Enzymatic efficacy of some types of Aspergillus fungi isolated from some manuscripts and its effect on some of the physical and chemical properties of the manuscripts

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Abstract:
The study has been conducted in the laboratory of Imam Hussein Center (peace be upon him) for the restoration and conservation of manuscripts and the care of researchers at the Imam Hussein Holy Shrine. 150 fungal isolates of Aspergillus spp. have been taken from manuscripts. Aspergillus, Aspergillus flavus, Aspergillus nidulans, Candidus, Apergillus oryzae, Apergillus niger were diagnosed. The enzymatic tests were performed to determine which species has the ability to produce analyzer of protein, cellulose, fat and starch enzymes on solid culture media of specific inspection.

The results showed that Aspergillus fungi were differentiated in its production of the studied enzymes. A. nigar gave positive result for all studied enzymes with varying level of efficacy. The highest enzyme activity of this species was amylase, lipase and cellulase. However, A. oryza recorded the highest efficacy of cellulase and amylase and medium efficacy with protease. A. candidus was producing high efficacy to protease, lipase and cellulase enzymes and gave a negative test with the amylase enzyme. A. flavus was the higher efficacy with protease, lipase, cellulase enzymes and medium efficacy with amylase, while A. nidulans was recorded as the highest efficacy with protease, lipase, and cellulase enzymes and medium efficacy with amylase enzyme.

The effect of growth of these fungi on the physical properties of the elongation strength, tension force and color change of paper was studied. The results showed that the effect of fungi on the manuscripts through the exposure of the damaged sample to the dark color compared to the standard sample (unaffected). The results demonstrated that the lowest values in terms of tensile strength and elongation for the affected sample compared to the non-affected sample, as well as the study of their effect on the chemical properties using X-ray diffraction analysis to determine the degree of crystallization of cellulose. The results showed an increase in the crystallization of cellulose. This means a change in the chemical and mechanical properties of cellulose. In addition, the infrared spectrum was used to determine changes in the functional groups of paper observing which revealed occurring a slight decrease in the OH group as a result of the loss of water molecules from cellulose. Also, we noticed an increase in C = O absorption due to cellulose oxidation processes.

1-Introduction:
Manuscripts and books are materials of organic origin (plant or animal), such as paper, leather, papyrus, cloth and sometimes wood. These substances are hydroscopic nature which mean their internal aquatic content changes by changing ambient moisture, particularly when the relative humidity in the environment rises, the organic material absorbs water, and then the water content of the materials leading the manuscripts and books to be susceptible to degradation fungi and also facilitates the adhesion of dust and other pendants in the air, causing contamination and fouling of the archaeological materials. The dust and the pendants in the air have a role in the infection of the manuscripts with fungi because they carry fungal spores that grow very fast especially if moisture and heat are available[1].
Fungi play the main role in the destruction of manuscripts as measured by bacteria and Actinomycines because of their ability to withstand the wide range of temperatures and lack of moisture, it has been found that fungi can grow to the degree of zero percentage (thermo phobic fungi) and can withstand in more than 60 °C. Regarding to humidity, the fungi grow well at humidity more than 60%, and the saprophytic fungi are related to the destruction and degradation of the manuscripts when the suitable conditions for growth are exist, they are 24-30oC, relative humidity more than 60% and the presence of the manuscript as a nutritional materia [1]. These fungi depend on the way they infect the papers and leather of the manuscripts produce specific enzyme that can break down the paper cellulose and the leather and vellum protein. The enzyme of cellulose is known as cellulase, while the a analysing enzyme for the leather and vellum is Lipase. These enzymes are just complex organic substances that are very sensitive to the change in temperature and (pH)[2].

This study was conducted for the purpose of identifying the most important fungi that cause different chemical and physical effects on the manuscripts and to make way for subsequent studies.

2-Materials and Procedures:-
2-1. Collection of samples:
One hundred and fifty samples were collected using sterile cotton swabs from the manuscripts preserved in the safe of Imam al-Hussain (peace be upon him) centre for the restoration and conservation of manuscripts. It was cultured on the culture medium Potato dextrose agar (PDA) with anti-bacterial additive (chloramphenicol250 mg/L). The incubation was done at temperature of 28 °C for (5-7) days. The collection was carried out periodically every month during the entire year of the experiment, taking into account the use of the same manuscripts each time.

2-2 Used Media:-
2-2-1-Medium of Potato Dextrose Agar (PDA):-
The medium was prepared using product of Himedia-India company by dissolving 39 g of medium powder in one liter of distilled water then autoclaved and used for fungi growth.

2-2-2-Starch Agar Medium:-
It was prepared by dissolving 15 g of starch, 1 g K2HPO4 and 0.5 g of 7H2OMgSO4. And 15 g of Agar in a liter of distilled water according to the method of [3] which was modified by removing yeast extract).

2-2-3-Skimmed-milk Agar:-
This medium was prepared by dissolving 5 g of skimmed milk in 50 ml distilled water, and 10 g of the Agar was dissolved in 450 ml distilled water in another jug, the pH was equalized to 7, the two solutions were sterilized separately then cooled to a degree 45 °C and then mixed together [4], used the medium to detect the susceptibility of fungi to the production of protease enzyme.

2-3-Celluloltic activity - Cellulase Enzyme:
The method described before was adopted by [5] and the medium consist of Potassium dihydrogen phosphate KH2(2 g/L) Ammonium Sulphate (1.4 g/L), Urea (0.3 g/L), Magnesium sulfate (0.3 g/L), calcium chloride (0.3 g/L), Peptone (1 g/L) and sodium salt for carboxy methyl Cellulose CMC (10 g/L). Urea was added after sterilization, CMC-Na salt (Carboxymethylcellulose sodium salt) was gradually added with stirring using a magnetic mixer and heating until it dissolved and then the other components of the Medium were added and the sterilization was made by a steam device on the pressure of 15 pounds/inch2 units and 115o C for 20 minutes, the reagent used to detect the cellulase enzyme is HCl-iodine solution, prepared by mixing 100 ml of acid (HC 0.1) and 500 ml (1%) + (2)% KI in terms of Weight/Volume[6], and the formation of an yellow halo around the fungal colony after the reaction of the reagent indicates to the fungus ability on the analysis of cellulose.
2-3-1-Lipolytic Activity - Lipase enzyme:
The efficacy of fungus production of Lipase enzyme was determined by method of [7], the medium consists of:

Peptone (8 g/L), calcium chloride (0.1 g/L), Tween 80 80 (10 ml/L), Ager (20 g/L) and distilled water (1 litre). The Tween (Sorbitan polyxythylene mono-oleic 80) was sterilized separately and then added to the alkaline medium after sterilization. The secretion of the lipase enzyme in the solid medium was detected either by a visible white residue under the growth or white crystals immersed in the medium around of the colony.

2-4- Diagnosis of Fungi

The following references were adopted in diagnosis of fungi associated with manuscripts [9],[10],[11],[12]. The following were calculated[12]: The total number of isolated Aspergillus isolates during each month of the 12 months of study. The percentage of frequency of Aspergillus spp is calculated from the following equation:

\[
% \ \text{Frequency} = \frac{\text{Number of single type isolates}}{\text{Total Number of all isolates}} \times 100
\]

1. The appearance percentage was calculated according to the following equation:

\[
% \ \text{Occurrence} = \frac{\text{a p p e a r e d i n}}{\text{Total Number of samples during the study}} \times 100
\]

2-5- Enzymatic activity study

Culture media were used for quantitative detection of the effectiveness in production of enzymes (Protease, Cellulase, Lipase, Amylase) of fungal species, which were selected in the study of five species of Aspergillus.

Corky-piercing tablets with 6 mm equal diameters were taken from pure cultures of developing fungal isolates on the PDA medium of three days of age; the nutrition containing dishes were injected with various enzymatic tests and with three repeaters per isolation, taking into account leaving a dish without injection as a control. They were incubated at 28 °c for 72 hours for all enzymes, after which the detection of the studied enzymes was made.

2-6 - Detection of cellulose analyzing fungi

The medium of agar-cellulose was injected from the pure and developing fungal species on the PDA. Medium has detect cellulose decomposition by using the HCl-Iodine reagent, where the reagent was added to the dish and left for 5 minutes and then poured the solution , and the dish left for 10 minutes. The appearance of a translucent halo around the fungal colonies was shown to indicate the conversion of cellulose into simple sugars by cellulose enzyme , the larger the diameter of the halo, the higher activity of fungi in the production of the enzyme[6].
2-6-1-For protein:
Skimmed milk Agar was poured into sterile Petri dishes, then inoculated with fungal species and then detected protein breakdown (Casein in milk) when a transparent halo appears around the fungal colonies[4].

2-6-2-Amylase:
The starch medium was inoculated with pure fungal species, it detected starch decomposition using iodine reagent. The reagent was added to the dish and left for 5 minutes, then the solution poured and the dish left for 5 minutes. A transparent halo around the fungal colonies was observed which indicates the production of the amylase enzyme, The higher the diameter of the halo, the greater the activity of fungus in production of the enzyme[13].
Table (1) was used to measure the diameter of the translucent halo around the fungal colonies of the above colonies to detect the effectiveness of degradation:

Table (1): Diameter of the translucent halo around the fungal colonies of the cellulose, protein and starch detection media.

| analyzing eff | analysis depth | code |
|---------------|----------------|------|
| not analyzing | zero           | -    |
| low effect    | less 10 mm     | +    |
| moderate eff  | 10-15 mm       | ++   |
| high effect   | more 15 mm     | +++  |

2-6-3-Lipase enzyme
The dishes containing the basic material (peptone supported by Tween 80) were injected with fungal isolates to test their ability in secretion of the enzyme. The results were recorded by observation of white sediment around the colonies or by the appearance of a transparent halo around the colony of the fungus [14].

2-7-Studying some physical properties of sound and affected manuscripts: - Color Change: Using (Brightness & Color Meter) The color change of the affected samples was measured comparing to the standard samples (unaffected) and was measured on the wavelength of the high light [15],[16]

2-8-Mechanical properties:
The properties of tensile strength and elongation force were measured by Tinius Olsen Compong device in order to identify the affected mechanical properties and compare them with standard samples (unaffected) [17],[18],[19].

2-8-1-Analysis using infrared spectrum:
The analysis was performed using far infrared spectra (Bruker-Tensor 27) to identify the functional groups of the affected samples and compare them with standard samples (unaffected) to determine the change in functional groups in paper cellulose [20],[21].
2-8-2-X-ray diffraction analysis:
The device (X-Ray: Diffract Meter-ADX2700) was used. To identify the change in the degree of cellulose crystallization for the affected samples compared to the standard samples that are not affected [22][23],[24].

3-Results and discussion

3-1-Isolated fungal species:

Table (2) shows the number of fungal isolated species of Aspergillus ssp: Table 2: Number of Aspergillus isolates isolated from manuscripts during 12 months of study for 5 days at 28 °C ± 2 °C.

| Fungus     | Isolates No |
|------------|-------------|
| A.niger    | 80          |
| A.flavus   | 74          |
| A.oryzae   | 50          |
| A.nidulans | 25          |
| A.candidus | 25          |
| Total      | 245         |

3-2-Percentage of appearance and frequency:
Many fungi were isolated on the manuscripts and five fungi species were selected of Aspergillus spp are A.niger, A.oryzae, A.flavus, A.candidus, and A.nidulans.

Figure 1: Species of fungal species of Aspergillus spp isolated from the manuscripts during 12 months of study for 5 days at a temperature of 28 ± 2 on the PDA medium.
The results of Table (2) showed that most of the samples were infected with \textit{A. niger} with an appearance of 80%, while \textit{A. flavus} ranked second with 74%, followed by \textit{A. oryzae} with 50% and the other two spp. \textit{A. nidulans} and \textit{A. candidus} by 25%, and \textit{A. niger} and \textit{A. flavus} showed the highest appearance of the studied manuscript samples. These results are consistent with [25]. The reason for the domination of \textit{Aspergillus} spp on manuscripts is due its wide spread in the inappropriate environment[26], as well as this result is consistent with what many researchers have mentioned [27], [28] as well as growth in wide ranges of heat and moisture. The species of \textit{Aspergillus}-spp are growing in ranges of temperatures ranging from (5–45\degree C) or higher [29]. These fungi also have the capability to grow at low humidity levels where aspergillus- spp predominate in moisture content ranging from (15-18)\% as well as other factors such as storing the manuscripts under unsuitable conditions for keeping, as the longer the duration increases the likelihood of infections and some of the vital factors of insects and spiders play an important role in the increase of fungal infections through by causing damage to the manuscripts help the storage fungi to cause infection[30] [31], the simple nutritional requirements of the \textit{Aspergillus} species and its bearing on critical environmental conditions have been the cause of the dominance of this species, as well as having a multiple enzymatic system that enables them to exploit various food sources[32]. Some of its species also have the ability to compete in inhibiting the growth of other species through the production of effective vital toxins such as Aflatoxin[33].

As for the percentage of frequency, the results of Table (3) showed that the fungus \textit{A. niger} had the highest frequency value of 31.50%, followed by \textit{A. flavus} (29.13%) followed by \textit{A. oryzae} (19.69%) while the lowest frequency was \textit{A. nidulans} and \textit{A. candidus} (9.84%) for each of them.

Table (3) The percentage of the appearance and frequency of some fungi isolates isolated from the manuscripts during 12 months of study for 5 days at 82 \degree C ± 2 \degree C.

| Fungus  | Appearance Percentage | Frequency percentage |
|---------|-----------------------|----------------------|
| \textit{A. niger} | %80 | 31.50 |
| \textit{A. flavus} | %74 | 29.13 |
| \textit{A. oryzae} | %50 | 19.69 |
| \textit{A. nidulans} | %25 | 9.84 |
| \textit{A. candidus} | %25 | 9.84 |

### 3-3-enzymatic decomposition

#### 3-3-1-Producing protease enzyme of protein decomposition

The results shown in Table (4) show that all fungal species of \textit{Aspergillus}, spp. have the ability to produce the protein decomposition enzyme (protease). The results of the test showed that \textit{A. niger}, \textit{A. candidus}, \textit{A. flavus} and \textit{A. nidulans} were highly effective where the diameter of the transparent halo was greater than 15 mm, while the \textit{a.oryzae} was average in effectiveness, the halo diameter ranged from (10-15) mm. These results are consistent with his findings [34] of protease produced by the fungal species \textit{A. niger}, \textit{A. flavus} and \textit{A. oryzae}. It was very effective as the diameter of the transparent halo was greater than (15 mm), while the \textit{a.oryzae} was average in effectiveness, the halo diameter ranged from (10-15) mm. also These results are consistent with Hussein's[35].
Also [36] was able to produce protease enzyme from A. niger, also [37] mention that A. niger was the optimum in the production of protease in Casein medium, these results was consistent with only what was reached [35] by that A.niger was the most efficient fungus in the production of protease enzyme. Proteins is created by binding of large molecules of about 20 different kinds of aminoacids, they are of great importance as components of cell membranes and structural elements of cells, so their degradation by protease enzyme greatly affects in the system and function of cells forming the leather of manuscripts.

![Figure 2](image_url)

**Figure 2** Effect of fungus in protein analysis on skimmed milk Agar medium at 28 ° C after three days of incubation.

**Table (4)** Analysis of protein by fungi on skimmed milk Agar medium at 28 ° C after three days of incubation.

| Fungi Spp   | Effectiveness of analysis mm | Diameter of analysis area mm | Effectiveness of analysis of Casein |
|-------------|------------------------------|------------------------------|-------------------------------------|
| *A. nidulans* | 15 > High Effectiveness       | 30                           | ++                                  |
| *A. candidus* | 15 > High Effectiveness       | 32                           | +++                                 |
| *A. flavus*   | 15 > High Effectiveness       | 25                           | +++                                 |
| *A. oryzae*   | 15-10 Moderate Effectiveness  | 10                           | ++                                  |
| *A. niger*    | 15 > High Effectiveness       | 25                           | +++                                 |
High Effectiveness +++
Moderate Effectiveness ++

3.3.2 Producing enzyme for cellulose analysis:
For cellulose, the results of Table (5) showed that all isolated fungal species had the ability to secrete the cellulose enzyme at high efficiency and were highly effective as the transparent halo diameter was greater than 15 mm (Fig. 3). These results are consistent with what reached by [38], where it was reported that the duration of (4-8) days was optimal for the production of the enzyme by Aspergillus spp when culturing it on filter paper as a sole source of carbon, as mentioned by [39] that he was able to isolate Aspergillus spp and other species that were the main cause of clothing damage and rot of various tissues, as confirmed by [35], that the fungus A. niger showed a very active activity in the secretion of cellulose, while cellulose is the basic component of the cell walls of the manuscript paper, a sugar made up of glucose molecules.

It is found in all high plants as a structural material in the form of a fine fibers, it is analyzed by the Cellulase enzyme, which consists of Exoglucanase, Endogolucanase and [40] (B-glucanase).

Figure (3) Fungi effectiveness in analysis of Cellulose on cellulose Agar medium at 28 ° C after three days of incubation.
Table (5) Analysis of cellulose by fungi on cellulose enzyme Agar medium at 28 °C after three days of incubation.

| Fungi Spp. | Effectiveness of analysis | Diameter of analysis area mm | Code |
|------------|---------------------------|------------------------------|------|
| *A. oryzae* | High Effectiveness | 35 15 > | +++ |
| *A. candidus* | High Effectiveness | 25 15 > | +++ |
| *A. nidulans* | High Effectiveness | 30 15 > | +++ |
| *A. flavus* | High Effectiveness | 30 15 > | +++ |
| *A. niger* | High Effectiveness | 37 15 > | +++ |

High Effectiveness Moderate +++
Effectiveness ++

3-3-3-Producing enzyme analyzer for Lipase

As for lipase, the results of Table (6) showed that all isolated fungal species had the ability to produce Lipase enzyme by forming white deposits visible under growth as in Fig (4). These results agreed with what [41] has reached, in study performed for the production of Lipase enzyme and protease by the cultivation of laboratory fungus. Lipase enzyme is an important enzyme in causing the disease because it attacks the second component of the cell surface, the plasma membrane [42]

These results agreed with the findings of [43], where he tested the fungus in lipid analysis and gave positive test, as Tween 80 was used as a reaction material for its properties to give visible test easy to observe firstly and their viscosity and easy to mix with the components of culture medium gives more contact between the enzyme and the lipid analyzer.
Fig. (4): Analysis of lipase by fungi on peptone Agar medium supported by Tween 80 and at 28 °C for 3 days incubation.

Table (6): Analysis of lipase by fungi on peptone Agar medium supported by Tween 80 and at 28 °C for 3 days incubation.

| Fungi Spp | Result | halo diameter |
|-----------|--------|---------------|
| A. oryzae | Formation of visible white precipitate under growth | 15 mm |
| A. candidus | of visible white precipitate under growth | 30 mm |
| A. nidulans | Formation of visible white precipitate under growth | 30 mm |
| A. flavus | Formation of visible white precipitate under growth | 33 mm |
| A. niger | Formation of visible white precipitate under growth | 18 mm |

High Effectiveness +++
Moderate Effectiveness ++

3-3-4-Producing enzyme analyzer for amylase:
The results of Table (7) showed the test of the effectiveness of the analysis of starch by amylase-producing fungus isolated from the manuscripts cultured on the medium of the starch Agar. The species A. oryzae, A. flavus, A. nidulans were highly effective with a transparent halo diameter greater than 15 mm as in (Fig. 5), while the A. niger species were moderately effective, the diameter of the halo was between 10-15 and A. candidus gave a negative test. These results agreed with [44]. A. oryzae species A. flavus and A. niger were highly effective in the analysis of starch on the medium of the starch Agar and differed with our results where A. niger had an moderate effectiveness.

Peptone is a source of nitrogen that is proper for the production of alpha-amylase[45], and the highest alpha-amylase production was obtained in the medium containing peptone and soluble starch, such as nitrogen and carbon sources, and has found that adding yeast extract or Peptone to the liquid culture medium will shortens the delay and increase of both the dry weight of the cell and the synthesis of the produced enzyme, As a result of that, the yeast and peptone extract is preferred for the growth and synthesis of amylase by living organisms [46] [47]. showed that peptone was the optimal nitrogen
source for synthesis of Amylase. The peptone is considered as one of the best sources of nitrogen because it is a mixture of amino acids, protein and mineral salts while the yeast is a source of growth factors and vitamins.

Figure (5) Fungi high effectiveness in production of amylase enzyme on pure starch Agar medium at 28 °C for three days of incubation.

Table (7) Analysis of starch by fungi on amylase enzyme Agar medium at 28 °C after three days of incubation.

| Fungi Spp. | Effectiveness of analysis | Diameter of analysis area mm | code |
|------------|---------------------------|-----------------------------|------|
| A. nidulans | High Effectiveness | Mm 15> | +++ |
| A. candidus |               | zero | |
| A. flavus  | High Effectiveness | Mm 20 | + |
|            |               | Mm 15> | + |
| A. oryza   | High Effectiveness | Mm 22 | + |
|            |               | Mm 15> | + |
| A. niger   | Moderate Effectiveness | Mm 12 | ++ |
|            |               | Mm 15-10 | |

High Effectiveness Moderate +++
Effectiveness ++

3-4 The results of physical analyzes of damaged and intact samples of manuscripts: Are as follows:
Table 8: Color change values for unaffected paper samples and paper samples affected by fungus growth.

| Sample No.          | elongation force | tensile str |
|---------------------|-----------------|-------------|
| unaffected paper sample | 9.87            | 3.90        |
| affected paper sample by fungi | 7.66            | 2.88        |

3-4-1-Chromatic change:
The color coordinates of L-a-b were recorded on the unaffected paper samples and paper samples affected by the growth of fungus for the same manuscript paper using a chromatography device. The color code was used to measure the damaged and non-damaged papers. The results were as in Table (8).

Table 9: Strength values of tensile strength and elongation force of unaffected paper samples and paper samples affected by fungus growth.

| Samples                  | L     | a       | b       |
|--------------------------|-------|---------|---------|
| unaffected paper sample   | 146.67| -27.11  | 16.92   |
| affected paper sample by fungi | 144.07| -26.38  | 16.79   |

L is the color brightness and its value ranges between (L = 0) and (L = 100) white and black color. a, b represents the color properties.
The results showed that the affected sample showed a dark color compared to the standard sample (non-affected) and the increase in the value of b indicates the yellowing of the samples.

3-4-2-Mechanical properties:
Where the processing lengths of 14 cm x 10 cm (4) lengths of each sample were made. Measurements of tensile strength and elongation were performed for each of them. The tensile strength and elongation force values were included in Table (9).

3-5-Analysis using infrared spectrum
Figure 6: Infrared light for standard paper (non-affected) paper.

| functional wav | absorption area | interpretation                                      |
|----------------|-----------------|---------------------------------------------------|
| -OH extend     | 3337,28 cm⁻¹    | Resulted from hydroxyl groups in cellulose         |
| -CH exten      | 2909,04 cm⁻¹    | Hydrocarbon group in each of cellulose, hemicellulose and lignin. |
| -C=O exten     | 1643,57 cm⁻¹    | Resulted from oxidation of cellulose & lignin turning it into carbonyl & carboxyl groups. |
|                | 1427,65 cm⁻¹    |                                                   |
|                | 1320,99 cm⁻¹    |                                                   |
| -C= C annular  | 1105,73 cm⁻¹    | Resulted from lignin existence.                   |
|                | 1157,73 cm⁻¹    |                                                   |
| -C-O exten     | 1046,00 cm⁻¹    | Resulted from Cellulose and Hemicellulose         |
| -CH ranging    | 881 cm⁻¹        | Resulted from Cellulose                           |

It is explained from the Infra Red spectrum of the standard sample, there is a C = O group in the area of 1320.99 - 1643.57 which is the result of the process of cellulose oxidation due to time limitation. A comparison was made between the infrared absorption spectra of the standard sample (non-affected) and the samples affected with the growth of fungi and the result was as follows:
Figure 7: Infrared spectrum of standard paper sample (unaffected) affected by fungi growth. We observe a slight decrease in the OH group in the region (3200 - 3300) due to the loss of water molecules from cellulose. We also note an increase in the C = O uptake area (1155 - 1361) due to cellulose oxidation studies resulting from the loss of water molecules.

3-6- *Analysis using x-ray diffraction:* 
Calcium crystallization was calculated in the damaged paper and standard sample (not affected) according to the formula of crystallization of cellulose.

\[
\text{Cr. I. \%} = \left( \frac{I(002) - I_{18^\circ}}{I_{18^\circ}} \right) \times 100
\]

The relative intensity of reflection at angle 22.6 is expressed in the crystallized part (I) 002 in cellulose.
I 18\(^\circ\) the relative intensity of reflection at angle 18 is expressed by the non-crystallized part of cellulose.
- The degree of Cellulose crystallization rate = 67.3%
- The degree of crystallization of cellulose for the affected sample is 69.7%, i.e. there has been an increase in the degree of crystallization of cellulose.
This means that there is a change in the chemical and mechanical properties of cellulose.

References
[1] Youssef, Mustapha Mustafa Sayed, 2012, Maintenance of the manuscripts science and work, the House of Arab writer for printing and publishing.
[2] Shaheen, Abdel Moez, 1980, Scientific foundations for the maintenance and restoration of vellum and papyrus, Egyptian Authority for Antiquities, Museums Sector.
[3] Sarhan T. A.,(2012). Mycology practical. Printed book by the City College of Science University of Baghdad, first edition, p 62. .(Arabic)
[4] Aaronson , S. (1973) . Enrichment culture , In : "CRC - Hand book Microbiology" . The chemical Rubber Co. press , Cleveland , U.S.A ; 1. PP725 - 735 .
[5] Reese. E.T. & Mandles, M.(1963). Enzymic hydrolysis of cellulose & its derivatives. Fungi of wheat and broad-bean staw composts-Mycopathology-78:161-168.

[6] Yoeh, H.H.; Khew, E. & Lin, G. (1985). Simple method for screening cellulytic fungi. Mycologia; 77: 161 – 162.

[7] Sierra, G. (1975). A simple method for the detection of lipolytic activity of microorganisms & some observation on the influence of contact between cells & fatty substrate. Antooni van Leeuwenhoek 23:15-22.

[8] Pitt, J.I. & Hoching, A.D. (1997). Fungi & food Spoilage. Blackie Academic & professional. 2nd ed. London. New York. Tokyo. Melbourne.

[9] Barnett, H.L. & Bary, B.H. (1972). Illustrated Genera of imperfect fungi. 3rd. Burgess publishing.com.

[10] Ellis, M.B. (1971). Dematiaceous by phomycetes common weather mycological Institute. Kew, Survey, England.

[11] Moubasher, A.H. (1993). Soil fungi in Qatar & other Arab countries. Dept of. Bota. 2nd ed.

[12] Booth, T.; Gorrie, S. & Mabsin, T.M. (1988). Life Strategies among fungal assemblages on Salicornia europase agg. Mycologia; 80: 176-191.

[13] Pandey A, Webb C, Soccol CR & Larroche C (2005). Enzyme Technology. 1st ed. New Delhi: Asiatech Publishers Inc., p. 760.

[14] Tako, M. Papp, T., Kotogan, A., Nemeth, B., Vagvolgyi, Cs.(2012). Extracellular lipase production of Zygomycetes fungi isolated from soil, Rev. Agric. Rural. Dev. 1(1): 62-66.

[15] Pellizzi, E., Lattuati-Derieux, Lavédrine, A., and Cheradame, H., Flexible Polyurethane Easter Foam Consolidation: Preliminary Study of Aminopropylmethyldiethoxysilane Reinforcement Treatment, Proceeding Adhesives and Consolidants for Conservation: Research and Applications, CCI Symposium 2011, Ottawa, Canada.

[16] Limbo, S., and Piergiovanni, Shelf life of minimally Processed Potatoes: Part 1. Effects of High Oxygen Partial Pressures in Combination with Ascorbic and Citric Acids on Enzymatic Browning, Postharvest Biology and Technology, Vol. 39, 2006.

[17] Jablonský, M., Botková, M. (2012) Accelerated Ageing of Wood-Containing Papers: Formation of Weak Acids and Deterioration of Tensile Strength. Wood Research, Vol. 57, Issue 3

[18] Zervos, S. (2010) Natural and Artificial Ageing of Cellulose and Paper: A Literature Review. In Cellulose: Structure and Properties, Derivatives, and Industrial Uses, Nova Science Publishers, Inc., NY.

[19] Zeng, X., Vishtal, A., Retulainen, E., Sivonen, E., Fu, S. (2013) The Elongation Potential of Paper-How Should Fibres be Deformed to Make Paper Extensible, BioResources, Vol. 8, Issue 1

[20] Alia, M; Emsley, A; Heywood, R.; Herman, H;" Spectroscopic studies of the ageing of cellulosic paper" Polymer 42 (2001)pp. 2893-2900.
[21] Agnès ,L ; Céline E; Martin, R; Bertrand,L& Yong-Jae, C;" Case study Characterization and degradation pathways of ancient Korean waxed papers” Journal of Cultural Heritage 10 (2009)pp. 422-427.
[22] C.M. Popescu, P.T. Larsson, C.M. Tibirna, C. Vasile, Characterization of fungal degraded lime wood by X-ray diffraction and cross-polarization magic-angle-spinning 13C nuclear magnetic resonance spectroscopy, Applied Spectroscopy, 64(9), 2010, pp. 1054-1060.
[23] Park, Sunkyu, John O Baker, and Michael E Himmel, . 2010. "RCeseealrlcuh lose crystallinity index: measurement techniques and their impact on interpreting cellulase performance." Biotechnology for Biofuels 3:10.
[24] Zugenmaier, Peter. 2008. Crystalline Cellulose and Derivatives. Characterization and Structures. Verlag Berlin Heidelberg: Springer.
[25] Varalakshmi KN, Kumudini BS, Nandini BN, Solomon J, Suhas R, Mahesh B and Kavitha AP.(2009) Production and characterization of α- amylase from Aspergillus niger JGI 24 isolated in Bangalore. Polish Journal of Microbiology; 58: 29-36.
[26] Alcamo,E.(1996).Fundamentals of microbiology.5thed.The Binjumin / cummings putlishing compang. pp: 451.
[27] Domach , K.H. Gams , W. & Enderson , aT. (1980).Compendium of soil fungi . Academic press, London ; PP:859 .
[28] Abdel – Hafez , A.I.I. (1976) . Some ecological studies on Jordanian soil fungi , Assiut Univ. Egypt. ( cited by Moubasher & Al – subai , 1987 ).
[29] Moubasher,A.H.;Abdel-Hafez,S.I.I.;Abdel-fattah,H.M.& Mohrran .(1982).
[30] Rustum, Y. S. I. (1997). Aflatoxin in food and feed occurance, Legistatation an activation by physical methods. Food chemistry ,59:57- 67.
[31] Agarwal, V. K. & Sinclair. J. B. (1997). Principles of seed pathology .2nd ed. Lewis publisheers.CRC press Inc.p: 539.
[32] Abu Hila, Abdullah Nasser.(1987). Basics of Mycology, Public Libraries - King Saud University, Riyadh. P.325.(Arabic)
[33] Mishra , R.R. & Kanaujia , R.S.(1973) . Observation on soil fungistasis in relation to soil depth , Seasonal changes soil Amendment & physico – chemical characteristics of the soil plant& soil ; 38 : 321 – 330
[34] Nakagowa , Y. (1970) . Alkaline proteinase from Aspergilluse . In : " Methods in enyzymology " . Acadmic press , Newyork & London ; 19 ; PP : 583 – 585 .
[35] Hussein, Ban Musa, 2006, Efficiency of some fungal species isolated from the soil of Karbala Governorate in the analysis of maize and whey residue, Master Thesis.
[36] Ichishima, E.; Yamani, A.; Nitta, T.; Kinoshita, M.; Nikkuni, T.; Oka, T. & Yokoyama, S. (1973). Production of a new type of acid carboxypeptidase of mold Aspergillus niger. Appl. microbiol.; 26: 327 – 331.

[37] Kantmen, sh. A. (2001). Study of some skins fungi & its activity to product protease enzyme, Biomas. Biochem. 22: 67 – 88.

[38] Garcia – Kirchner, O.; Segura, G.M. Robledo; B.I. & Duran, P.E. (2000). Screening of potential antibiotic action of Cellulolytic fung. Dep Bioprosess, Mexico; 84: 69 – 78.

[39] Christov, L.P.; Szakacs, G. & Balakrishnan, H. (1997). Production, Partial characterization & use of fungal Cellulase – free xylanase in pulp leaching. Process. Biochem; 32: 511 – 517.

[40] Bhat, M.K. & Bhat, S. (1997). Cellulase degrading enzymes & their potential industrial application. Biotech. Adv. 15: 58-620.

[41] Rivera – Munoz, G; Tinico – Valencia, J.R. Sanchoz, S. & Farres, A. (1991). Production of microbial lipases in solid state Fermentation system Biotechnol Lett; 13 : 277 – 280.

[42] Griffin, H.A. (1980). Fungal physiology. John wiley & sons. New York & Toronto.

[43] Rifai, Faten Nouri, 2005, Isolation and Diagnosis of Fungi associated with Sesame Seeds, Journal of Education and Science, Volume (17) Issue (1).

[44] Hussein, Sarab Fadhil, 2014, Effect of Some Food Ingredients on the Enzyme Effectiveness of Some Isolates of Aspergillus, Master degree thesis.

[45] Hsu, W. H., Long L. L. Chyau C. C. (1998). Production and properties of a raw-starch-degrading amylase from the thermophilic and alkaliophilic Bacillus sp. TS-23. Biotechnol. Appl. Biochem. 28: 61–68.

[46] Martins M. L and Santos E.O. (2003). Effect of the medium composition on formation of amylase by Bacillus sp. Braz. arch. biol. technol. 46(1):57 – 61.

[47] Martins M. L. and Teodoro C. E. (2000). Cutlure condition for the production of thermostable amylase by Bacillus sp. Braz. J. Microbiol. 31(4):982-302.