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MOLECULAR AND ENZYMATIC PROPERTIES OF FUNGI ISOLATED FROM HISTORICAL MANUSCRIPTS PRESERVED AT THE AL-HUSSEIN HOLY SHRINE

Alaa A. Jasim[1], Ban T. Mohammed[1*] and Adnan A. Lahuf[2]

[1,2]Department of Biology, University of Kerbala, Iraq.
[2]Department of Plant Protection, University of Kerbala, Iraq.
*e-mail: bantmh@gmail.com

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ABSTRACT : A total of 300 samples were collected from manuscripts preserved in the Al-Hussein Holy shrine. Fungal species were isolated and identified by both traditional and molecular methods. The results showed that seven fungal species are the first record in Iraq as a general and in the manuscripts: *Alternaria atra*, *Aspergillus ustus, Cladosporium exasperatum, Chaetomium globosum*, *Microdochium nivale*, *Penicillium tardochrysogenum*-1, *Penicillium tardochrysogenum*-2. The fungal were registratied in the International Genbank and have accession numbers: MK503427, MK503428, MK504425, MK504424, MK503439, MK504426, MK504427, respectively. The phylogenetic tree of ITS rRNA sequences showed that the*Alternariaatra* strain alaa1-A. isolate from multiple sequence alignment of 18S rDNA gene sequences were identical to the international isolates ex: Qatar, Iranetc, so *Aspergillus ustus* is identical 100% to the international isolates Egypt, Brazil, China, USA and Netherlands. *Cladosporium exasperatum* is identical 100% to the international isolates India and Kenia. *Microdochium nivale*, it was identical with the international isolates such as China isolates. *Penicillium tardochrysogenum*-1, *Penicillium tardochrysogenum*-2 are identical 100% to the international isolates Slovakia, China, South Africa, India and Spain. The*Aspergillus ustus* had ranked first in terms of occurrence, frequency and distribution density coefficient. *Chaetomium globosum* strain ala11-C.gl from multiple sequence alignment of 18S rDNA gene sequences and identical to the international isolates ex: China and Egypt, while *Cladosporium exasperatum* was in the last rank in terms of occurrence, frequency and distribution intensity. The degradation of cellulose, protein, starch and fat because of the activity of fungi on the manuscripts were detected for the purpose of identifying the effects of fungi and their damage to manuscripts. The results of the enzymatic tests of the seven fungal fungi, that the *Alternaria atra* and *Aspergillus ustus* recorded a higher efficacy of the cellulose enzyme in terms of the mean halo zone diameter of 47.44 and 47.00 mm respectively, which did not differ significantly between them. The *Microdochium nivale* was more effectiveon protease activity, the halo zone was 63.78 mm and did not differ significantly from both *Aspergillus ustus* and *Chaetomium globosum*, so the amylase showed high efficacy of the *Microdochium nivale*, with a diameter of halo zone was 60.22 mm, which was not significantly different from the *Aspergillus ustus* with an average diameter of 59.33 mm. *Chaetomium globosum* was the highest effective enzyme Lipase which dimeter halo zone was 68.56 mm, which was not significantly different from the *Microdochium nivale* at 63.22 mm. The enzymes activity is considered as a good response to confirms the contamination and the need to address for the purpose of conservation and preservation of the legacy of civilization.

Key words : Opportunistic fungi, manuscripts fungi, air fungi, fungal enzymes.

INTRODUCTION

Manuscripts constitute an important part of the intellectual, cultural and Islamic heritage, regardless of the various environmental factors contributing to its damage, there were many biotic factors involvedto damage the manuscripts (Al-Sayed, 2001). The fungi were more important causal agent to damage the manuscript, so there wereten species of fungus belonging to six genera isolated from the manuscript, as well as the isolation of the sterile white fungus as the first study in Iraq (Mohammed *et al*, 2018). The fungus differs in terms of enzymatic activity, which is due to the type of fungus, environmental factors and substrate types (Hussein and Mohammed, 2013; Romaní *et al*, 2006). The exogenous enzyme produced including Cellulase, Protease, amylase and lipase by fungi were studied in many articles (Delabona *et al*, 2012; Yike, 2011; Krishnan *et al*, 2016 and Griebeler *et al*, 2011), but theactivity of these enzymes on manuscripts was first thestudied in Iraq by Al-Waily (2017), which included the role of degradation enzymes that caused a true cases to damage the manuscripts materials. Because the manuscripts differ instructure and types of material that consist (Al-Sayed, 2001). Therefor,

there weremore than one types of enzymes produced to degradation the material of the manuscripts (Mohammed *et al*, 2018).

Because of leastof studies on the fungus of manuscripts, this study is targeted to isolate and diagnose the fungus associated with the manuscripts and causes the damage and study the effectiveness of enzymatic analysis of the components of the manuscripts, as well as registration in genbank, study the Phylogenetic relationship and homology with the international species.

MATERIALS AND METHODS

Agricultural media were used :

Sabouraud's dextrose agar medium : was prepared as listed in Kwon-Chung and Bennett (1992), melted 65 g of Sabouraud's dextrose agar powder with one liter of distilled water and pH was adjusted at 6.5.

Czapex agar (CA) : It was given by (Ramirez,1982), which included the following composition:1g K_2HPO_4, 0.5g KCl, 2g $NaNO_3$, 0.5g $MgSO_4.7H_2O$, 0.05g $FeSO_4.7H_2O$, 20g Sucrose, 18g Agar then added to a liter of distilled water.

Potato dextrose agar (PDA) : Preparation ofthe PDA was according to Collee *et al* (1996) by pouring 39g of medium powder per one liter of distilled water.

Cellulose agar medium : (Bååth and Söderström, 1980) method which included the following materials : 2g$NaNO_3$, 1g $(NH_4)_2SO_4$, 1g KH_2PO_4, 0.5g $MgSO_4.7H_2O$, 0.5g KCl, 0.05 $CaCl_2$, 0.01g$FeSO_4.7H_2O$, 0.01 g $CaSO_4.5H_2O$, 0.005g $MnSO_4.4H_2O$, 0.001 g $ZnSO_4.7.H_2O$, 15 g Agar. Dissolved in a liter of distilled water and added to the 5 g of pure cellulosic acid 85% (Tansey, 1971). The medium was used to detect the susceptibility of fungi to the production of cellulose enzyme.

Skimmed Milk medium : Five grams of skimmed -milk was poured in the 50 ml distilled water. Dissolved 10 g of sugar in 450 ml distilled water in another flask. Equilibrate the pH to 7. Sterilize the two solutions separately and then cool to 45°C. Then mixed together (Hankin and Anagnostakis, 1975). This medium was used to detect the susceptibility of fungi to protease production.

Starch agar medium : A fifteen grams of starch, 1 g K_2HPO_4, 0.5 g of $MgSO_4.7H_2O$ and 15 g of sugar in a litter of distilled water (Mohammed *et al*, 2018).

Tween 80 agar medium : This medium was prepared from the following ingredients, 10 g peptone, 5 g NaCl and 0.1 g $CaCL_2$, per liter of distilled water, 5 ml of Tween80 was added and the pH was adjusted at 6.8. This medium was used to investigate the susceptibility of fungi and yeasts to production of lipase enzymes (Slifkin, 2000).

To all of the above mentioned Media, 100 mg / L of Amoxicillin antibiotic was added after sterilization, theywere sterilized by autoclave at 121°C under 1.5 pressure for 20 min. Then cooled andpoured into plastic Petri dishes.

Collection of samples : The study included the collection of 300 samples; the samples were taken from beginner, middle and end of manuscripts. The samples were taken from the manuscripts by smear and planted on special media, and then transferred to the laboratory.

The inoculations were planted in Sabouraud's Dextrose Agar medium (SDA). They were placed in the incubator for growth at 28^0C and for 5-7 days. The dishes were examined first for the purpose of counting the growing colonies on the growing media. The fungal species were purified onto the SDA and incubated at 28°C for 5-7 days to obtain pure colonies. The fungus was also re-grown on Czapex Dox Agar and Potato Dextrose Agar medium. The isolated fungus was firstly identified according to morphological and microscopic characteristics recorded in different mycological keys (Ellis, 1971; Barnett and Bary, 1972; Pitt and Hoching, 1997 and Moubasher, 1993). Consecutively, the identification was confirmed at molecular level after DNA extraction and purification as described in Al-Tamimi (2019), using ribosomal internal transcribed spacer (ITS) region with universal primers ITS1 and ITS4 and then PCR reaction was then sent to the Korean company Macrogen to sequence the DNA using DNA sequencing system. Then the fungal were registration in International Genbank. The results werethen compared with the international isolates using Omega program phylogeny.

Total number of isolates and fungal species isolated from each manuscript were calculated. The percentage of occurrence, Frequency and Distribution Intensity Index (DII) were calculated from the following respective equations (Booth *et al*, 1988):

$$\% \text{ Occurrence} = \frac{\text{Number of specimens that appeared genus or species}}{\text{The total number of samples during the study}} \times 100$$

$$\% \text{ Frequency} = \frac{\text{Number of single isolates}}{\text{Total number of isolates}} \times 100$$

$$\text{DII} = \% \text{ occurrence} \times \sqrt{\%} \text{ Frequency}$$

Detection of analyzing fungi : In order to investigate the activity of fungi and their damage to the manuscripts, fungi were studied. Analyzing enzymes of Cellulose, Protein, Starch and lipids were examined.Each

experiment included three replicates for each fungal species and each enzyme typesin addition to the control (without inoculation).

The center of selective media was inoculated with 5 mm tablet of pure fungus that have been developed on PDA medium, three days age at 28°C for 72 hours.

Cellulose : Use the medium agar- cellulose media. Cellulose decomposition was detected by using the HCl-Iodine reagent. Addition ofthe detector to the dish and left for 5 minutes, then pour the solution and leave the dish for 10 minutes. The appearance of a translucent circle around the fungal colonies showed that cellulose was transformed into simple sugars by the enzyme cellulose. The diameter of the circle, means the higher activity of the fungus in the production of the enzyme (Yeoh *et al*, 1985).

Protein : Use the medium Skimmed milk- agar media. Protein breakdown (casein in milk) was detected when a translucent circle appeared around the colonies. The higher diameter of circle means higher activity of the fungus in the production of the enzyme (Hankin and Anagnostakis, 1975).

Starch : Use the medium starch agar.Detection of starch decomposition using reagent. Addition of the detector to the dish and left for 5 minutes and then poured the solution and left the dishes for 5 minutes. The appearance of a transparent circle around the fungal colonies indicates the production of the enzyme amylase and the greater the diameter of the circle whenever evidence of fungal activity in the production of the enzyme (Pandey *et al*, 2000).

Lipidenzyme : Use the medium Tween 80-supported peptide. The results were recorded by observation of white sediments around the colonies or by the appearance of a transparent circle around the colony of fungi (Takó *et al*, 2012).

The following methods depend on the enzymes activity to select only seven species of fungi as listed below.

Table 1 : The number of isolates and the percentage of total numbers of fungal species isolated from the three sites of the manuscripton the PDA at 28 ° C and for a period of 3 days incubation.

Place of isolates sampling of manuscript	Number of isolates	Percentage of total numbers
Beginning	239	43.76
Center	102	17.96
End	216	37.96
Total	557	99.68

Table 2 : The isolate was first identified on the manuscripts, registered with the genbank and accession numbers.

No.	Isolates of fungal	Accession numbers
1	*Alternaria atra* strain alaa1-A.	MK503427
2	*Aspergillus ustus* strain alaa4-A.us 5.	MK503428
3	*Chaetomium globosum* strain ala11-C.gl	MK504425
4	*Cladosporium exasperatum* strain ala14-C.ex	MK504424
5	*Microdochium nivale* strain alaa6-M.ni 5.8S	MK503439
6	*Penicillium tardochrysogenum* strain ala15-P.ta	MK504426
7	*Penicillium tardochrysogenum* strain ala13-P.ta	MK504427

RESULTS AND DISCUSSION

Isolation and diagnosis of fungi isolated from manuscripts. Five hundred and fifth six species of fungus belonging to seven genera were isolated from three sites of the manuscripts representing the beginning, center and end of the manuscript (Table 1).

The beginning of the manuscripts recorded the highest number of isolates that constitute 43.76 percentage of the total numbers, the reason for this difference may be due to the nature of the beginning cover of these manuscripts, so many covers which are the main constituent of the manuscript are leather or the beginning was more prone and flipping pages than other parts. The center of the manuscript consists of paper, whose basic structure is cellulose, although, cellulose forms is the bulk of the manuscript, fungi find it difficult to analyze the components Cellulose, as well as other materials of inks, adhesives, etc. and their effect on changing pH and humidity, which is a determinant of enzymatic effectiveness with appropriate temperatures (Silva *et al*, 2001).

Seven types of fungi were isolated during this study and were the first recorded in the manuscripts as they were the first record in Iraq. This was confirmed by the results of the registration of the GenBank of the National Center for Biotechnology Information (NCBI National Center of Biotechnology) and had accession numbers as shown in Table 2. All fungi were found under the Kingdom Eukaryota; Fungi; Dikarya and the Division of Ascomycota and under division pezizomycotina.

The first one of isolates was *Alternariaatra strain alaa1-A*., which had accessionnumber in Genbank MK503427 (Table 2). In Fig. 1: A,B, *Alternaria atra* morphology characterized by colonies begin as grayish white, rapidly turning dark and fluffy.Colonies showd concentric circles of dark-brown. The reverse is black. Colonies are not wrinkle. Microscopic hyphae are highly septate, dark walled with dark septa, conidiophores are produced directly from hyphae with septation. The conidiophoresare often foot shaped at the terminal cell, producing conidia. The conidia are large, brown, unique,

semi ovule shaped with multiple septations (8–16 × 23–50 ìm) with transverseand and longitudinal septations, frequently (Fig. 1:C). These characteristies were similar to the general described in Carmen (2017). The phylogenetic tree was constructed for *Alternaria atra* strain alaa1-A. isolate from multiple sequence alignment of 18S rDNA gene sequenceswere identical to the international isolates ex: Qatar, Iran etc. (Fig. 1D).

Also,these condisolate was *Aspergillus ustus* strain alaa4-A.us 5., which had accession number in Genbank MK503428 (Table 2). In Fig. 2 A, B, *Aspergillus ustus*. The colony begins as white colony, but rapidly developcolors of brown to black. Colonies are velvet like and aremature in 3–5 days. Form concentric circles of uniform in color. The reverse issmooth, black. Colonies are not wrinkle. Fig. 2: A, B. Microscopic hyphae are septate and branching at the long conidiophores arise from hyphae via a foot cell, ending in a swollen, round, uniseriate. Vesicle from the phialides emerge the conidia that form chains. The conidia are round with a smooth surfac (Fig. 2C.) These characteristics were similar to the general describe in Carmen (2017). The phylogenetic tree was constructed for *Alternaria atra* strain alaa1-A. isolate from multiple sequence alignment of 18S rDNA gene sequencesthe *Aspergillus ustus* is identical 100% to the international isolates Egypt, Brazil, China, USA and Netherlands (Fig. 2D).

So, the third isolate was *Chaetomium globosum* strain ala11-C.gl, which had accession number in Genbank *MK504425* (Table 2). In Fig. 3 A, B, *Chaetomium globosum* strain ala11-C.gl colonies begin as white and cottony, spread, to white with age. The reverse is yellow to orange and are not wrinkle, (Fig. 3 A, B). Microscopichyphae are dematiaceous, septate and wavy. Large (90–170 × 110–250 µm) flask shaped perithecia are produced. Perithecia with the appearance of a pineapple, with hyphae radiating outward in all directions. The hyphae may be wavy or long and pointed at the ends. Asci are not observed (Fig. 3 C). These characteristics were similar to the general describe in Carmen (2017). The phylogenetic tree was constructed for *Chaetomium globosum* strain ala11-C.gl from multiple sequence alignment of 18S rDNA gene sequences and identical to the international isolates ex: China and Egypt (Fig. 3D).

The fourth isolate was *Cladosporium exasperatum* strain ala14-C.ex, which had accession number in Genbank MK504424 (Table 2). In Fig. 4 A, B), *Cladosporium exasperatum* strain ala14-C.ex. Colony first appears white, then rapidly becomes dark brown.

The surface is rough and convoluted, with a smooth to powdery texture. The reverse is dark brown to black (Fig. 4 A, B). Microscopic hyphae are hyaline, septate, and rough. Conidiophores are produced from the hyphae, with a large swollen oval shaped cell from which branching occurs. Separate conidiophores bearing flask shaped phialides. Conidia are produced in chains from these terminal phialides. Conidia are round, remain in distinct chains. These characteristics were similar to the general describe in Carmen (2017). The phylogenetic tree was constructed for *Cladosporium exasperatum* strain ala14-C.ex from multiple sequence alignment of 18S rDNA gene sequences and identical to the international isolates ex: India and Kenya (Fig. 3D). The fifth isolate was *Microdochium nivale* strain alaa6-M.ni, which had accession number in Genbank MK503439 (Table 2). In Fig. 5 A, B, *Microdochium nivale* strain alaa6-M.ni. Colony first appear white, then rapidly becomes universal color dark creamy to dark green todark olive. Mycelium inter- and intra-cellular, hyaline, hyphal strands running parallel to the surface of the media as shown in Fig. 5A. The reverse is dark brown to black (Fig. 5 B). Microscopic hyphae are hyaline, septate and rough. Conidiophores are produced from the hyphae, with a large swollen oval shaped cell from which branching occurs. Separate conidiophores bearing flask shaped phialides. Conidia are produced in chains from these terminal phialides. Conidia are round, remain in distinct chains. These characteristics were accepted by Galea *et al* (2009). The phylogenetic tree was constructed for *Microdochium nivale* strain alaa6-M.ni from multiple sequence alignment of 18S rDNA gene sequences and identical to the international isolates ex: China (Fig. 5D).

The sixth and seventh isolates were *Penicillium tardochrysogenum* strain ala15-P.ta which has accession number MK504426 and *Penicillium tardochrysogenum* strain ala13-P.ta, there accession number was MK504427 (Table 2).

Colonies developed rapidly and were olive green with white margin. Reverse was yellow. Surface of the colony was powdery to wooly velvety (Fig. 6 A1, A2, B1, B2, respectively). Microscopic hyphae were hyaline and septate. Phialides were formed on short branched. Secondary branching occurs via metula. Phialides produced singly. Branching via metula formation. This gives a brush like appearance referred to as a penicillus. Phialides were wollen at their bases descending into a neck. Conidia (ameroconidia) were produced from phialides in chains and were smooth, ellipsoidal, hyalineas described in ('New Penicillin-Producing Penicillium Species and an Overview of Section Chrysogena' 2012) and Carmen (2017). The phylogenetic tree was

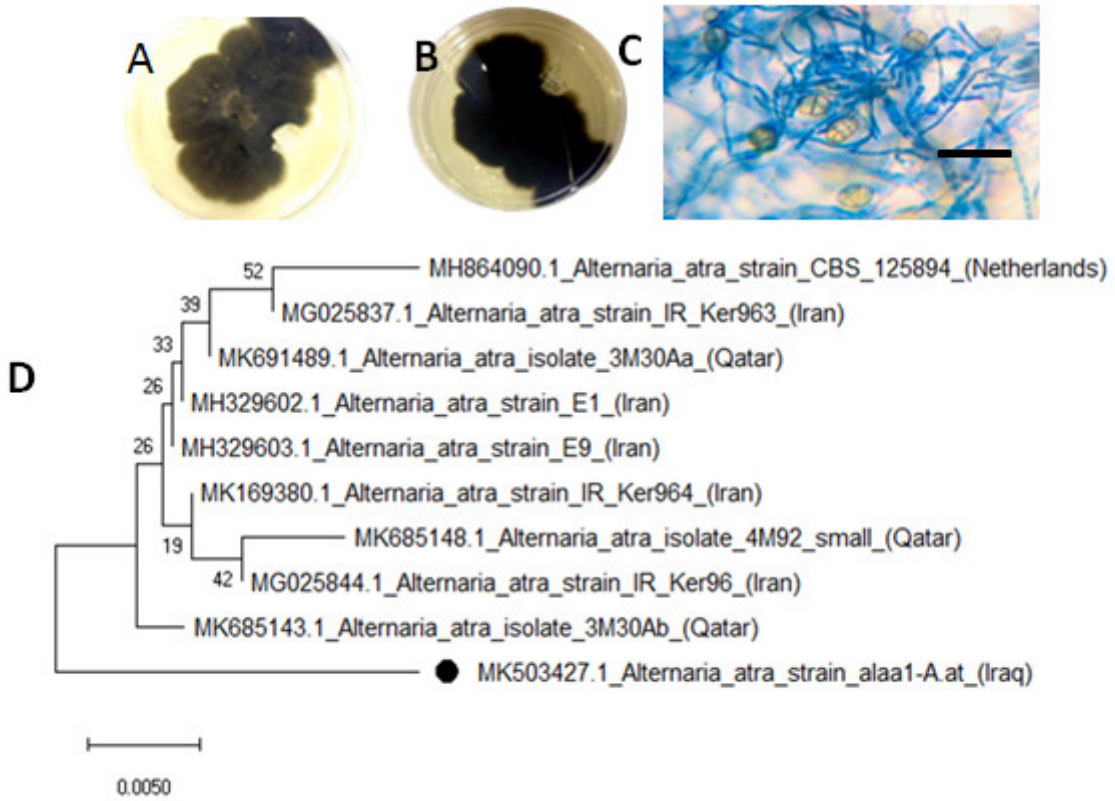

Fig. 1 : *Alternaria atra* strain alaa1-A. (A) 5 days face old culture at 28°C on PDA medium; (B) reverse culture; (C) Microscopic features showing conidia and conidiophores (Scale bar = 20µm); (D) Phylogenetic tree of *Alternaria atra* strain alaa1-A with reference strains. Bar indicates genetic distance due to sequence variation.

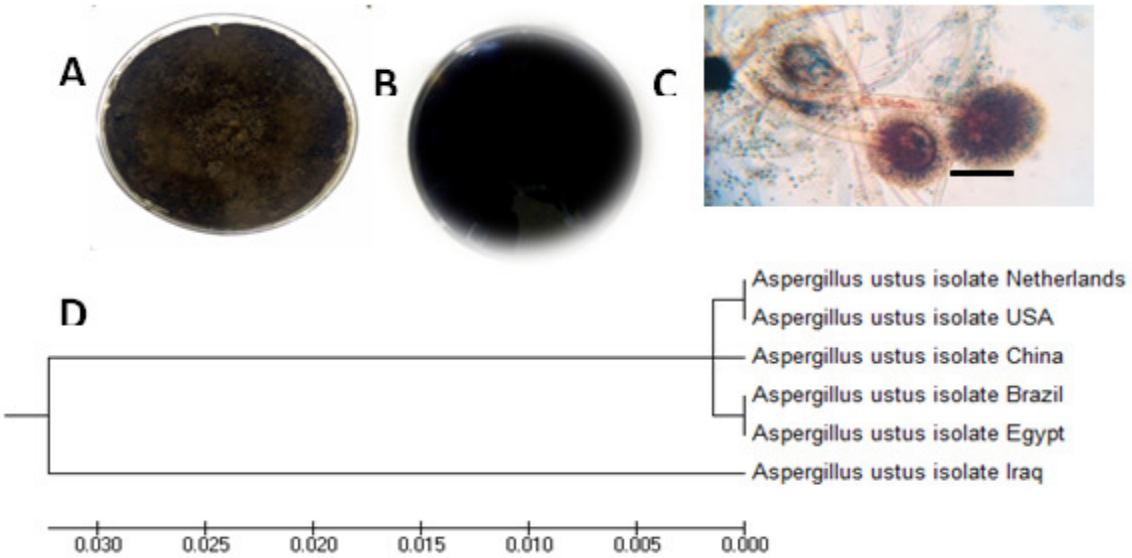

Fig. 2 : *Aspergillus ustus* strain alaa4-A. (A) 5 days face old culture at 28°C on PDA medium; (B) reverse culture; (C) Microscopic features showing conidia and conidiophores (Scale bar = 20µm); (D) Phylogenetic tree of *Aspergillus ustus* strain alaa4-A with reference strains. Bar indicates genetic distance due to sequence variation.

constructed for were identical 100% to the international isolates Slovakia, China, South Africa, India and Spain.

Aspergillus ustus recorded the highest percentage of occurrence, frequency and distribution density coefficient, followed by *Alternaria atra* as shown in Table 3. These resultesare accepted with Mohammed *et al* (2018) that these fungi can grow and reproduction in a broad spectrum in temperature, humidity, acidity and many adverse condition and can reproduce and separate more than one method (Ruisi *et al*, 2007).

Detection of fungi analyzed

Cellulose : The seven fungal isolates were ableto produce cellulose degradation enzyme at different

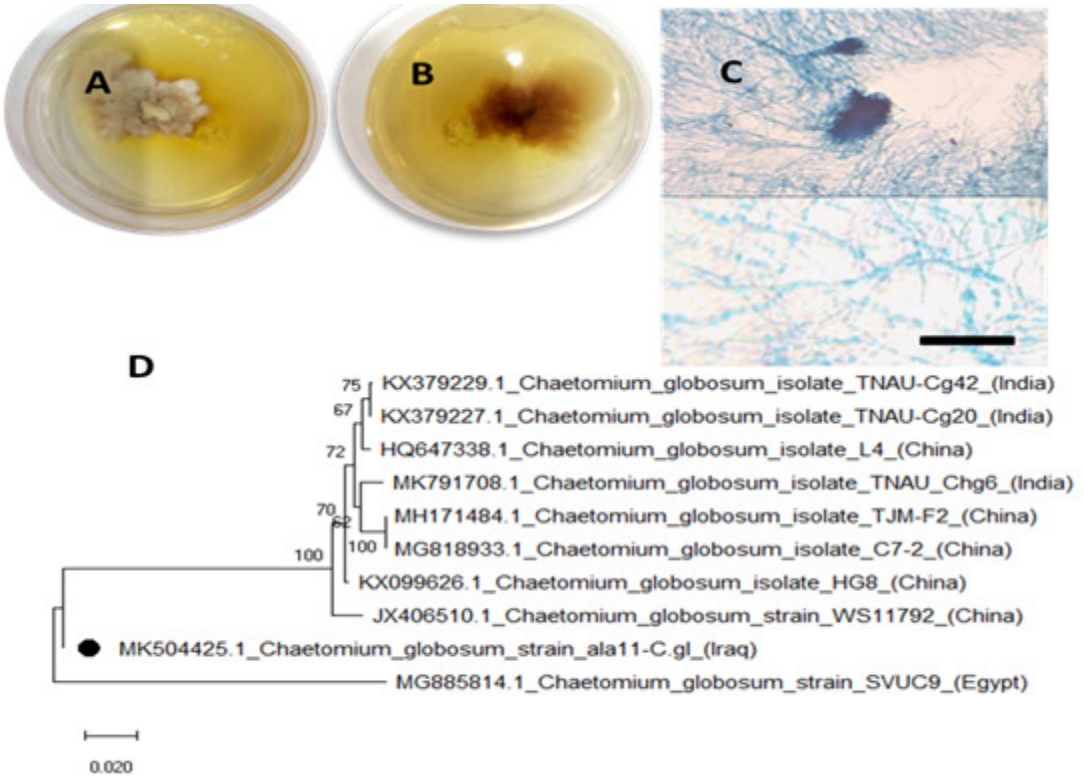

Fig. 3 : *Chaetomium globosum* strain ala11-C.gl (A) 5 days face old culture at 28^{0}C on PDA medium; (B) reverse culture ; (C) Microscopic features showing conidia and conidiophores (Scale bar = 20 µm); (D) Phylogenetic tree of *Chaetomium globosum* strain ala11-C.gl with reference strains. Bar indicates genetic distance due to sequence variation.

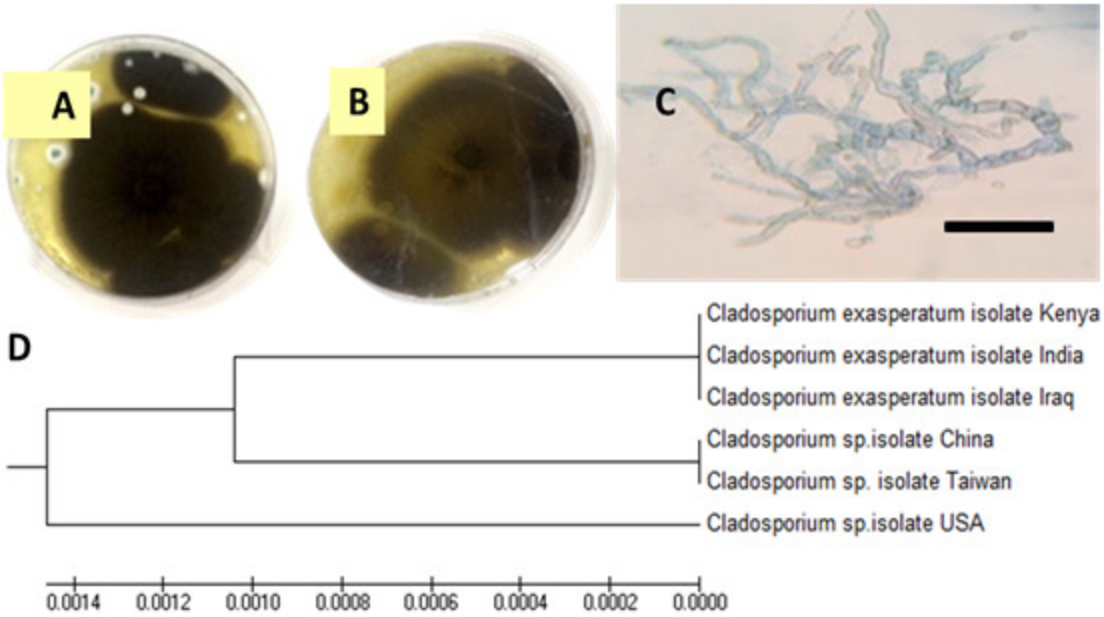

Fig. 4 : *Cladosporium exasperatum* strain ala14-C.ex : (A) 5 days face old culture at 28^{0}C on PDA medium; (B) reverse culture; (C) Microscopic features showing conidia and conidiophores (Scale bar = 20 µm); (D) Phylogenetic tree of *Cladosporium exasperatum* strain ala14-C.ex with reference strains. Bar indicates genetic distance due to sequence variation.

efficiencies (Table 4). The *Alternaria atra*, which was not differ significantly from the *Aspergillus ustus* and *Chaetomium globosum* while, *Cladosporium exasperatum* effective and penicillium tardochrysogenum -1 were the lower activity compared with the rest (Fig 7 A, B, C, D). The table also showed that there were interaction between the fungus type and the incubation period. ex: *Alternaria atra* at nine days of incubation was give the highest value, which was not significantly different from *Aspergillus ustus*. This is the fact that most of the fungi have the ability to degradation natural cellulose for the purpose of growth and reproductive

Table 3 : Percentage of total occurrence, percentage of frequency and distribution density coefficient of fungi isolated from manuscripts.

Isolates offungal	Percentage of occurrence	Percentage of frequency	Distribution intensity coefficient
Alternaria atra	%10.0	14.23	37.72
Aspergillus ustus	11.6%	14.93	44.82
Chaetomium globosum	%7.0	12.47	24.71
Cladosporiu mexasperatum	%7.1	13.18	25.77
Microdochium nivale	%8.33	14.93	32.18
Penicillium-1 tardochrysogenum	9.33%	14.41	35.41
Penicillium-2 tardochrysogenum	9.0%	15.81	33.18

Table 4 : Degradation of cellulose by fungus on the cellulose agar at 28°C and for a period of 3,6,9 days incubatione.

Isolats of fungal	Diameter of halo zone (mm)			Average fungi
	Incubation period (day)			
	3	6	9	
Alternaria atra	22.33	55.00	65.00	47.44
Aspergillus ustus	23.67	52.33	65.00	47.00
Chaetomium globosum	23.33	50,67	58.33	44.11
Cladosporium exasperatum	19.67	21.67	29.00	23.44
Microdochium nivale	23.67	47.67	54.33	41.89
Penicillium-1 tardochrysogenum	19.33	28.33	48.33	32.00
Penicillium-2 tardochrysogenum	22.67	45.00	55.00	40.89
Average incubation period	22.10	42.95	53.57	

$L.S.D._{0.05}$, Incubation period 4.167, Interaction 7.218, Fungi 2.728

Table 5 : Protein degradation by fungus on skimmed milk at 28°C and for a period of 3, 6, 9 days.

Isolats of fungal	Diameter of halo zone (mm)			Average fungi
	Incubation period (day)			
	3	6	9	
Alternaria atra	21.67	36.67	55.00	37.78
Aspergillus ustus	50.00	65.00	73.67	62.89
Chaetomium globosum	48.33	65.67	71.67	61.89
Cladosporium exasperatum	35.00	50.00	55.00	46.67
Microdochium nivale	54.33	63.33	73.67	63.78
Penicillium-1 tardochrysogenum	25.00	35.67	53.67	38.11
Penicillium-2 tardochrysogenum	40.00	44.00	54.00	46.00
Average incubation period	39.19	51.48	62.38	

$L.S.D._{0.05}$, Incubation period 4.728, Interaction 8.189, Average fungi 3.095

through the production of cellulosic enzymes (Armstrong, 1989). Soutilize a set of hydrolytic enzymes typically composed of endoglucanase, cellobiohydrolase and β-glucosidase, in some species, because many species grow on dead wood or litter, in environment rich in cellulose and the Fungal cellulolytic systems differ from the complex cellulolytic systems of bacteria (Baldrian and Valášková, 2008).

Protein : As shown in Table 5, fungi were different in the rate of the halo diameter represented by the efficacy of protease and the *Microdochium nivale* recorded the highest efficacy of protease, which was not significantly different from the enzyme efficacy of *Aspergillus ustus* and *Chaetomium globosum* (Fig. 7 E, F, G). While, *Penicillium tardochrysogenum*-1 was less effective for protease, which did not differ significantly from the effectiveness of the protease enzyme for *Alternaria atra*. In terms of the incubation period, the enzyme's effectiveness increased with increasing incubation period until the end of the 9th-day experiment. The interaction between types of fungi and the period of incubation, *Microdochium nivale* was the

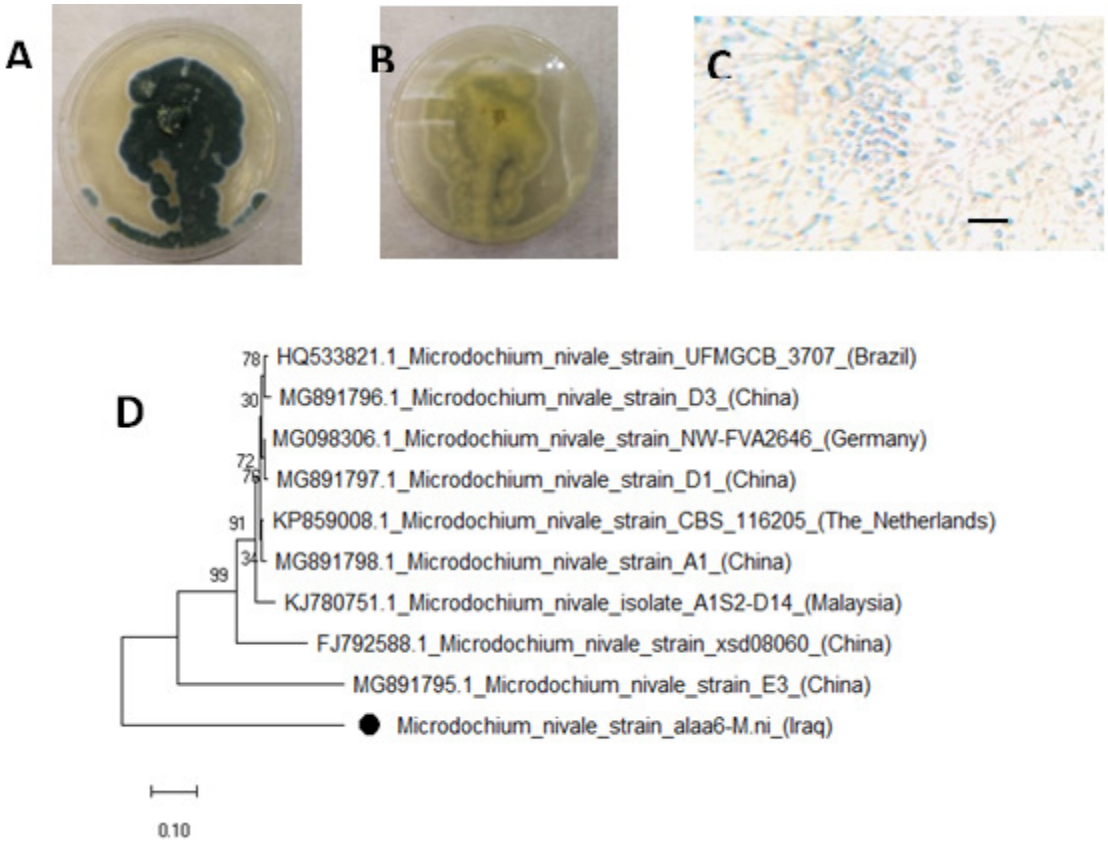

Fig. 5 : *Microdochium nivale* strain alaa6-M.ni*:* (A) 5 days face old culture at 28°C on PDA medium; B) reverse culture; (C) Microscopic features showing conidia and conidiophores (Scale bar = 20µm); (D) Phylogenetic tree of *Microdochium nivale* strain alaa6-M.ni with reference strains. Bar indicates genetic distance due to sequence variation.

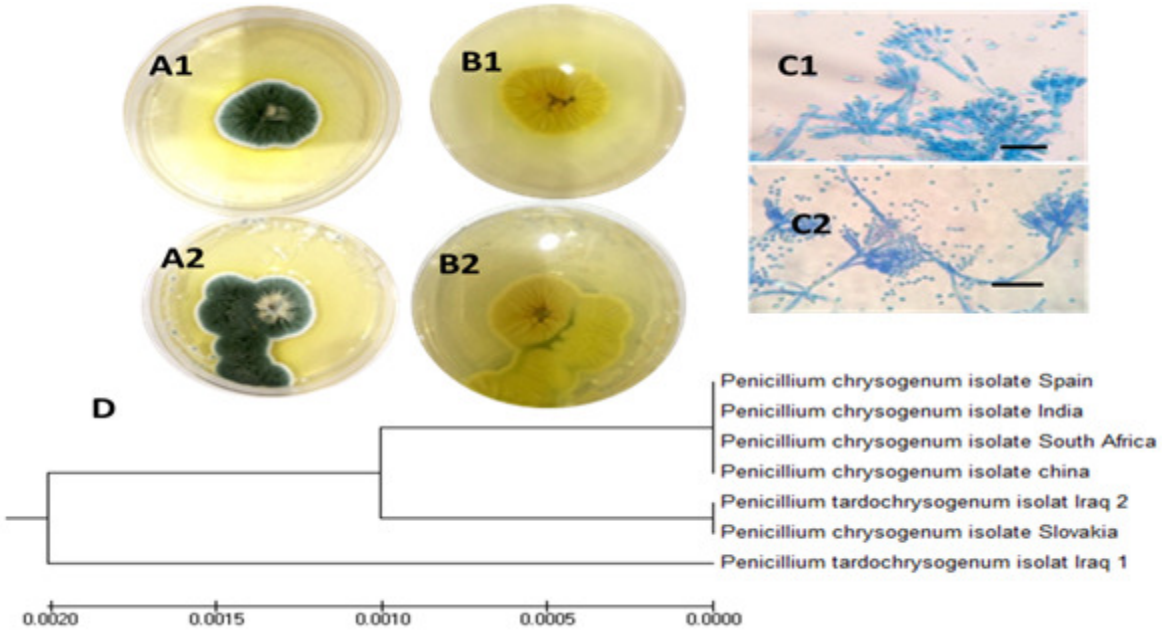

Fig. 6 : (A) *Penicillium tardochrysogenum* strain ala15-P.ta after 15 days face old old culture at 28°C on PDA medium; (A2) *Penicillium tardochrysogenum* strain ala13-P.ta after 5 days face old culture at 28^{0}C on PDA medium. (B1, B2) reverse cultures respectively; (C1, C2) Microscopic features showing conidia and conidiophores respectively (Scale bar = 20 µm); (D) Phylogenetic tree of *Penicillium tardochrysogenum* strain ala15-P.ta and *Penicillium tardochrysogenum* strain ala13-P.ta with reference strains. Bar indicates genetic distance due to sequence variation.

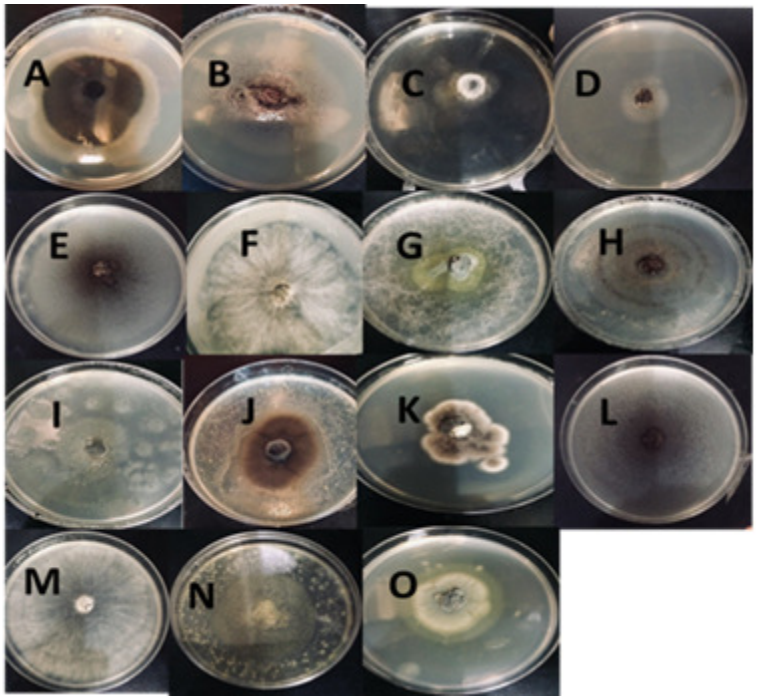

Fig. 7 : A = Degradation of cellulose by *Alternariaatra* on the cellulose agar at 28°C andfor a period of 9 days incubation. **B** = Degradation of cellulose by *Aspergillususstus* on the cellulose agar at 28°C and for a period of 9 days incubation. **C**= Degradation of cellulose by *Chaetomium globosum* on the cellulose agar at 28°C and for a period of 9 days incubation. **D**= Degradation of cellulose by *Cladosporium exasperatum* on the cellulose agar at 28°C and for a period of 9 days incubation. **E**= Protein degradation by *Aspergillususstus*on skimmed milk at 28°C and for aperiod of 9days. **F** = Protein degradation by *Chaetomiumglobosum*on skimmed milk at 28°C and for a period of 9 days. **G**= Protein degradation by f*Microdochiumnivale*on skimmed milk at 28°C and for a period of 9 days. **H**= Starch degradation by *Aspergillus ustus* on starch at28°C for 3 days incubation. of 9 days. **I**= Starch degradation by *Microdochium nivale* on starch at 28 ° C for 3 days incubation. of 9 days. **J** = Lipid degradation by *Alternaria atra* on agar - tween 80at 28°C for 3 days incubation of, 9 days. **K** = Lipid degradation by *Aspergillus ustus* on agar - tween 80 at 28°C for 3 days incubation. of 9 days. **L**= Lipid degradation by *Chaetomium globosum* on agar - tween 80 at 28°C for 3 days incubation. of, 9 days. **M**= Lipiddegradation by *Cladosporium exasperatum* on agar –tween 80at 28°C for 3 days incubation of 9 days. **N**= Lipid degradation by *Penicillium tardochrysogenum* -1 on agartween 80 at 28°C for 3 days incubation of 3, 6, 9 days. **O** = Lipid degradation by *Penicillium tardochrysogenum* -2 on agar -tween 80 at 28°C for 3 days incubation of 3, 6, 9 days.

Table 6 : Starch degradation by fungus on starch at 28°C for 3 days incubation of 3, 6, 9 days.

Isolats of fungal	Diameter of halo zone (mm)			Average fungi
	Incubation period (day)			
	3	6	9	
Alternaria atra	0.00	0.00	47.67	15.89
Aspergillus ustus	50.00	55.00	73.00	59.33
Chaetomium globosum	0.00	0.00	70.67	23.56
Cladosporium exasperatum	28.33	35.00	34.00	32.44
Microdochium nivale	53.33	58.33	69.00	60.22
Penicillium-1 tardochrysogenum	23.00	25.00	39.00	29.00
Penicillium-2 tardochrysogenum	27.67	34.33	34.33	32.11
Average incubation period	26.05	29.67	52.52	

$L.S.D._{0.05}$, Incubation period 4.40, Interaction 7.63, Fungi 2.88

highest efficacy of the enzyme protease in the 9th day of incubation, which was not significantly from the *Aspergillus ustus*. The role of protease to analyze the protein was studied previously (Maheshwari *et al*, 2003), but the type of fungus depended on more than one cause such as the types of protein constituents, the lather and Parchment paper the lather and parchment paper that used for manuscripts cover which is more suitable to attack by microorganisms especially the saprophytic fungi or weak pathogenic fungi (Baldrian and Valášková, 2008).

Although, the *Microdochium nivale* was thrives in a cold climate (Kuwabara *et al*, 2002), so attach many

Table 7 : Lipid degradation by fungus on agar -tween 80 at 28°C for 3 days incubation of 3, 6, 9 days.

Isolats of fungal	Diameter of halo zone (mm)			Average fungi
	Incubation period (day)			
	3	6	9	
Alternaria atra	26.67	48.33	48.33	41.11
Aspergillus ustus	41.67	63.33	67.67	57.56
Chaetomium globosum	62.67	70,00	73.00	68.56
Cladosporium exasperatum	25.00	35.00	41.67	33.89
Microdochium nivale	53.00	65.00	71.67	63.22
Penicillium-1 tardochrysogenum	35.00	41.67	41.67	39.44
Penicillium-2 tardochrysogenum	35.00	45.00	68.33	49.44
Average incubation period	39.86	52.62	58.90	

$L.S.D._{0.05}$, Incubation period 5.43, Interaction 9.41, Average fungi 3.43

plant as a pathogen (Ren *et al*, 2014), but it was found in manuscript it was the first record, that means the fungus is regenerated or still active in residue during the manufacturing the paper for manuscript or contamination during keeping it.

Starch : In Table 6, *Microdochium nivale* gave the largest diameter of the amylase enzyme, which did not differ significantly from the effect of the *Aspergillus ustus* and differed significantly from the rest of the isolated fungi (Fig. 7 H, I). The *Alternaria atra* was less effective than the rest fungi. The same table shows that the enzyme increases with the period of incubation increases significantly until the end of the experiment on the ninth day of inocubation. *Aspergillus ustus* was the highest activity of the enzyme and did not differ significantly from the *Chaetomium globosum*, noting that this fungus did not register any effectiveness in the third and sixth days and this may be due to the slow growth due to the environmental conditions (Beales, 2004). This may be anounced that the differ composition on manuscript and role of some substances used as to maintain and kepping the manuscript can causes to utilize from fyngi for feeding and reproduction (Mohammed *et al*, 2018).

Lipids : The results showed, that, all isolates are capable to degradation amylase. As shown in Table 7, *Chaetomium globosum* showed the largest diameter of the halo zone which did not differ significantly from the *Microdochium nivale* (Fig. 7 J, K, L, M, N, O). While the *Cladosporium exasperatum* recorded a lower diameter of the halo zone. In the same table it is noted that the enzyme increased with the incubation period increases significantly until the end of the experiment on the ninth day of incubation. Within the period of inocubation the halo zone diameter remains the same value *Alternaria atra* and *Penicillium-1 tardochrysogenum*. The interactin between the fungus and period of incubation was significant and *Chaetomium globosum* was the most effective of the enzyme and did not differ significantly from the *Microdochium nivale*. With note that on the ninth day of the incubation period, the diameter in both the *Alternaria atra* and *Penicillium tardochrysogenum-1* were still conistant, where the emergence of white deposits around the fungal colony, which confirms the ability of the fungus on the secretion of the enzyme continuously and conduct a second course of activity when the entry of food, as the course of feeding on one of the components of the other food medium. This means the ability of the fungi to adapt and survive despite the entry of the basic material of food (Park *et al*, 2013). These results were in accordance with Pandey *et al* (2000) and with Al-Waily (2017), when isolated and identified the fungi from manuscripts, but in different species.

CONCLUSION

The work on manuscripts has many aspects, most important of which is the identification of the most important factors affecting the life of manuscripts, especially fungi, which have the most important role in the analysis of the components of the manuscripts and dealing with manuscripts as a food used by organisms for reproduction and growth. The fungi that were isolated and diagnosed for the first record, all of them related to the Ascomycetes. Although, some of them are saprophyte but the rest are parasite and weak parasite, but they have enzyme activity enzyme activity needs further study and in any case, the presence of these fungi is a reason to deepen the study of the manuscripts and try to deal with the case of injury and restoration of manuscripts.

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