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Use of the Fungal Extracts in the Conservation of Mural Paintings and Inscriptions with Application on a Selected Object

Submitted By

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Summary

Microbial growth causes loss of strength and elongation, oxidation state, discoloration, changes in appearance, degree of polymerization, and breakdown of molecular structure of mural paintings, so the biodeterioration control has been performed using alternatives as the rational use of natural products that come from microorganisms. The use of natural products (fungal extracts) attains strong interest that can be used as an alternative to synthetic chemicals in order to prevent and reduce the dangerous effects of microorganisms on historical artifacts as well as the dangers of using chemicals.

Therefore, trends in biodeterioration control have indicated the need for biocide procedures using non-harmful and non-toxic compounds whose efficiency is kept over time and without adverse effects on cultural heritage and human health. Particularly, due to their antimicrobial-repellent properties, well known since ancient times. The fungal extracts contain several secondary metabolites able to inhibit the growth of bacteria and fungi. They act directly on the microbial cell by inhibiting its growth, inducing the deterioration of the cytoplasmic membrane, regulating intermediary metabolism, activating or inhibiting enzymatic reactions, or affecting the enzyme synthesis, and biocontrol appears to be a reliable alternative to chemical fungicides, because the applied fungicides are highly toxic and impose environmental hazards for both the treated objected and conservators, so the new trends used environmentally safe methods of fungi extracts and its derivatives to control fungi colonizing cultural heritage objects. These substances have high potent antimicrobial activity and low toxicity for human and the environment.

From this point of view, the thesis of the letter includes the use of fungal extracts produced from fungi isolated from the archaeological Stelae and their use in inhibiting the growth of fungi and cleaning.

The study included four chapters divided into:

Chapter 1: Study of Fungal Extracts and their Chemical Composition

This chapter discussed the Structure of Fungal Cell are composed of filaments called hyphae. Hyphae may contain internal cross walls, called septa that divide the hyphae into separate cells. Coenocyte hyphae lack septa. Also, this chapter contained the painting techniques from tempera technique, fresco, and secco techniques, where

the techniques of Stela (cases study) are tempera technique and this chapter contained the study of biodeterioration of mural paintings. Biodeterioration is the alteration of organic and inorganic materials induced growth of microorganisms. The biological activity of microorganisms like bacteria, fungi, algae and lichens, contributes to the deterioration of cultural heritage, particularly if they are exposed to open air. The interaction of these agents with physico-chemical properties of the materials is considered central to understand the long term deterioration, and Microbial induced deterioration processes can cause different kinds of alterations such as discoloration of materials, the formation of crusts on surfaces, and the loss of material, which leads to structural damage, microorganisms can cause severe problems due to the excretion of aggressive metabolic products such as organic or inorganic acids. Secondly, material components can be used as substrates for microbial metabolism, and biodeterioration of Stone.

We discussed the control of microbial deterioration through mechanical methods, physical methods and we discussed chemical methods that are toxic and dangerous to the environment and to public health. Also we discussed the search for alternatives to commercial biocides .So, Natural biocides are considered safer for human beings and greener for the environment and have been used both for organic and inorganic materials. Several of these products are derived from plants and could be employed in their pure form as well as crude extracts or as essential oils. The use of antifungal natural extracts was always a viable alternative to the use of harmful chemicals (that often affects the rest of the environment or even human health). For example, nettle or olive leaf extracts are used since ancient times as antifungals. The increasing interest on natural products that can be used as an alternative to synthetic chemicals in order to prevent and reduce the deterioration of microorganisms on historical artifacts.

We discussed characteristics of fungal extracts and its derivatives to control fungi colonizing mural paintings. These substances have high potent antimicrobial activity and low toxicity for human and environment and friendly method of the environment, cheap and studied biosynthesized, and we studied the major groups of antimicrobial compounds from fungal extracts. **Use the *fungal* extracts to Many reasons**, some advantages include (1) the fungal cells remain viable; (2) the metabolite's extraction is simple and easy; and (3) large-scale production is convenient.

Chapter 2: (**Materials and Methods**) Deals with the experimental study on the method of preparation of fungal extracts from fungi and their use in inhibiting and cleaning. Where, it included taking swabs of Stelae (cases studies) to isolation and identifying of fungi then preparation fungal extracts from fungi and examined the fungal extracts by the GS-MS and determination of total antioxidant capacity (TAC) and determination of total phenolic content (TPC), and used them in inhibition of fungi and cleaning and applied some consolidation materials in the mural painting by making experimental samples and exposing them to the microbiology aging and thermal aging process in order to understand and assess the influence of microorganisms on causing a physical and chemical change in mural paintings, and then conducting different examination and analysis of the experimental samples.

Then we dealt with making experimental samples and used them in the inhibiting, cleaning process, and consolidation. The First step included the application of the fungal extracts (fungal extract from *Aspergillus flavus*, fungal extract from *Aspergillus caespitosus*, fungal extract from *Paecilomyces variotii*) on the experimental samples after infection of experimental samples with fungal isolates. In the consolidation process were chosen nanomaterial (nano palaroid - nano $CaCO_3$) with their concentrations and then exanimated nanomaterial by using (TEM) microscope to confirm the size of nanomaterial granules are in the size of nanomaterial and confirm the homogeneity process in the compound nanomaterial ,then applied nanomaterial to experimental samples, finally we examined and analyzed the samples to confirm the penetration of these materials within the samples and observed their effect on the surface of the sample and its ability to cover the surface and followed by thermal aging cycle and set the discoloration values of the samples to ensure that these materials do not affect colors.

Chapter 3: (**Results and Discussion**) This chapter includes all the results of the examined and analysis that were dealt with during the experimental study and it begins to identify the species fungus through the Molecular Identification (*Aspergillus flavus,Aspergillus caespitosus, Sarocladium terricola,* and *Paecilomyces variotii*) then preparation of the fungal extracts and GC-MS investigation of fungi extracts.

Fungal extract from *Aspergillus flavus:* The main detected compounds are 9 Hexadecanoic acid, methyl ester (17.57%), 9,12-Octadecadienoic acid (Z,Z), methyl ester (16.24%),9-Octadecenoic acid, methyl ester, (E) (7.17%),1,2-Dioctylcyclopropene (2.27%), fungal extract from *Aspergillus caespitosus*: The main

detected compounds are Hexadecanoic acid, 15-methyl, methyl ester (8.72%), 2-Pentenal, 2-methyl (8.57%), 9,12-Octadecadienoic acid, methyl ester, (E,E) (7.75%), 4,4-dimethyl-1-octene (7.34%), Undecane, 2,3-dimethyl (5.66%), and Fungal extract from *Paecilomyces variotii*: The main detected compounds are 9-Octadecenoic acid (Z) (9.07%), 1-Tetradecanol (7.22%), Pentadecanoic acid, 14-methyl, methyl ester (5.75%), Sulfurous acid, isohexyl 2-pentyl ester (6.06%).

Also, study of the selected Stelae, the first case study is a painted limestone rectangular funerary Stela of Henu, son of Sobek-Hetep (Cairo CG 20212). This is the most common name in the Middle Kingdom "Sobekhotep".The painted Stela was discovered in 1861 during the excavations by A. Mariette (director of the Egyptian Antiquities Service) in North Abydos, more specifically in the "Northern Cemetery." The Stela dated back to the dynasties 12 (Middle kingdom). The object's dimensions are 26 cm in height and 15.5 cm in width. The second case study is a painted limestone rectangular funerary Stela with cornice of Imeny son of Neb-Ieyou [Cairo CG 20594]. The provenance is identical to the previous one. A. Mariette dated it to the same period. The painting dimensions are 26 cm in length and 26 cm in width. The third case study a mural Stela made of limestone that known as limestone slab dating back to the reign of King Ramses II of the nineteenth dynasty from the New Kingdom era. The dimensions of the Sela are as follows: Length: 3.5 meters, Width: 25 cm, Height: 70 cm, and Weight: 3 tons, then recording and documenting the Stelae in a different way whether recording by AutoCAD program and examined the Stelae by (stereomicroscope – light microscope (USB) – scanning electron microscope, and multispectral imaging) to identify the deterioration manifestations of Stelae such as cracks, salts, and fungal spots.

We used SEM.EDX, pXRF, XRD, FTIR techniques and technical photograph, in order to identify the pigments and stone used on three painted limestone Stelae. The results showed that the pigments used were Egyptian blue, red ochre (hematite), yellow ochre (goethite) and carbon black, Egyptian green, and calcium carbonate. In some areas of Stela GC 20212, a mixture of pigments was employed in order to obtain a distinct hue as orange pigment. The animal glue was used in the different painting surfaces as the medium. The findings of this study are in accordance with previous analyses of ancient Egyptian pigments, which indicate the continuous use of artificial and natural earth pigments. By Visible-induced infrared luminescence (VIL),

Egyptian blue appeared as white or very pale areas in the VIL image, while all other materials appeared black or dark grey as could be seen in Stela CG 20594.

Also, fungal extracts have evaluated on the experimental samples, and the best results for The fungal extracts (fungal extract from *Aspergillus flavus*, fungal extract from *Aspergillus caespitosus*, and fungal extract from *Paecilomyces variotii*) applied on the experimental samples infected by fungal isolates (*Aspergillus flavus*, *Aspergillus caespitosus*, *Sarocladium terricola*, and *Paecilomyces variotii*), The fungal extract (fungal extract from *Aspergillus flavus*) concentration at 0.05% has given good result in inhibiting and cleaning the fungi, while the concentration at 0.03% left traces of mycelium fungi on the surface. And fungal extracts (fungal extract from *Aspergillus caespitosus* and fungal extract from *Paecilomyces variotii*) have given the best result by concentration at 0.03% in inhibiting the fungi while concentration the at 0.05% leaved residue of fungal spots on the surface of color, also, The best result of consolidate materials are nano paraloid at 3% and nano calcium carbonates at 2%. These materials proved their ability to penetrate well into the pores, with no change in color of the samples and giving a good strength of samples.

Chapter 4: (**The Applied Study**) includes the application of fungal extracts in inhibiting and cleaning the fungi from the selected Stelae. The fungal extracts (fungal extract from *Aspergillus flavus*, fungal extract from *Aspergillus caespitosus*, and fungal extract from *Paecilomyces variotii* which proved to be successful in the experimental study as mentioned above, was used for treating the deteriorated mural paintings in (cases studies). Then, the application of nano paraloid at 3% and nano calcium carbonates at 2% on the surface of Stela through Japanese paper using spray and soft brushes were used in the distribution of the nano calcium carbonates.