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Fumigation is the ideal method in treating damaged archaeological paper using *Ceratophyllum demersum L* extract: A case study

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Abstract

All materials of cultural heritage, including paper degradation over time. Microbial contamination with fungi and bacteria can cause a significant damage to old manuscripts as well as a health threat to the librarians. Most of the biological damage is started in poor environmental conditions for storage and display. However, conservation slows down the rate of microbial deterioration.

This work aimed to eliminate the effect of microbial deterioration on old manuscripts. The effect of the extract of *Ceratophyllum demersum L*. Using the fumigation method was studied. The applied doses of the plant extract did not cause any observable alterations or color changes to the old manuscripts. A dose of 200 ppm of the plant extract was the efficient concentration in eliminating microbial growth. Brushing, sparing and fumigation methods were tested for treating microbial deterioration of the old manuscripts. Fumigation using plant extract was found to be the ideal method for its application on damaged archeological papers.

Keywords: Biological damage; *Ceratophyllum demersum* L.; Old manuscripts; Fumigation
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1. Introduction

Archeological materials like textiles, wood and old paper attack by microbial causing very fast deterioration [1-3]. Fungal degradation of library materials causes different kinds of damage depending on the species of organism responsible for the attack and the characteristics of the substratum. Damage can occur because of mechanical stress, production of staining compounds or enzymatic action [4-8]. The deterioration of documents made of proteins is a complex process, which involves the chemical oxidative degradation of amino acid chains and hydrolytic cleavage of the peptide structure. Microbes can hydrolyze collagen fibers and other collagen -based materials, but can also modify the inorganic components, or produce organic acids which discolor the parchment. Bacteria displaying proteolytic activities play a major role in the deterioration of ancient documents and books made of parchment. Species belonging to the genera *Bacillus, Staphylococcus, Pseudomonas, Virgibacillus* and *Micromonospora* have been isolated from deteriorating manuscripts [9]. In recent decades, the dogma has changed and it is now generally agreed that microbial not only cause serious aesthetical destruction of books and manuscripts, but also inhabit and penetrate into the materials, resulting in material loss, due to acid corrosion, enzymatic degradation and physical alteration. Moreover, decontamination of the infected artifacts, exhibition rooms and depots may result in high expenditure for museums.

Aquatic plants have an array of bioactive compounds which reveal antimicrobial activities [10-12]. In the present study, natural extracts from *Ceratophyllum demersum L*. Is considers has quality timber species (mahogany group), many uses for this wood from the fact that it combines such desirable characteristic as attractive appearance, good dimensional stability, excellent finishing qualities and a high degree of material durability. This valuable trace contains certain components which have some biological activity as fungi and bacteria [13]. Aquatic plants are of special interest, unlike the terrestrial plants, because they are capable of bio concentrating many metals in large quantities [14,15]. Extracts are the compounds present in plants that can be extracted by organic solvents. They are found in higher concentrations in the plants and are generally considered to be biosynthesized in order to slow or prevent pathogen invasion. In connection with are interested in biologically active material, products [16]. We investigated the chemical constituents of the extract of *Ceratophyllum demersum L*. Large number of sesquiterpene lactones and tetrahydrofurofazonlignaros were previously reported from over hundred species of microbes [17]. Therefore, the present study was designed to identify microbial invading this manuscript and searching for simple, cheap, non-toxic and eco-friendly control against causative fungi. This will participate effectively in the development of

national and international strategy for the collection and storage of rare books and manuscripts in libraries.

In the present study, one invasive species of aquatic plants, *Ceratophyllum demersum*, was used for screening its protective effect against microbial deterioration of old manuscripts. Analysis of chemical composition of *Ceratophyllum demersum L.* Using GC/MS revealed a variety of bioactive compounds [18].

Brushing, sparing and fumigation methods are the prevailing methods used for treating microbial deterioration. In the current study, we evaluated the fumigation method for application of plant extracts on damaged archeological paper.

2. Material and Methods

2.1 Experimental sites

The study was carried out in the Microbiology Laboratory, Conservation center, The Grand Egyptian Museum, Egypt.

2.2. Collection of swabs

Swabs were obtained from rare books from the parliament of the Arab Republic of Egypt. Most of books suffer from severe microbial infections, which are manifest in the presence of clear microbial lesions in the form of brown spots, which have been combined in many pages of books to cover the entire pages in brown color (Fig. 1). Microbial swabs were cultured on plates of cellulose, protein and nutrient agar media.

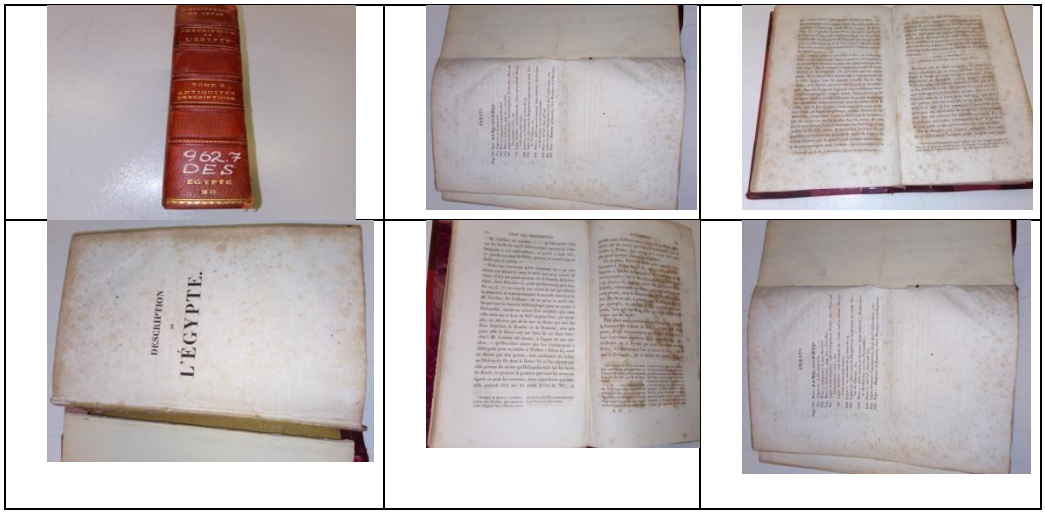


Fig (1): The biodeterioration of the manuscripts.

2.3. Isolation and purification

The growths that emerged in the Petri dishes were taken each growth separately and cultured on the same previous media in order to obtain the microorganisms in pure forms to complete the steps of identification.

2.4. Characterization and identification

Microbial colonies that grew on the incubated plates were subculture into fresh separate sterile potato dextrose agar (PDA) and nutrient agar plates and incubated to obtain pure cultures of pathogens. The purified isolates were kept in slants and stored for characterization. Microscopic examination and morphological characteristics were noted and compared with existing authorities.

2.5. Collection and extraction of plant material

The plant was collected from the main stream of the River Nile (N: 24º 04.646'; E: 32º 52.701'). Part of the collected samples were authenticated and voucher specimens were sent to the Herbarium. The other part of the samples were washed properly, left for air-drying and ground to a fine powder. About 100 g of the ground material was extracted with methanol (100%) as described in [19].. The plant extract was used for antimicrobial assays.

2.6. GC/MS analysis of the extracts

Analysis of chemical compositions of the studied extracts was carried out using GC/MS at Institute of Marine Sciences, Alexandria, Egypt. [18].

2.7. Assay of plant extracts against isolated microorganism

The bacterial and fungal cultures were incubated for 24 hours at 37 °C on nutrient agar and PDA media, respectively. The zones of growth inhibition around the walls were measured after 18 to 24 hours of incubation at 37 °C for bacteria and 48 to 96 hours for fungi at 28 °C. The sensitivities of the microorganism species to the plant extract were determined by the sizes of inhibition zones (including the diameter of the wheel) on the agar surface around the wells. Values <15 millimeters were considered as not active against microorganisms. The MIC of plant extract was determined by measuring the sizes of inhibitory zones on the agar surface around the wells. The controls were the solvents used for every extract.

2.8. Evaluation of different methods for treatment of deteriorated manuscripts

The plant extract was applied using the fumigation method to treat deteriorated manuscripts. A concentration of 1000 ppm of plant extract was used to test the inhibition of microbial growth for 6 months. Microbial growth was examined by taking of swabs from each treated specimen

after 48 hours, 3 months and 6 months. The swamps were cultured in Dox's medium for fungi, starch nitrate for *actinomycetes* and nutrient agar for bacteria.

3. Results and Discussion

The isolated microorganisms from the rare books were identified as: *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Penicillium citrinum, Fusarium flocciferum,G+ve bacilli, G+ve short bacilli* and *G+ve Microbacilli* colonies (Fig. 2). From the results, it can be seen that the genus *Aspergillus* was the dominant genus in five books having 60% of the total fungal isolates, followed by *Penicillium* and *Fusarium*.

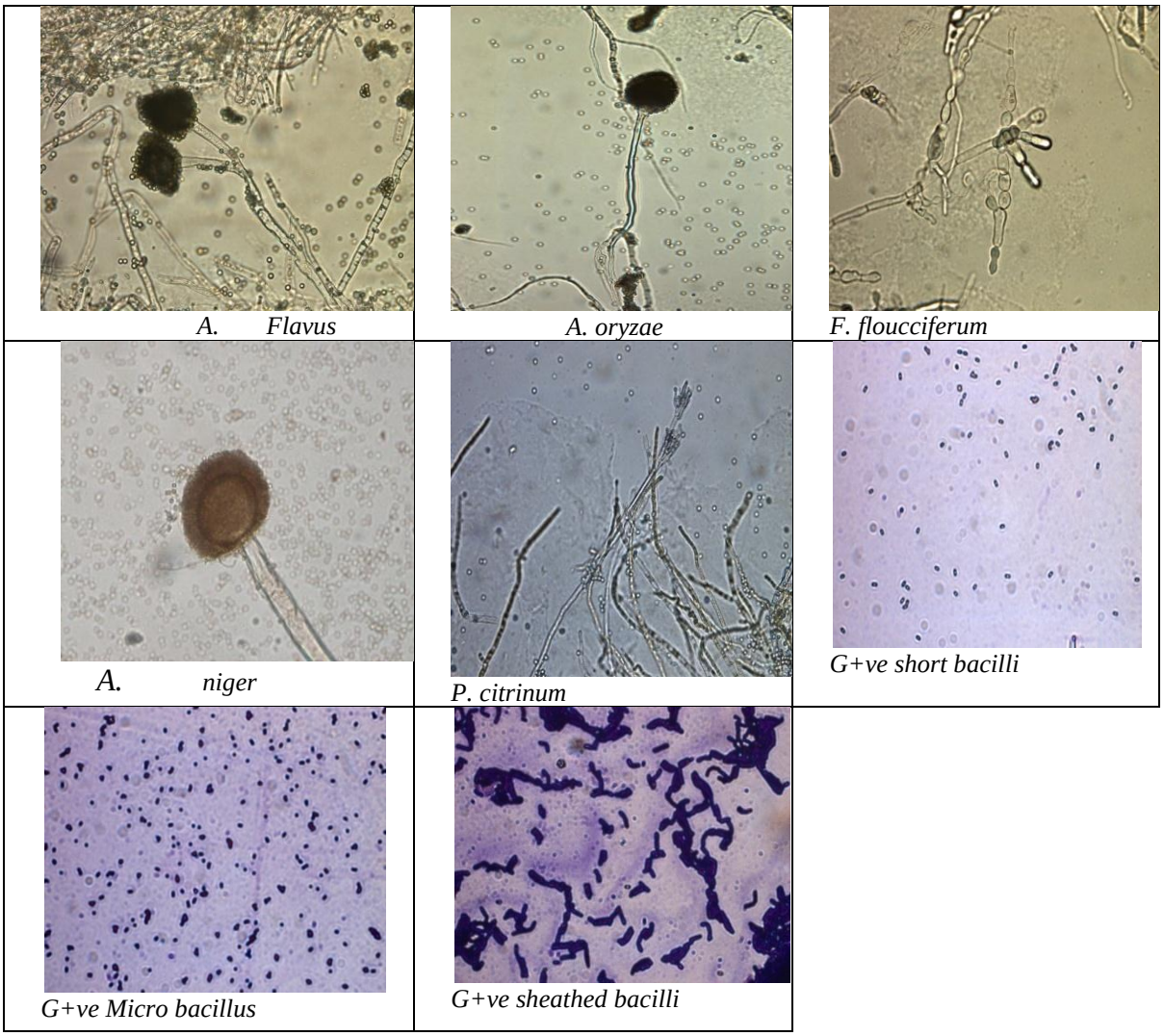


Fig (2): A photo showing isolated microorganisms.

These results were in line with those of [19]**.** Where the following genera: *Aspergillus niger, Aspergillus flavus* and *G+ve short bacilli* were isolated from ancient manuscripts. These isolated spores forming bacteria are frequently being detected on historical articles [20, 21].

3.1. Assay of plant extracts against isolated microorganism

Five fungal species *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Penicillium citrinum, Fusarium flocciferum* and three bacterial strains *G+ve bacilli, G+ve short bacilli* and *G+ve Microbacilli* were assessed in terms of zone of inhibition of microorganism growth. The results of the antimicrobial activities in the plant extract are shown in Table 1.

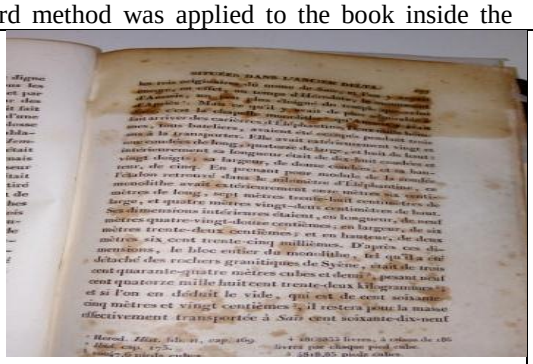
Data depicted in Table 1. Showed that the MIC of the plant extract inhibiting growth of all isolated microorganisms was 1000 ppm when comparing inhibition zones for *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Penicillium citrinum, Fusarium flocciferum* and three bacterial strains *G+ve bacilli, G+ve shourt bacilli* and *G+ve Microbacilli* where values were equal to 35, 36, 40, 30, 50, 33, 25 and 24 mm, respectively.

Table (1): Inhibition zone (mm) four different concentrations (800-1000 ppm) of the plant extract

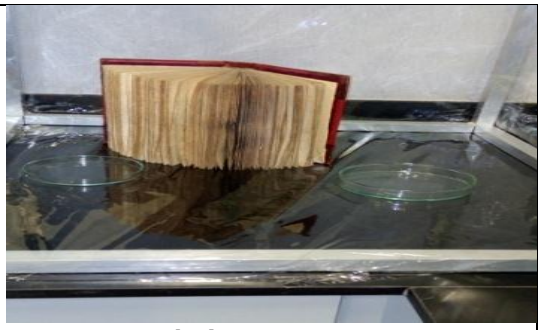
Microorganism	Concentration of plant extract (ppm)		
	800	900	1000
Aspergillus niger	0.0	24	35
Aspergillus flavus	24	31	36
Aspergillus oryzae	0.0	25	40
Penicillium citrinum	0.0	26	30
Fusarium flocciferum	28	39	50
G+ve bacilli	20	26	33
G+ve short bacilli	0.0	0.0	25
G+ve Microbacilli	0.0	0.0	24

3.2 Evaluation of Ideal method for treatment of deteriorated manuscripts

There are various methods that can be used for applying the microbial treatment to the books by using the MIC of plant extract. These methods differ based on the condition of the books. Different methods were used for this purpose brushing method, sparing method and fumigation method. The first and second method was applied in small scale, but the third method was applied to the book inside the sealed tight as shown in **Fig. 3**. After Application, we found the suitable method to apply is fumigation within the place of tightly sealed. This is in fact a new method for eradicating paper destroying organisms. These so-called "fumigation" methods require the use of sealed chambers and are sometimes a good alternative to microcides.

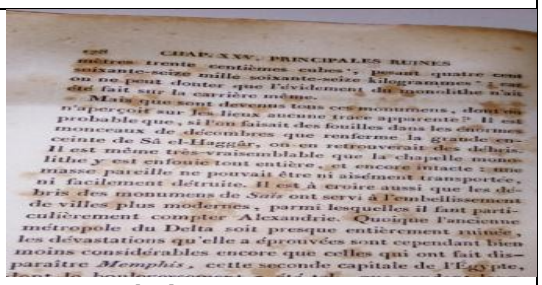


Brushing method



Fumigation method

Fumigation method



Sparing method

Fig (3): Different methods applying to the microbial treatment.

3.3 Evaluation of fumigation method for treatment of deteriorated manuscripts

Treatment of the infected books was applied by a fumigation method with *Ceratophyllum demersum* extract at 1000 ppm to prevent microbial growth. Effect of antimicrobial activity of the plant extract at 1000 ppm was examined by taking of swabs from each treated book after 48 hours, 3 months and 6 months as described in section 2.5. The results showed that using of *Ceratophyllum demersum* at their MIC concentration of 1000 ppm was sufficient to completely prevent the growth of all microbial isolates for up to 6 months (Table 2).

Table (2): The effect of applying of *Ceratophyllum demersum L*. Extract at 1000 ppm on infected books

Growth detection time	Growth of the infectious isolates
	Plant extract (*Ceratophyllum demersum* L.) at 1000 ppm
48 hours	-
3 months	-
6 months	-

(-) = No growth

These results were similar to those of [22] who evaluated the antimicrobial activity of piperine against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* and found out that the active ingredient in *Piper nigrum* has an inhibitory effect on these pathogens. [23]. Used *C. papaya*; *C. adorata* and *Acalypha ciliata* to control pawpaw fruit rot fungi. [24]. Reported that *Carica papaya* leaf extracts controlled incidence of foliar mycopathogens of groundnut (*Arachis hypogea*). In the same context, [25]. Found that mycelia growth of *Alternaria solani* which cause yam rot was inhibited by using leaf extracts of *Carica papaya*.

4. Conclusion

The findings showed the potential of *Ceratophyllum demersum L*. Extract at their MIC (1000 ppm); where it was sufficient to completely prevent the growth of all microbial isolates. Consequently, it could be used to control the microbial deterioration of historical books when applied using the fumigation method. The use of plant products will reduce over dependence on the use of synthetic chemicals in controlling microbial pathogens as well as reducing cost of management and conservation of library staff.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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