



## ISOLATION AND IDENTIFICATION OF CONTAMINATED FUNGI OF BOOKS AND MANUSCRIPTS IN THE LIBRARIES OF A NUMBER OF IRAQI UNIVERSITIES AND HOLY SITES

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### Abstract

The study covered the isolation and diagnosis of contaminated fungi of books and manuscripts in the libraries of a number of Iraqi universities and holy sites. The study included (21) library, sources of Isolation represented in the covers and inside books and manuscripts, shelves, windows near the shelves and Interior and exterior doors. The results of the study revealed the presence of fungal contamination of books and manuscripts in all studied sites with a clear contrast in the preparation and types of isolated fungi. A total of (16) different fungi were isolated and *Aspergillus sp.* recorded the highest percentage in number and diversity with (85.4%), followed by *Mucor sp.* (6.6%), *Rhizopus sp.* (3.3%), *Alternaria sp.* (1.3%) followed by *Fusarium sp.* (0.9%), *Curvularia sp.* (0.7%) and *Penicillium sp.* (0.3%).

As for the studied sites, there was a difference in the number and type of fungus according to the location. The results showed that the Iraqi Books and Documents Library was the most Contaminated by (8.5%) and the library of the Biotechnologies College at the Qadisiyah University was the least contaminated by (1.6%).

**Keywords :** Books fungi, manuscripts fungi, opportunist fungi, *Aspergillus sp.*

### Introduction

The library is one of the most important sources of human knowledge and cultural heritage. It is a repository for books, documents and manuscripts. Paper has always been the tool used to document human knowledge. Studies have shown that the majority of people use print materials. Although the electronic version is available (Bankole, 2010). The raw material used for the manufacture of paper is organic plant material such as herbs, fibers, wood pulp and organic animal materials such as leather. The nature of these materials is protein, carbohydrate, in addition to other materials such as inks and preservatives such as acids. These materials are characterized by a property called hydroscopic nature, That mean the water content of these substances changes as a result of their absorption into water, which facilitates the adhesion of dust, particulates and stuck in the air, which causes the library assets to be infected with fungus as they carry the tiny spores (Youssef & Sayed, 2002). Many fungi grow on the cover of folders made of leather, parmesan or cotton fibers. They are mainly spread over the exposed parts of the folders, and the most important *Aspergillus* genus (Pinzari & Montanari, 2011). and the most important sources of contamination books with fungi is the outer air, which enters the library through the doors, windows and other ventilation outlets, which dont not escape fungal spores, especially saprophytic fungi (Joseph *et al.*, 2005).

Some fungi involved in the deterioration of library materials may be a danger to the Incoming of the library of workers and visitors, Because of its ability to produce fungal toxins in addition to it is the cause of many systemic diseases (Crook & Burton, 2010) Where they enter the body by inhalation of spores sick or direct contact through the skin, including respiratory diseases such as allergies and asthma (Bowyer *et al.*, 2006).

Based on the foregoing and the lack of studies dealing with this subject, this study was conducted to investigate the fungal species contaminated the books and manuscripts and to test their sensitivity to antibiotics and sterilizers.

### Materials and Methods

#### Collection of samples

Samples were collected from libraries in (15) colleges belong to Al-Qadisiya University, Central Library of Al-Qadisiyah University, Central Library in Al-Kufa University, Central Library of Karbala University, Iraqi Books and Documentation Library and Iraqi Museum Library, In addition to the Libraries in Holy Sites in Imam Hussein Shrine and Imam Ali Shrine, Some of the fungi were isolated from the books displayed in these libraries as well as the old manuscripts preserved in the Iraqi Museum and the upper thresholds. The samples were taken from six areas in the library: books, shelves, nearby walls, windows, the inner door and the outer door using sterile cotton swabs containing a liquid media BHIB (Brain Heart Infusion Broth) To maintain the vitality of fungi until they are transferred to the laboratory (Chadeganipour *et al.*, 2013). In addition, air samples were collected using petri dishes containing a media (SDA) distributed at different locations in the library at a height of ( 1.5) meters and left open for five minutes (Shadzi *et al.*, 1993). The samples were then transferred to the laboratory.

#### Cultivation of samples

The samples were cultivate in sterile Petri dishes and using different media prepared for this purpose: Sabrod Dextrose Agar, melt Extract Agar and potatoes Dextrose agar, using the streaking method, the dishes were marked according to the library and the location taken from them, which were previously established on the cotton swabs during the collection process of the samples, And incubated at a temperature of (28°C) for a period of ( 3-5 days), then removed dishes to inspect colonies and promised and start the necessary procedures for purification and diagnosis (Mohammed *et al.*, 2018).

#### Phenotypic diagnosis of fungus

The fungal species were purified by the transfer of part of the developing colonies in the first form by a Loop To

Petri dishes containing media (SDA) and incubate at 28°C for (7 days), repeating this process several times to obtain pure colonies (Kown-Chung & Bennett, 1992). The diagnosis was based on microscopic characteristics by preparing slides by moving a portion of the colony by a sterile loop into the slide and by adding a drop of blue dye. The samples were then examined under a microscope to examine the hyphae whether they are divided or not, the Conidia and the morphological characteristics of the colony such as shape, color and size, and the diagnosis was based on the following taxonomic Keys (Moustafa, 1982; Afzal *et al.*, 2013; Kidd *et al.*, 2016; Carmen & Sciortino, 2017).

The Percentage of fungal frequency and the percentage of Combined frequencies of species were calculated using the equation:

$$\text{Percentage of frequency} = \frac{\text{Number of isolates of one type}}{\text{Total number of isolates}} \times 100$$

## Result and Discussion

### Total number of fungi distributed by location

The results showed a difference in the number and types of fungi isolated from books, manuscripts and old documents according to the studied sites. The percentage of each site was calculated and showed a clear difference. The highest percentage was recorded in the Iraqi Books and Documents Library (8.5%). While the lowest percentage recorded at the Biotechnology College Library At the University of Al-Qadisiyah University by (1.6%), and the rest of the sites were included in different percentages as shown in Table (1).

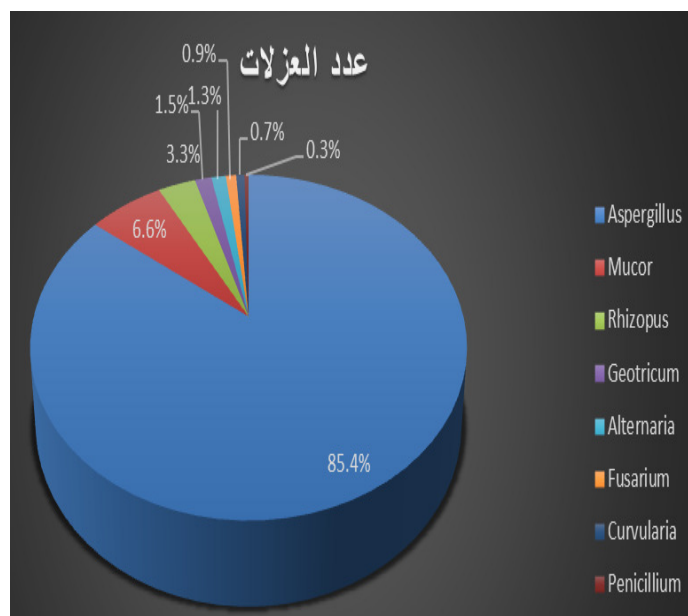
**Table 1 :** Number of fungal isolates distributed by location of study

| %    | Number of Isolates | Location of library                 | S  |
|------|--------------------|-------------------------------------|----|
| 2.9  | 16                 | Immam Ali holy shrine               | 1  |
| 2.5  | 14                 | Imamm Hussein holy shrine           | 2  |
| 7.4  | 41                 | Iraqi Museum                        | 3  |
| 8.4  | 39                 | Al-qufa University/central          | 4  |
| 8.5  | 47                 | Iraqi Books and Documents           | 5  |
| 4.9  | 27                 | Karbala University/central          | 6  |
| 6.9  | 38                 | Al-qadisia University/central       | 7  |
| 6.5  | 36                 | College of Education                | 8  |
| 4.0  | 22                 | College of Veterinary Medicine      | 9  |
| 5.7  | 32                 | College of Science                  | 10 |
| 4.0  | 23                 | College of Medicine                 | 11 |
| 4.0  | 22                 | College of Engineering              | 12 |
| 5.2  | 29                 | college of Literature               | 13 |
| 3.6  | 20                 | College of Law                      | 14 |
| 2.9  | 16                 | College of Education for Girls      | 15 |
| 2.9  | 16                 | College of Archeology               | 16 |
| 1.6  | 9                  | College of Biotachnology            | 17 |
| 4.0  | 22                 | College of Computer Science         | 18 |
| 3.0  | 18                 | College of Agriculture              | 19 |
| 5.4  | 30                 | College of Physical Education       | 20 |
| 5.7  | 32                 | College of Management and Economics | 21 |
| 100% | 549                | Total                               |    |

The reason for the difference in the fungal numbers in the studied sites for several reasons, including the age of the library and geographical location, as well as the design of the library in terms of the existence of ventilation outlets that facilitate the entry of fungus spores (Chadeganipour *et al.*, 2013) In addition to the different storage conditions and the different materials made from the library assets of books, documents and manuscripts (Mesquita *et al.*, 2009).

### Types of fungal isolates

(16) species of fungi were isolated from all the sites of the study. These species belong to (8) Genus, with the genus *Aspergillus sp.* (85.4%), followed by *Mucor sp.* (6.6%), *Rhizopus sp.* (3.3%), Followed by genus *Geotrichum sp.* (1.5%), and the emergence of the genus *Alternaria sp.* by (1.3%), Followed by the genus *Fusarium sp.* (0.9%), the genus *Curvularia sp.* (0.7%) and finally the genus *Penicillium sp.* (0.3%). Table (2).



**Table 2 :** Types of fungus and number of isolates according to the sites studied

| Type                                       | Number of Isolates |                  |                      |                   |                     |                      |                   |                 |                        |                    |                   |                   |                 |                    |                   |                    |
|--|--------------------|------------------|----------------------|-------------------|---------------------|----------------------|-------------------|-----------------|------------------------|--------------------|-------------------|-------------------|-----------------|--------------------|-------------------|--------------------|
| Location                                   | <i>A. niger</i>    | <i>A. flavus</i> | <i>A. versicolor</i> | <i>A. terreus</i> | <i>A. fumigatus</i> | <i>Mucor indicus</i> | <i>A. glaucus</i> | <i>Rhizopus</i> | <i>Mucor racemosus</i> | <i>A. clavatus</i> | <i>Geotrichum</i> | <i>Alternaria</i> | <i>Fusarium</i> | <i>A. candidus</i> | <i>Curvularia</i> | <i>Penicillium</i> |
| Immam Ali holy shrine                      | 7                  | 3                | 2                    | 2                 | -                   | 1                    | -                 | 1               | -                      | -                  | -                 | -                 | -               | -                  | -                 | -                  |
| Imamm Hussein holy shrine                  | 5                  | 3                | 1                    | 2                 | 1                   | -                    | -                 | -               | 1                      | 1                  | -                 | -                 | -               | -                  | -                 | -                  |
| Iraqi Museum                               | 20                 | 8                | 6                    | 1                 | 1                   | -                    | -                 | 1               | -                      | -                  | -                 | -                 | -               | 1                  | 3                 | -                  |
| Al-qufa University/central                 | 19                 | 3                | 6                    | 3                 | 1                   | -                    | -                 | -               | 4                      | -                  | 1                 | -                 | 1               | -                  | -                 | 1                  |
| Iraqi Books and Documents                  | 12                 | 14               | 4                    | 3                 | 4                   | 2                    | -                 | 1               | 2                      | 2                  | 1                 | 1                 | 1               | -                  | -                 | -                  |
| Karbala University/central                 | 11                 | 2                | 3                    | 1                 | 2                   | 2                    | -                 | 1               | 2                      | -                  | 2                 | 1                 | -               | -                  | -                 | -                  |
| Al-qadisia University/central              | 21                 | 4                | 5                    | 1                 | 1                   | 4                    | 1                 | -               | -                      | -                  | 1                 | -                 | -               | -                  | -                 | -                  |
| College of Education                       | 20                 | 4                | 2                    | 2                 | -                   | -                    | 3                 | -               | 1                      | 2                  | -                 | -                 | 1               | 1                  | -                 | -                  |
| <b>College of Veterinary Medicine</b>      | 9                  | 2                | 2                    | 2                 | 1                   | 1                    | 2                 | 2               | -                      | -                  | -                 | -                 | -               | -                  | 1                 | -                  |
| College of Science                         | 8                  | 10               | 2                    | 3                 | 5                   | -                    | 2                 | 1               | -                      | 1                  | -                 | -                 | -               | -                  | -                 | -                  |
| College of Medicine                        | 7                  | 7                | -                    | -                 | 3                   | 1                    | 2                 | -               | -                      | -                  | 1                 | 1                 | 1               | -                  | -                 | -                  |
| College of Engineering                     | 6                  | 3                | 4                    | 1                 | -                   | 2                    | -                 | 3               | -                      | 1                  | -                 | 1                 | -               | 1                  | -                 | -                  |
| college of Literature                      | 4                  | 6                | 6                    | 8                 | 1                   | -                    | -                 | 2               | 2                      | -                  | -                 | -                 | -               | -                  | -                 | -                  |
| College of Low                             | 2                  | 6                | 3                    | 2                 | 1                   | 3                    | -                 | 1               | -                      | -                  | -                 | 1                 | -               | 1                  | -                 | -                  |
| <b>College of Education for Girls</b>      | 3                  | 8                | -                    | -                 | -                   | 1                    | 2                 | 1               | -                      | -                  | -                 | -                 | 1               | -                  | -                 | -                  |
| College of Archeology                      | 3                  | 3                | -                    | -                 | -                   | -                    | -                 | 2               | -                      | 1                  | -                 | -                 | -               | -                  | -                 | -                  |
| <b>College of Biotachnology</b>            | 6                  | 6                | -                    | -                 | 2                   | -                    | -                 | -               | -                      | -                  | -                 | 2                 | -               | -                  | -                 | -                  |
| <b>College of Computer Science</b>         | 13                 | -                | 4                    | 1                 | -                   | -                    | 1                 | 1               | -                      | -                  | 1                 | -                 | -               | -                  | -                 | 1                  |
| College of Agriculture                     | 9                  | 3                | 2                    | -                 | -                   | 1                    | 3                 | -               | -                      | -                  | -                 | -                 | -               | -                  | -                 | -                  |
| <b>College of Physical Education</b>       | 11                 | 6                | 6                    | 1                 | 2                   | 1                    | 1                 | 1               | 1                      | 2                  | -                 | -                 | -               | -                  | -                 | -                  |
| <b>College of Management and Economics</b> | 9                  | 3                | 3                    | 2                 | 6                   | 4                    | 2                 | -               | -                      | -                  | 1                 | -                 | -               | -                  | -                 | -                  |

### Frequency of Species

The percentage of frequencies of all fungal species isolated during the study were calculated and the results showed a significant variation between them Table (3). The highest frequency was *Aspergillus niger* (37%), followed by *A. flavus* (19%), *A. versicolor* (11%), followed by *A. terreus* (6%), *A. fumigatus* (6%) and *Mucor indicus* (4%), followed by *A. glaucus* (3.4%) while the *Rhizopus* sp. (3%), *Mucor racemosus* (2.3%), *A. clavatus* (2%), *Geotrichum* sp. (2%), followed by *Alternaria* sp. (1%), the lowest recorded species *Fusarium* sp., *A. candidus*, *Curvularia* sp., *Penicillium* sp., Which are respectively (0.9%, 0.7%, 0.7%, 0.3%).

**Table 3 :** Percentage of species frequency

| Percentage% | Number of Isolates | Types of Fungi           | S  |
|-------------|--------------------|--------------------------|----|
| 37          | 205                | <i>Aspergillus niger</i> | 1  |
| 19          | 104                | <i>A. flavus</i>         | 2  |
| 11          | 61                 | <i>A. versicolor</i>     | 3  |
| 6           | 35                 | <i>A. terreus</i>        | 4  |
| 6           | 31                 | <i>A. fumigatus</i>      | 5  |
| 4           | 23                 | <i>Mucor indicus</i>     | 6  |
| 3.4         | 19                 | <i>A. glaucus</i>        | 7  |
| 3           | 18                 | <i>Rhizopus</i> sp.      | 8  |
| 2.3         | 13                 | <i>Mucor racemosus</i>   | 9  |
| 2           | 10                 | <i>A. clavatus</i>       | 10 |
| 2           | 8                  | <i>Geotrichum</i> sp.    | 11 |
| 1           | 7                  | <i>Alternaria</i> sp.    | 12 |
| 0.9         | 5                  | <i>Fusarium</i> sp.      | 13 |
| 0.7         | 4                  | <i>A. candidus</i>       | 14 |
| 0.7         | 4                  | <i>Curvularia</i> sp.    | 15 |
| 0.3         | 2                  | <i>Penicillium</i> sp.   | 16 |

### Combined Frequencies

The results showed that some fungal species recorded combined frequencies and appeared together in the same sample for more than one time at different rates. *Aspergillus niger* and *A. flavus* recorded a combined frequency of (29%), *A. niger* and *A. versicolor* (16%), *A. niger* and *A. terreus* (15%), *A. niger* with *A. glaucus* by (13%), *A. niger* with *Mucor racemosus* (9%), *A. niger* with *A. fumigatus*, (9%), *Rhizopus* sp. with *Mucor indicus* (7%) and finally *A. niger* with *curvularia* sp. (2%) Table 4.

**Table 4 :** Percentage of combined frequencies

| Percentage % | Number of Isolates | Types of Fungi                             | S |
|--------------|--------------------|--|---|
| 29           | 16                 | <i>A. niger</i> + <i>A. flavus</i>         | 1 |
| 16           | 9                  | <i>A. niger</i> + <i>A.versicolor</i>      | 2 |
| 15           | 8                  | <i>A.niger</i> + <i>A.terreus</i>          | 3 |
| 13           | 7                  | <i>A.niger</i> + <i>A. glaucus</i>         | 4 |
| 9            | 5                  | <i>A. niger</i> + <i>mucor racemosus</i>   | 5 |
| 9            | 5                  | <i>A.niger</i> + <i>A. fumigatus</i>       | 6 |
| 7            | 4                  | <i>Rhizopus</i> sp. + <i>Mucor indicus</i> | 7 |
| 2            | 1                  | <i>A.niger</i> + <i>Curvularia</i> sp.     | 8 |

*Aspergillus* sp. showed a very high frequency percentage compared to other isolated species and its appearance in all samples. Most isolated species belong to this species, while most other species appeared in one species due to several factors including their ability to reproduce and grow under different conditions Such as the temperature of (5-45 °C), Sometimes it reaches more than that (Moubasher *et al.*, 1982) As well as the production of large numbers of reproduction units and Sclerotia and resistance to

environmental conditions inappropriate and ability to analyze cellulose, protein and fat through the secretion of the enzymes analyzed and use of these materials as sources of nutrition (Flannigan & Sellars, 1977). It also has the ability to grow and reproduce in a low humidity ratio of up to 15-18% (Rustum, 1997).

The fungal species that belong to the genus *Aspergillus sp.* are among the most important fungi that infect books, documents and manuscripts. These species were isolated from ancient manuscripts in the city of Fez as well as the genus *Mucor sp.* and the genus *Penicillium sp.* (El-Bergadi *et al.*, 2014). *Aspergillus sp.* was isolated by (41%) from the books and manuscripts contaminated with fungi were stored in the library of the Astan Qods Museum in Mashhad, Iran (Shamsian *et al.*, 2006). Thus, the percentage is similar to the results of this study in addition to the genus.

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