar plates were prepared and incubated at 28^{o}C for 5 days. The fungal colonies were purified, identified, and kept as stock cultures in sterile Czapek's Dox agar slants at 4 ± 1^{o}C.

Identification of isolated fungi

The pure isolates were identified based on cultural, morphological features such as colony colour and growth, spore coloration, and microscopic features of hyphae and spore structures described in standard mycology books **(Booth, 1971; Raper and Fennell, 1977; Moubasher, 1978; Domsch *et al.*, 1980; Kitch and Pitt, 1992).**

Screening for cellulolytic activity based on clear zones using solid medium

Using Czapek's agar medium with carboxymethyl cellulose (CMC) as the only carbon source rather than sucrose, the diameter of clear zones surrounding growing fungal colonies was used to evaluate the cellulolytic activity of fungal isolates **(Hasanin *et al.*, 2018).** The medium was prepared and sterilized at 121°C for 15 min before being poured into the sterilized Petri dishes. Separate discs of fungal isolates (5mm) were inoculated after solidification and incubated at 30±2°C for 5 days. Following incubation, 10 ml of Congo red (0.1 % w/v) was added to each dish for 30 minutes before being washed with 1M NaCl for 20 min. After that, dishes were treated with 5% acetic acid for 5min before being washed with distilled water **(Darwesh *et al.*, 2020).** The clear zone is an indicator of cellulose degradation.

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

Modified Mandel Weber medium **(Jasani *et al.*, 2016)** was used to test the positive isolates that gave a clear zone around the fungal colony in the previous step. Ingredients were (g/l): 10.0 carboxymethyl cellulose; 2.0 KH_2PO_4; 1.4 $(NH_4)_2SO_4$; 0.2 $MgSO_4.7H_2O$; 0.3 $CaCl_2$; 0.03 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. pH was adjusted at 4.8. Each fungal isolate was inoculated with one disc (5 mm) in a 100 ml flask containing 25 ml sterilized media and cultured for seven days at 30°C. For each fungus, three replicas were used. Filtration was done using Whatman No. 1 filter paper after incubation. All fungal filtrates were centrifuged for 10 minutes at 10000 rpm, and the supernatants were collected to measure carboxymethyl cellulase (CMCase), β- glucosidase (βGase), and filter-paper cellulase (FPase) activities **(Nathan *et al.*, 2014).**

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)**. Under standard assay conditions, one unit of filter-paper cellulase (FPase), β- glucosidase (βGase), and carboxy-methyl cellulase (CMCase) was defined as g of reducing sugars released per minute per gram of dry weight. A pure glucose standard curve was created using concentrations ranging from (0.05 to 0.5 mg/ml).

Carboxymethyl cellulase (CMCase) assay

Carboxy-methyl cellulase (CMCase) activity was determined as described by **(Mandels *et al.*, 1976)**. In this method 0.5 ml of fungal filtrate was added to 1% CMC dissolved in 0.05M Na-citrate buffer (pH 4.8) and incubated for 45 minutes at 45°C, then 1ml of dinitrosalicylic acid reagent was added to each tube and immersed in a boiling water bath at 95-100°C for 10 minutes before cooling after that the amount of reducing sugar was measured at 540 nm. This assay was performed on each of three fungal suspension replicas.

β- glucosidase (βGase) assay

β- glucosidase (βGase) activity was determined using the method described by **(Mandels *et al.*, 1976)**. In this method 0.5 ml of fungal filtrate was added to 1% D-salicin dissolved in 0.05M of Na-citrate buffer (pH 4.8) and incubated for 45 min of incubation at 45°C then 1ml dinitrosalicylic acid of reagent was added to each tube and immersed in a boiling water bath at 95-100°C for 10 min after cooling the amount of reducing sugar was measured at 540 nm. This assay was performed on each of three fungal suspension replicas.

Filter-paper cellulase (FPase) assay

Filter-paper cellulase (FPase) activity was determined as described by **(Mandels *et al.*, 1976)**. In this method 0.5 ml of fungal filtrate was added to 50 mg of filter paper (Whatman No. 1) in 1 ml of 0.05M Na-citrate buffer (pH 4.8) and incubated for 45 min at 45°C, then1ml of dinitrosalicylic acid reagent was added to each tube and immersed in a boiling water bath for 10 min after cooling the amount of reducing sugar was measured at 540 nm. This assay was

performed on each of three fungal suspension replica. The amount of reducing sugar (glucose) was measured at 540 nm after cooling. This assay was performed on each of three fungal suspension replicas.

Statistical analysis

The data were subjected to one way analysis of variance (ANOVA) using the SPSS 19.0 software program, and the significance of the mean difference was determined using **(Duncan, 1955).** Values are very highly significant when $p < 0.001$, highly significant when $p < 0.01$ and significant when $p \leq 0.05$. Results were reported as mean value ± SD.

Results

Isolation of Fungi from different collected samples

Twenty fungal species were isolated from different places in Al-Gharbia governorate. These fungal species were found to belong to seven different genera "*Trichoderma, Asperagillus, Rhizopus, Cephalosporium, Fusarium, Penicillium, Mucor".*

Identification of isolated fungi

The fungal isolates were purified and identified using 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

Isolate Code	Fungal species	Source	localization of plant rhizosphere
Rs-1 Rs-2 Rs-3 Rs-4	***Aspergillus nidulans* (Eidam,1884)** ***Aspergillus sydowii* (Thom and Church,1910)** ***Cephalosporium* spp (Corda, 1894)** ***Trichoderma viride* (person,1794)**	Rice soil	Al-Gharbia governorate, Egypt
Ws-1 Ws-2 Ws-3 Ws-4	***Aspergillus candidus* (link.1809)** ***Aspergillus flavus* (link,1809)** ***Fusarium oxysporum* (Schlechtendal,1840)** ***penicillium itilicum* (link,1809)**	Wheat soil	
Bs-1 Bs-2 Bs-3	***Aspergillus nidulans* (Eidam,1884)** ***Aspergillus ochraceus* (K. Wilhelm,1877)** ***Mucor racemosus* (Micheli,1729)**	Bean soil	
Sbs-1 Sbs-2 Sbs-3	***Aspergillus tamarii* (Thom,1910)** ***Penicillium corylophilum* (Link,1809)** ***Mucor* (Micheli,1729)**	Suger beet soil	
Ps-1 Ps-2	***Aspergillus niger* (Van Tieghem,1867)** ***Trichoderma harzianum* (Rifai,1969)**	Pea soil	
Ms-1 Ms-2 Ms-3 Ms-4	***Aspergillus clavitus* (link,1809)** ***Aspergillus niger* (Van Tieghem,1867)** ***Rhizopus* (Ehrenberg,1833)** ***Fusarium solani* (Synder,1940)**	Mixed soil	

Rs: Rice soil, Ws: Wheat soil, Bs: Bean soil, Sbs: Suger beet soil, Ps: Pea soil, Ms: Mixed soil

Screening for cellulolytic activity using solid culture

Among 20 fungi, 17 were found to have cellulolytic activity as indicated by the clear zones, ranging from 1.2 to 9.3 cm in diameter as shown in Fig. 1. The highest clear zone (9.3 cm) was recorded by *Trichoderma virdi* (Rs-4). While the lowest clear zone diameter (1.2 cm) was recorded by *Aspergillus nidulans* (Ps-1) as shown in Table 2

Table (2): Screening for cellulolytic activity using solid culture

Isolate Code	Fungal species	Clear zone diameter (cm)
Rs-4	***Trichoderma virdi***	9.3 ± 0.2^{A}
Ps-2	***Trichoderma harzianum***	7.3 ± 0.1^{C}
Ms-2	***Aspergillus niger***	6.6 ± 0.2^{E}
Ws-3	***Fusarium oxysporum***	4.1 ± 0.1^{H}
Ws-2	***Aspergillus flavus***	3.3 ± 0.2^{J}
Ws-1	***Aspergillus candidus***	6.7 ± 0.1^{E}
Bs-1	***Aspergillus nidulans***	1.9 ± 0.1^{I}
Rs-3	***Cephalosporium***	5.3 ± 0.1^{F}
Rs-2	***Aspergillus sydowii***	8.6 ± 0.1^{B}
Sbs-2	***Penicillium corphilum***	2.5 ± 0.2^{K}
Sbs-1	***Aspergillus tamari***	7.1 ± 0.1^{CD}
Ws-4	***penicillium italicum***	2.3 ± 0.2^{K}
Ps-1	***Aspergillus nidulans***	1.2 ± 0.1^{M}
Bs-3	***Mucor racemosus***	0.0 ± 0.0^{N}
Bs-2	***Aspergillus ochraceus***	3.0 ± 0.1^{J}
Ps-1	***Aspergillus niger***	7.0 ± 0.1^{D}
Ms-1	***Aspergillus clavitus***	5.0 ± 0.1^{G}
Ms-3	***Rhizopus***	0.0 ± 0.0^{N}
Ms-4	***Fusarium solani***	4.0 ± 0.2^{H}
Sbs-3	***Mucor***	0.0 ± 0.0^{N}
F-value		**1031.37*****

Rs: Rice soil, Ws: Wheat soil, Bs: Bean soil, Sbs: Suger beet soil, Ps: Pea soil, Ms: Mixed soil

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 $P \leq 0.001$. Different capital letters indicate significant differences at $p \leq 0.05$ using Duncan's test

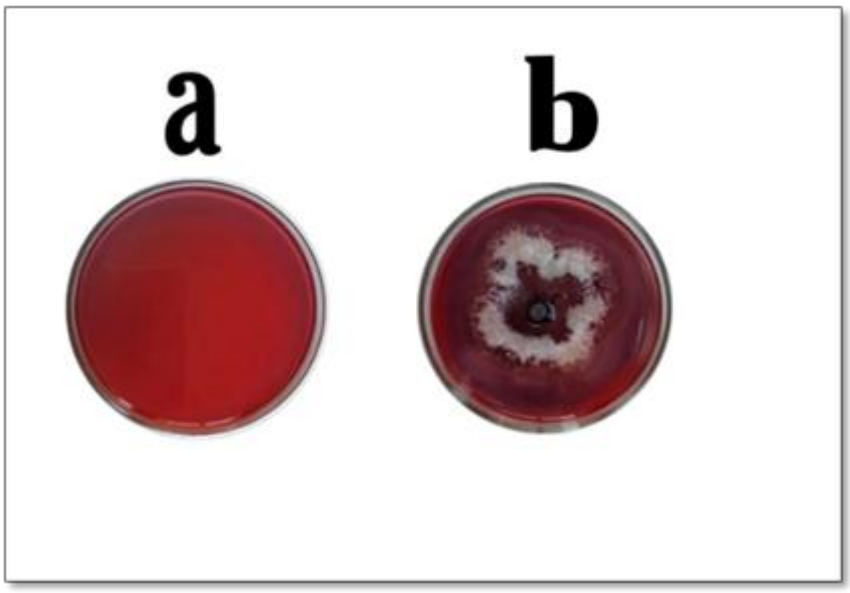

Fig.: (1): Cellulolytic activity based on clear zone: a) Control plate, b) Tested fungus (*Trichoderma virdi* Rs-4).

Screening for cellulase activity using liquid culture

Data represented in Table 3 indicated that the maximum carboxymethyl cellulase (CMCase) activity was recorded in *Trichoderma virdi* (Rs-4) (27.7 U/ml) followed by *Aspergillus candidus* (Ws-1) and *Aspergillus niger* (Bs-1) with 24.0 and 22.7 U/ml respectively. The activities of other remaining isolates ranged from 22.7 to 10.0 U/ml. Also, the maximum β glucosidase (βGase) activity was recorded in *Trichoderma virdi* (RS-1) (30.1 U/ml) and the remaining isolates ranged from 22.4 to 10.0 U/ml. Filter-paper cellulase (FPase) activity was the highest when *Aspergillus niger* (Ps-1) was applied and recorded (11.9 U/ml) and the remaining isolates ranged from 9.4 to 3.4 U/ml. Also, Table 3 indicated that the total cellulolytic activity was recorded in *Trichoderma virdi* (Rs-4), (67.2 U/ml), and the remaining isolates ranged from 52.1 to 26.8 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
Rs-4	***Trichoderma virdi***	27.7 ± 0.4^{A}	30.1 ± 0.3^{A}	9.4 ± 0.3^{B}	67.2 ± 0.9^{A}
Ps-2	***Trichoderma harzianum***	19.9 ± 0.3^{F}	17.8 ± 0.3^{F}	7.5 ± 0.3^{E}	45.3 ± 0.2^{E}
Ps-1	***Aspergillus niger***	18.0 ± 0.2^{H}	16.3 ± 0.4^{H}	11.9 ± 0.1^{A}	46.2 ± 0.6^{D}
Ws-3	***Fusarium oxysporum***	15.5 ± 0.2^{J}	19.2 ± 03^{E}	3.5 ± 0.2^{K}	38.2 ± 0.5^{H}
Ws-2	***Aspergillus flavus***	10.0 ± 0.3^{M}	17.1 ± 0.6^{G}	6.5 ± 0.1^{FG}	33.6 ± 0.7^{K}
Ws-1	***Aspergillus candidus***	24.0 ± 0.1^{B}	18.7 ± 03^{E}	9.0 ± 0.1^{C}	51.7 ± 0.3^{B}
Rs-3	***Cephalosporium***	19.0 ± 0.2^{G}	15.1 ± 0.4^{I}	6.3 ± 0.1^{G}	40.4 ± 0.5^{G}
Bs-1	***Aspergillus niger***	22.7 ± 0.1^{C}	21.1 ± 0.7^{C}	8.1 ± 0.1^{D}	52.0 ± 0.7^{B}
Sbs-2	***Penicillium corphilum***	12.0 ± 0.2^{K}	20.0 ± 0.6^{D}	4.5 ± 0.1^{J}	36.5 ± 0.8^{I}
Sbs-1	***Aspergillus tamari***	21.5 ± 0.2^{D}	22.4 ± 0.1^{B}	5.9 ± 0.0^{H}	49.7 ± 0.1^{C}
Ws-4	***penicillium italicum***	11.0 ± 0.1^{L}	9.1 ± 0.2^{K}	6.7 ± 0.1^{F}	26.8 ± 0.3^{L}
Bs-2	***Aspergillus ochraceus***	17.0 ± 0.2^{I}	10.0 ± 0.2^{J}	7.7 ± 0.1^{E}	34.8 ± 0.3^{J}
RS-2	***Aspergillus sydowii***	21.0 ± 0.2^{E}	22.3 ± 0.6^{B}	8.8 ± 0.1^{C}	52.1 ± 0.4^{B}
Ms-1	***Aspergillus clavitus***	18.1 ± 0.4^{H}	22.2 ± 0.3^{B}	3.4 ± 0.1^{K}	43.7 ± 0.7^{F}
Ms-4	***Fusarium***	12.4 ± 0.4^{K}	16.6 ± 0.3^{GH}	5.6 ± 0.0^{I}	34.6 ± 0.7^{G}
F-value		1160.66***	455.32***	769.50***	1175.29***

Rs: Rice soil, Ws: Wheat soil, Bs: Bean soil, Sbs: Suger beet soil, Ps: Pea soil, Ms: Mixed soil

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 $P \leq 0.001$. Different capital letters indicate significant differences at $p \leq 0.05$ using Duncan's test.

Discussion

Plants produce approximately 180 billion tons of cellulose per year, which is the world's largest reservoir of organic carbon. Cellulose is the most abundant and renewable polymer resource on the earth today. It is expected that 1012 tons are synthesized annually by photosynthesis in a relatively pure form, for example, in the seed hairs of the cotton plant, but are most common with lignin and other polysaccharides in the cell wall of woody plants. Cellulose is a structural component of the primary cell wall of green plants, oomycetes, and many types of algae. Cellulose is the most common organic compound in the world **(David and Ray, 2008).** Fungi are the most effective decomposition agents for organic matter in general and cellulosic substrate. To break down the lignocellulosic substrate, these fungi can secrete a variety of extracellular enzymes. Cellulase is one of the most important enzymes for lignocellulosic material decomposition. **(El-Sheekh *et al.*, 2009; Gahfif *et al.*, 2020; Pandey *et al.*, 2020).**

In this study, isolated fungal species from various sources were identified and tested for cellulase activity. Twenty species were identified based on macromorphological characteristics such as colony diameter, colony colour, colony reverse, margin, texture, and exudates. The slide culture technique was used to characterize fungal isolates using micromorphological parameters such as conidia size and shape, hyphal structure, conidiophore structure organization, phialides,conidia arrangement, and so on **(Londhe *et al*., 2019).**

Cellulase-producing fungi were screened on CMC agar plates flooded with Congo red and washed with NaCl. Seventeen fungal species were identified as cellulase-producing fungi based on the diameter of the clear zone around the colony. The highest cellulase-producing fungal species were *Trichoderma virdi* (Rs-4) and *Aspergillus sydowii* (Rs-2), followed by *Trichoderma harzianum* (Ps-2), *Asperagillus Tamarii* (Sbs-1), *Asperagillus niger* (Ms-2), and *Asperagillus candidus* (Ws-1). **Updegraff (2004) and Kuczek-Turpeinen *et al.*, (2005)** obtained similar results, reporting that cellulolytic activity of tested *Trichoderma* and *Asperagillus* species was relatively higher. According to **Lynd *et al.*, (2002)** *Trichoderma* spp. and *Asperagillus* spp. were two potential cellulase producers.

The results of a screening of cellulase activities by isolated fungi using a liquid medium indicated that *Trichoderma viride* Rs-4 had the highest carboxymethyl cellulase (CMCase) activity, followed by *Asperagillus candidus* (Ws-1) and *Asperagillus niger* (Ps-1). Also, the maximum β glucosidase (βGase) activity was recorded by *Trichoderma viride* (Rs-4). Filter-paper cellulase (FPase) activity recorded with *Asperagillus niger* (Ms-2) was the highest. The highest total cellulolytic activity was recorded by *Trichoderma viride* (Rs-4).

According to **Li *et al.*, (2010),** *Trichoderma viride* is a cellulase

producer, and crude enzymes produced by these microorganisms are commercially available for agricultural use. *Trichoderma* spp. and *Aspergillus* sp. were the most common and effective cellulase producers, according to **Yalpani (1987).**

Conclusion

This study revealed that *Trichoderma viride* and *Aspergillus niger* 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 and biohydrogen.

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فحص نشاط التحلل السيليلوزى لبعض فطريات التربه المحيطة بالجذر والمعزولة من مصادر مختلفة

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الانزيمات السيليلوزية هى انزيمات تساعد على تحويل السليلوز الى سكريات مختزلة وجلوكوز.وتعمل معظم الإنزيمات السليلوزية الفطرية على تحلل السليلوز والعديد من السكريات الأخرى إلى السكريات الأحادية. في هذه الدراسة تم عزل عشرين نوعا فطريا من عدة مواقع بمحافظة الغربية. تم التعرف على هذه الفطريات من سبعة أجناس مختلفة التريكوديرما والاسبراجيلس والريزوبس والسيفالوسبوريم والفيوزريم والبنيسيليم والميوكر. وتم فحص النشاط السيليلوزى لجميع هذه الفطريات التى تم تعريفها فى الوسط الصلب واوضحت النتائج انه حولى ١٧ فطر لديهم القدرة على تكسير السليلوز، وتم تسجيل اعلى نشاط للتريكودريما فيريدى والاسبراجيلس سيدوى وتم اختبار انزيم الكربوكسى مثيل سليلولاز والبيتا جليكوسيداز والسليولاز المحلله لورق الترشيح فى الوسط السائل واظهرت التريكوديرما اعلى نشاط اجمالى للثلاث انزيمات السليلوزية.