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EXTRACTION OF BIO-NANOMATERIALS FROM FUNGI ISOLATED FROM THE ARCHAEOLOGICAL BUSHEL FOR THE TREATMENT OF MICROBIAL INFECTIONS

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Abstract

The research paper aims to isolate and resist fungi as they are one of the factors of microbial damage to the archaeological bushel. The archaeological bushel understudy dates back to the era of King Farouk. It was one of the measuring tools that have been used for decades in measuring grains, especially wheat. The study helped identify the signs of damage because the bushel was extracted from a moist environment and was covered by clay calcifications and salts. There were morphological changes on the surface of the archaeological bushel, so the biological activities of the isolated microorganisms were investigated and the causative microorganisms from the archaeological bushel were isolated and characterized as Aspergillus flavus and Aspergillus fumigatus. Nanomaterials were created for the sterilization process of surfaces. Nano-silver and nano-gold materials were extracted from identified fungi and a comparison was made between them to choose the best in the treatment process. It was found that nano-gold 1% concentration is sufficient to inhibit all isolated microorganisms.

Keywords*: Fungi; Iron; Wood; Nano Material; SEM; XRD*

Introduction

A bushel is "a measure of capacity for dry things, such as grains, pulse and dry fruits" [1]. With the introduction of the bushel, it was made from pottery in ancient times. With the beginning of the manufacture of wooden measures, the bushel was made by digging the wooden block, tightening it with nails and placing a collar at the bottom to protect it from arrows or mats [2].

Non-separable iron/wood composite artifacts recovered from a moist environment are complex objects and require a good knowledge of conservation issues related to both materials before planning treatments. Their conservation involves compromises but intends first to treat the most fragile component while preventing alteration of the associated materials [3, 4]. Conservators face challenges when they treat objects made from more than one material. This is especially true if the object cannot be taken apart. Treating both materials at the same time can be a problem since standard treatments for one material may be harmful to another [5]. The diffusion of water in wood structures results in the invasion of microorganisms, which readily occurs if chemical degradations have already started to weaken the cell network [6-8].

The bushel under study was made from wood, laminated with metal and fixed with nails along the length of the body. It was in the form of a cone made of wood, covered along the hull

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with iron and fixed with nails along the hull to connect the iron to the wood from the outside. Moreover, the base was made of wood and fixed to the hull by two pieces of iron in the shape of the letter (L) and fixed with screws for support. It is noted that the seal of King Farouk was on the surface of the base from the inside, where the stamping process was carried out to define that the piece is the official balance used to reduce fraud and theft in weights. Its dimensions are 19.2cm in height, 17.8cm base diameter, 9.8cm nozzle diameter and 2cm base thickness.

This study aims to determine the bushel materials and identify the fungi and extract nanomaterials from them to provide necessary information for suitable future conservation works.

Material and Methods

Optical Microscopy (OM)

Transverse, tangential longitudinal and radial longitudinal sections were used for wood identification. These three thin sections were mounted on glasses to be observed under transmitted light using Optika microscopy (Italy) equipped with an Optika B9 digital camera. The observed anatomical features were then compared to reference wood anatomy atlases and databases [9-11].

Transmission electron microscope

It was used to determine the shape and size of silver and gold nanoparticles. Transmission microscopy used was a JEOL (TEM) device, Japan (JEM-1010), at an acceleration voltage of 80kV after drying a drop of aqueous silver and gold nanoparticles on copper-coated carbon. The TEM grid was kept under vacuum in desiccators before loading the sample and the particle size distribution of silver and gold nanoparticles was examined and evaluated using ImageJ 1.45s 1493 software [12].

Isolation of fungi

Sterile swabs were taken of the affected and eroded part of the archaeological scoop. The experimental study depends on the use of biosynthetic nanomaterials (silver-gold) in the treatment of microbial damage on archaeological holding. To isolate the microorganisms that grow on the item and lead to its damage, the media was used to grow fungi. The samples were cultivated on PDA. The media were prepared in the following proportions: Potato starch (200g), agar (18g), glucose (40g) [13-16] and distilled water (1000mL). At pH = 5.5 to 5.6, Bengal red was used at a concentration of (30mg/L) with half of the dishes prepared for the experiments. To reduce the overgrowth of the isolated fungi, as for the rest of the dishes, this substance was not used. The plates were incubated at 28°C for 5-7 days. Then, they were examined at the Microbiology Laboratory, the National Research Centre, to diagnose the type of microbial infection [17, 18].

Results and discussion

Wood identification

It was discovered to be made of pine wood. Pines are softwoods, but commercially they may be designated as soft pines or hard pines. Soft pines, such as white, sugar and piñon pines, have relatively soft timber, stalked cones with scales lacking prickles and little resin. Their wood is close-grained, with thin, nearly white sapwood. Pine trees are usually conical, with whorls of horizontal branches. Older trees may have round, flat, or spreading crowns. Most species have thick, rough furrowed bark [19-22].

Transverse section (TS)

Growth rings boundaries distinct - late wood tracheids thick walled (Fig. 1a) axial intercellular resin canals present with epithelial cells thin walled.

Tangential longitudinal section (TLS)

Rays uniseriate and its average height is medium (5-15 cells) (Fig. 1b) - radial intercellular resin canals present.

Radial longitudinal section (RLS)

Ray tracheid commonly present with dentate cell walls (Fig. 1c).

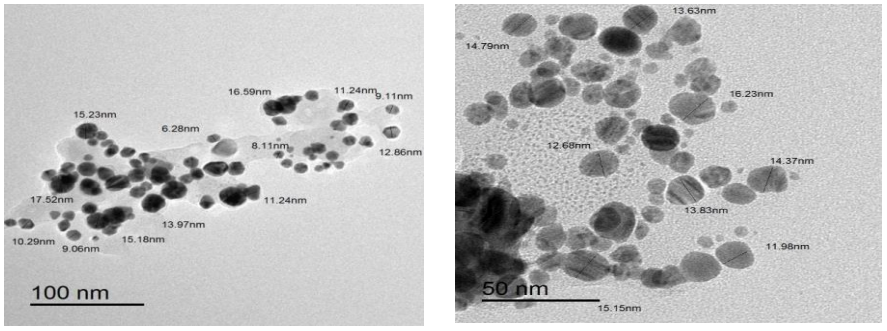
a **b** **c**

Fig. 1. The anatomical characteristics of sample 6 by OM in transmitted light**:** a - Transverse section (TS); b - Tangential section (TLS); c - Radial section (RLS)

Investigation by Transmission Electron Microscope

It was discovered that the generated silver nanoparticles have a spherical shape and their diameter ranges from 12 to 16nm by utilising a transmission electron microscope to examine the nanoscale samples of silver and gold made using fungal filter. In contrast, the gold nanoparticles have a polygonal shape with a diameter that varies from 9 to 17.5nm.

The morphology of nanoparticles is very variable. When such images were observed, these clusters were found to form aggregates of silver nanoparticles in the size range of 5-50nm. The nanoparticles were not in direct contact even within the blocks, which indicates the stability of nanoparticles by the capping agent when using *Trichoderma Reesei* filtrate [23].



a **b**

Fig. 2. Study of the shape and size of silver nanoparticles using transmission electron microscopy: a - nanosilver; b - nanogold

Electron microscopy shows the size and shape of silver nanoparticles formed by the fungus *Pleurotus ostreatus*, which shows that the particles are uneven in a spherical shape with a size difference of 10-30nm [24].

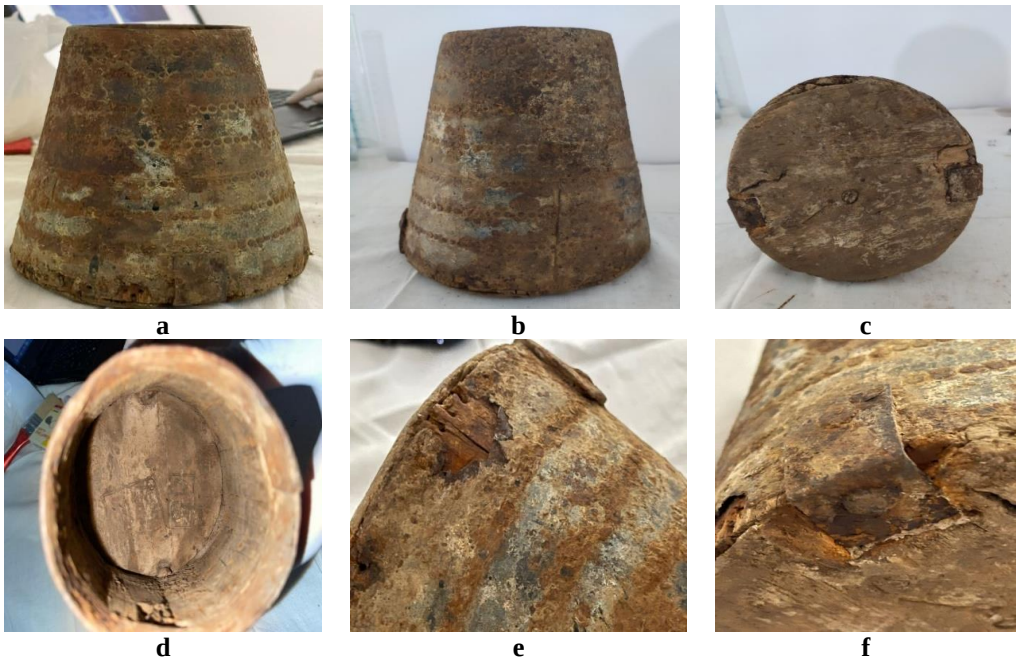


Fig. 3. The bushel: a and b - before restoration; c - the base of the bushel and the appearance of calcifications of dust; d - the bushel from the inside before the restoration; e - rust on the surface; f - layers of rust on the metal bracket

Graphic Documentation

The bushel's dimensions are signed, considering everything that appears on the body of the measure, starting with the fixing screws, as well as the sediments on the surface and distinguishing them on the drawing with a specific pattern, whether they are clay calcifications, rust, or cracks on the body and the trail.

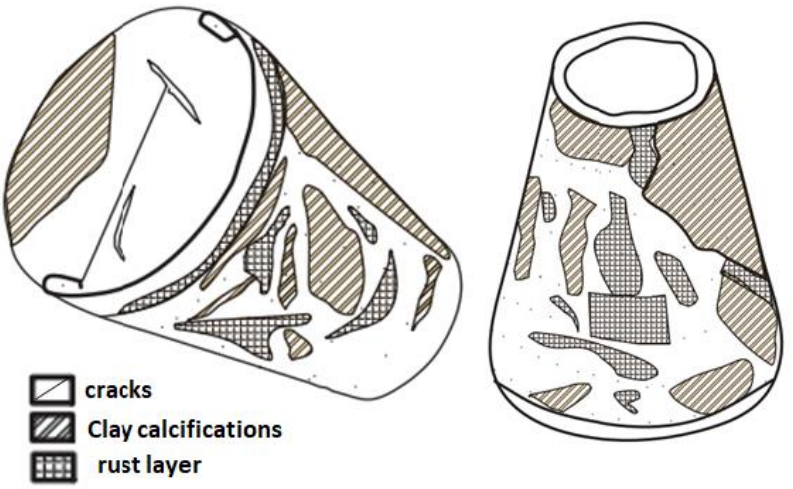


Fig. 4. An illustration of the of damage area of the bushel

3D Documentation

Three-dimensional documentation was done using AutoCAD to document the bushel and determine its damage manifestations [25].

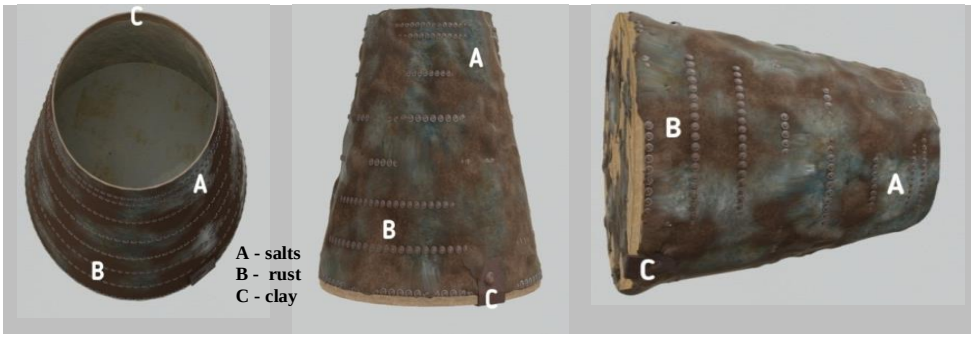


Fig. 5. 3D documentation of the bushel: a - the upper hole of the weight, showing the signs of damage; b - the sides of the bushel, showing the manifestations of damage; c - the base of the bushel instrument, showing the manifestations of damage

Qualitative detection of fungal isolates to produce cellulase enzymes using the solid environment

The carboxymethylcellulose agar medium was used for cultivation of fungi which isolated from the bushel. And the media consisted of (g/L) 10g peptone, 10g carboxymethylcelluloses, 2g Dipotassium phosphate, 3g. aqueous magnesium sulfate, 2g ammonium sulfate, 15-20g agar, 1 liter water and cellulose agar and Congo red environment consisting of 5g monopotassium phosphate, 25g anhydrous magnesium sulfate, 2g cellulose,15g agar, 2g red agar Congo, 2g gelatin, 1-liter distilled water [26], pH = 6.8-7.2. The culture of the fungi was infected from the infected trace and the plates were incubated at a temperature of 25-30℃ for 48 to 72 hours. The growth was observed and then the set of dishes was immersed in iodine and red solution Congo on the environment of carboxymethyl cellulose and cellulose, respectively [27]. The results are shown in Table 1 (Figs. 6 and 7), illustrating the cultivation of fungi isolated from the infected bushel in an environment containing carboxymethyl cellulose and verifying the ability of these fungi to produce cellulase enzymes by immersing them in an iodine solution. The results showed that these isolates could produce cellulose in varying proportions. It was noted that isolates 11, 12, 16 and 25 showed a higher transparency area than the rest, while isolates 1, 4, 6, 10 and 14 showed moderate activity. Isolates 3, 7, 20 and 24 showed less activity and isolates 2, 5 and 15 showed no cellulase enzyme activity.

Table 1. Qualitative survey of fungi isolates grown on carboxymethyl cellulose and cellulose in the solid environment using iodine solution as a reagent

Media	Cellulose	CMC
1	+	++
2	+	-
3	-	+
4	+	++
5	-	-
6	+++	+++
7	+	+
10	+++	++
11	++++	++++
12	+++++	++++
14	++	++
15	-	-
16	++++	++++
20	+	+
24	+	+
25	++++	+++++

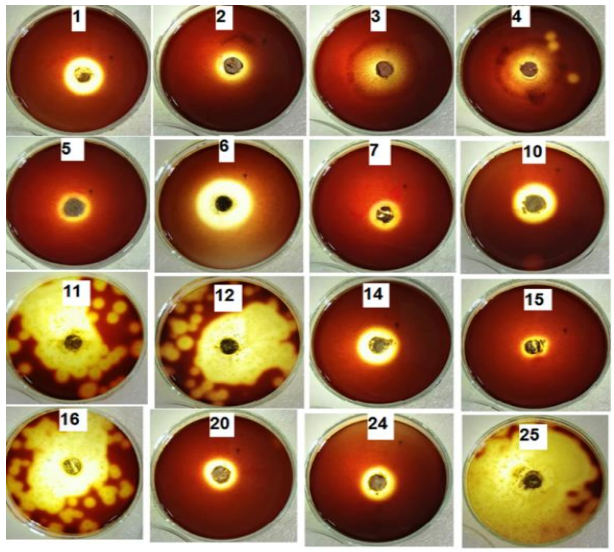


Fig. 6. Qualitative survey of fungi isolates grown on carboxymethyl cellulose in the solid environment using iodine solution as a reagent

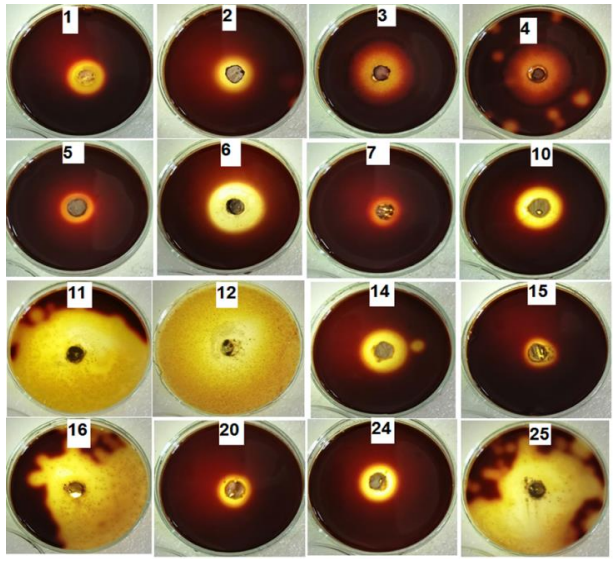


Fig. 7. Qualitative survey of fungi isolates grown on cellulose in the solid environment using iodine solution as a reagent

Table 2. Qualitative survey of fungi isolates grown on carboxymethyl cellulose and cellulose in a solid environment using Congo red solution as a reagent

Media	Cellulose	CMC	Media	Cellulose	CMC
1	+	+	11	+++	+
2	-	+	12	+++	+++
3	+++	+++	14	+	++
4	++	+++	15	-	+
5	+	+	16	++	+
6	++	++	20	+	++
7	+	+	24	+	++
10	+	++	25	++++	+++

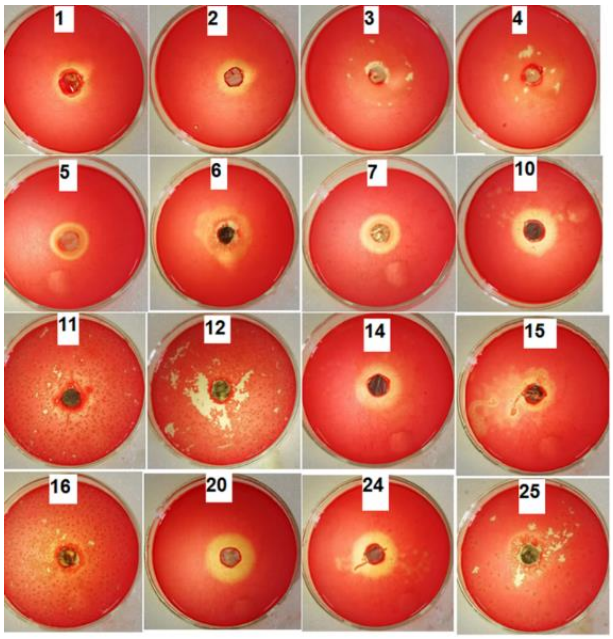


Fig. 8. Qualitative survey of fungi isolates grown on carboxymethyl cellulose in a solid environment using Congo red solution as a reagent and 1 M NaCl solution to remove color

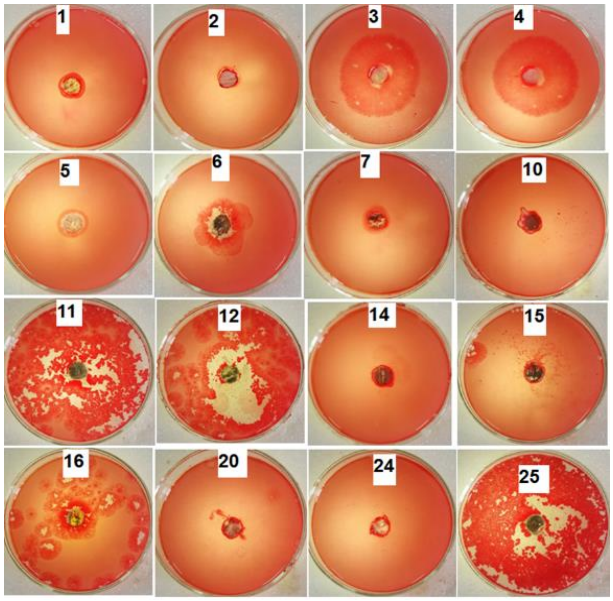


Fig. 9. Qualitative survey of fungi isolates grown on cellulose in a solid environment using Congo red solution as a reagent and 1M sodium chloride solution to remove color

Quantification detection of fungal isolates to produce cellulase enzymes

Fungi isolated from archaeological wood were cultivated in a liquid environment containing cellulose for 6 days, after which the fungi were removed by centrifugation at 4000rpm for 5-10 minutes. Then, the filtrate was used as a source of cellulase enzyme.

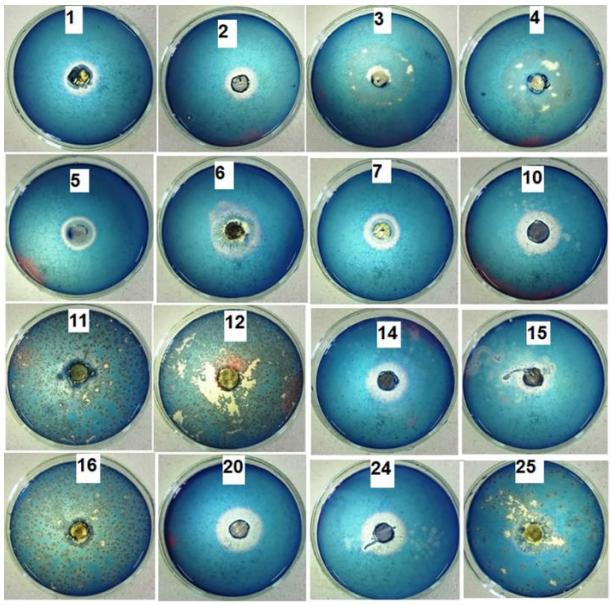


Fig. 10. Qualitative survey of fungi isolates grown on carboxymethyl in a solid environment using Congo red solution as reagent and using 1 M hydrochloric acid solution to remove color

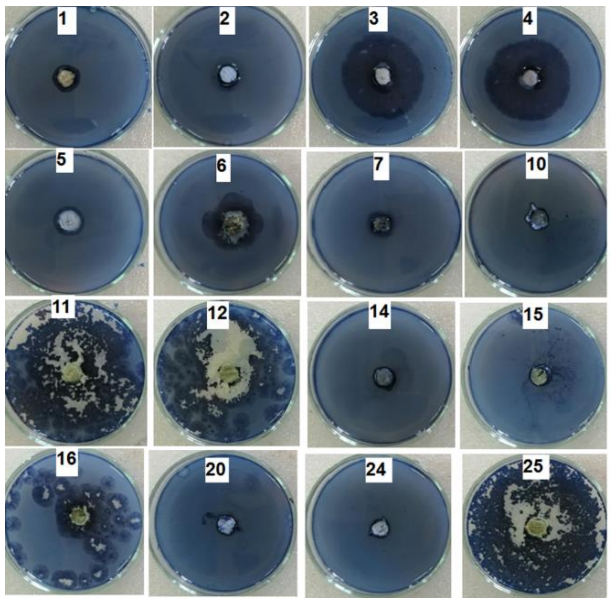


Fig. 11. Qualitative survey of fungi isolates grown on cellulose in a solid environment using Congo red solution as reagent and using 1 M hydrochloric acid solution to remove colour

Determination of cellulase enzyme activity using filter paper: (Exoglucanase, FPase)

The FP-ase activity was measured by mixing 1.0mL of fungal filtrate from each fungal isolate and +1.0mL of citrate buffer (0.05M + Whatman filter paper) at pH = 4.8 [28]. Then, they were incubated at 50℃ for an hour and the reducing sugars were measured as glucose [29]. The presence of glucose was detected using (DNS) as a reagent using a colorimetric device where the absorption strength was measured at 540nm and using the empty reagent, which

consisted of a cellulose environment not inoculated with isolates and to which the reagent was added. One unit of the enzyme was defined as the amount of enzyme that released 1 micromole of reducing sugars, expressed in glucose, per minute under reaction conditions.

To evaluate the degrading enzymatic activity the filter paper was used on the isolated fungi. Table 3 It was found that isolates (20, 14 and 2) showed the maximum enzymatic activity with values of (1.533, 1.599 and 1.48), isolates (25,16,11,5 and 4) showed average enzymatic activity with values (1.05, 1.40, 1.14, 1.05 and 1.33) and isolates (24, 15, 12, 10, 7, 6 and 3.1) showed weak enzymatic activity with values (0.380, 0.418, 0.493, 0.854, 0.392, 0.689, 0.593 and 0.413).

Table 3. Quantitative survey of fungi isolates using filter paper: (Exoglucanase, FPase)

No	swab NO	The hydrolyzed enzyme activity of the filter paper (units/mL)
1	1	0.413±0.060
2	2	1.488±0.155
3	3	0.593±0.006
4	4	1.337±0.049
5	5	1.058±0.090
6	6	0.689±0.068
7	7	0.392±0.097
8	10	0.854±0.096
9	11	1.144±0.100
10	12	0.493±0.048
11	14	1.599±0.104
12	15	0.418±0.039
13	16	1.409±0.006
14	20	1.533±0.041
15	24	0.380±0.058
16	25	1.053±0.071

Determination of cellulase enzyme activity using carboxymethylcellulose: (Endoglucanase, CMCase)

Carboxymethylcellulose (CMC-ase) activity was measured by mixing 1mL of fungal filtrate from each fungal isolate and +1.0mL of citrate buffer (0.05M +1.0mL of carboxymethylcellulose) at pH = 4.8. Then, they were incubated at 50°C for an hour [29] and the reducing sugars were measured as glucose. The presence of glucose was detected using (DNS) as a reagent using a colorimetric device where the absorption strength was measured at 540nm and using the empty reagent. One unit of the enzyme was defined as the amount of enzyme that released 1 micromole of reducing sugars, expressed in glucose, per minute under reaction conditions.

Table 4 shows the enzymatic activity of carboxymethylcellulose. It was found that isolates (25, 20, 16, 14 and 11) showed the highest enzymatic activity with values of (2.242, 2.521, 2.099, 2.226 and 2.087), isolates (10, 6, 4 and 2) showed average activity with a value of (1.152, 1.763, 1.439 and 1.768) and isolates (24, 15, 12, 7, 5, 3 and 1) showed weak activity with values (0.677, 0.500, 0.256, 0.190, 0.963, 0.200 and 0.521).

Determination of cellulase enzyme activity using salicin: (β-glucosidase, salicinase)

β-glucosidase or salicinase activity was measured by mixing 5mL of fungal filtrate from each fungal isolate and +1.0mL of citrate buffer (0.05M + salicin 1%) at pH = 4.8. Then, they were incubated at 50°C for an hour [30, 31] and the reducing sugars were measured as glucose. The presence of glucose was detected using (DNS) as a reagent using a colorimetric device

where the absorption strength was measured at 540nm. One unit of the enzyme was defined as the amount of enzyme that released 1 micromole of reducing sugars, expressed in glucose, per minute under reaction conditions.

Table 4. Quantitative survey of fungi isolates grown on carboxymethylcellulose hydrolyzing enzyme liquid medium

Serial	Isolation Number	Enzymatic Activity of Carboxymethylcellulose
1	1	0.512±0.185
2	2	1.768±0.169
3	3	0.200±0.007
4	4	1.439±0.115
5	5	0.963±0.086
6	6	1.763±0.129
7	7	0.190±0.017
8	10	1.152±0.102
9	11	2.242±0.216
10	12	0.256±0.096
11	14	2.521±0.090
12	15	0.500±0.096
13	16	2.099±0.135
14	20	2.268±0.045
15	24	0.677±0.194
16	25	2.087±0.084

Table 5 shows the activity of the enzyme degrading salicin. It was found that isolates (20, 6 and 4) showed the highest enzymatic activity with values of (2.468, 1.132 and 1.651), solates (16, 14, 11, 7, 5 and 1) showed medium activity with values of (0.955, 0.338, 0.510, 0.506, 0.942 and 0.537) and isolates (25, 24, 15, 12, 10, 3 and 2) showed weak activity with values of (0.338, 0.453, 0.489, 0.000, 0.000, 0.451 and 0.253).

Table 5. Quantitative survey of fungi isolates grown on salicin-dissolving enzyme liquid medium

Serial	Isolation Number	Enzymatic Activity of Salicine
1	1	0.537±0.092
2	2	0.253±0.028
3	3	0.451±0.43
4	4	1.651±0.127
5	5	0.942±0.114
6	6	1.132±0.051
7	7	0.506±0.046
8	10	0.000
9	11	0.510±0.099
10	12	0.000
11	14	0.937±0.053
12	15	0.489±0.092
13	16	0.955±0.027
14	20	2.468±0.281
15	24	0.453±0.074
16	25	0.338±0.046

Determination of cellulase enzyme activity using Xylanase

Xylanase activity was measured by mixing 1ml of fungal filtrate from each fungal isolate and +1.0mL of citrate buffer (0.05M + 1% xylan) at pH = 4.8. Then, they were incubated at 50°C for 15 minutes [32-34]. The reducing sugars were measured as glucose. The unit (Xylanase) is the amount of enzyme that releases 1 micromol of reducing sugar per minute.

Table 6 shows the enzymatic activity of the salicin. It was found that the isolates (25, 20, 16, 11, 10, 6 and 1) showed the highest enzymatic activity with values of (26.858, 27.380, 27.918, 33.977, 22.386, 20.114 and 34.209), isolates (14 and 4) showed moderate activity with values of (13.572 and 17.678) and isolates (24, 15, 12, 5, 3 and 2) showed weak activity with values of (5.057, 3.709, 5.242, 0.000, 3.547 and 2.847).

Table 6. Quantitative survey of fungi isolates cultivated **in** the liquid environment of xylan degrading enzyme

Serial	Isolation Number	Enzymatic Activity of Xylanase
1	1	34.309±2.130
2	2	34.309±2.130
3	3	13.572±1.208
4	4	3.547±0.711
5	5	17.678±0.555
6	6	00.000
7	7	20.114±1.026
8	10	0.422±2.456
9	11	2.380±33.977
10	12	0.763±5.242
11	14	0.504±2.847
12	15	1.208±3.709
13	16	1.386±27.918
14	20	0.943±27.380
15	24	0.707±5.057
16	25	1.863±36.858

Molecular Identification of Isolates

The fungal isolates of wood artifacts O14 and O20 were molecularly identified by the 18SrRNA protocol. The nucleotide sequences of 592 bp and 577 bp of the complete 18SrRNA gene were determined for isolates O14 and O20 (respectively) in both strands. In contrast, BLAST research showed that isolate O14 showed 100% similarity with Aspergillus flavus strain GFRS16 and O20 showed 100% similarity with Aspergillus fumigatus isolate MEBP0074. AB1 for isolated microorganisms (O14 and O20, respectively). A phylogenetic tree was constructed for these fungi (Figs. 20 and 21). These microbes were identified as Aspergillus flavus O14 and Aspergillus fumigatus O20 with GeneBank entry numbers MW0484411.1 and MW048521.1 for O14 and O20, respectively.

Conclusions

This paper aims to investigate the bushel, identify the microbial infestation on the bushel, and choose the best methods for sterilization and treatment. It used graphic documentation to determine the deterioration phenomena of the bushel and optical microscopy (OM) to identify the wood. Moreover, it did 3D documentation using AutoCAD to document the bushel and determine its damage manifestations. It utilized TEM to determine the shape and size of silver and gold nanoparticles. The results revealed that the generated silver nanoparticles

had a spherical shape, with a diameter ranging from 12 to 16nm. Additionally, wood was identified as pines, and the fungi isolated from the bushel were *Aspergillus flavus and Aspergillus fumigatus.*

The qualitative detection of fungal isolates to produce cellulase enzymes using the solid environment showed that these isolates could produce cellulose in varying proportions. The quantification detection of fungal isolates in the filtrate was used as a source of cellulase enzyme after the fungi were isolated and cultivated containing cellulose after 6 days. The fungi were removed by centrifugation at 4000rpm for 5-10 minutes. Carboxymethylcellulose (CMC-ase) activity was measured by mixing 1mL of fungal filtrate from each fungal isolate and +1.0mL of citrate buffer (0.05M +1.0mL of carboxymethylcellulose) at pH = 4.8. The results revealed that the enzymatic activity of carboxymethylcellulose. Moreover, it was found that isolates (25, 20, 16, 14, and 11) showed the highest enzymatic activity with values of (2.242, 2.521, 2.099, 2.226, and 2.087), isolates (10, 6, 4, and 2) showed an average activity with values of (1.152, 1.763, 1.439, and 1.768) and isolates (24, 15, 12, 7, 5, 3, and 1) showed weak activity with values of (0.677, 0.500, 0.256, 0.190, 0.963, 0.200, and 0.521). The determination of cellulase enzyme activity using salicin: (β-glucosidase, salicinase) illustrated that the presence of glucose was detected using (DNS) as a reagent using a colorimetric device where the absorption strength was measured at 540nm. One unit of the enzyme was defined as the amount of enzyme that released 1 micromole of reducing sugars, expressed in glucose, per minute under reaction conditions**.**

After testing the ability of nano-silver and nano-gold to influence the growth of fungi, the results illustrated that 1% nano-gold was sufficient to inhibit all isolated microorganisms.

References

[1] R.S. Ferguson, *The Carlisle Bushel*, **Archaeological Journal, 42**(1), 1885, pp. 303-311, DOI: 10.1080/00665983.1885.10852178.

[2] J. H. Salomon, The Book of Indian Crafts and Indian Lore: The Perfect Guide to Creating Your Own Indian-Style Artifacts. Simon and Schuster, 2015.

[3] A.M. Abdelmoniem, N. Mahmoud, W.S. Mohamed, *Isolation and Characterization of Microbial Infection of Polychrome Wooden Coffin from the 26th Dynasty, Egypt*, **Scientific Culture**, **6**(3), 2020, pp. 1-6, DOI: 10.5281/zenodo.3956802.

[4] S. Elsa, *Testing corrosion inhibitors for the treatment of marine iron/waterlogged wood composite artifacts in Polyethylene glycol solution*, **Bachelor Thesis**, Mémoire de diplôme HES: Haute Ecole Arc Conservation-Restauration, 2008, p. 13.

[5] C. Degrigny, L. Spiteri, *Electrochemical monitoring of marine iron artefacts during their storage/stabilisation in alkaline solutions*, **Proceedings of Metal**, National Museum of Australia Canberra ACT, 2004, pp. 315-331.

[6] R.A. Zabel, J.J. Morrell, **Wood Microbiology: Decay and Its Prevention**, Academic press, 2012.

[7] R.A. Blanchette, *A review of microbial deterioration found in archaeological wood from different environments*, **International Biodeterioration & Biodegradation**, **46**(3), 2000, pp. 189-204. DOI: 10.1016/S0964-8305(00)00077-9.

[8] J. A. Martín, R. López, Biological deterioration and natural durability of wood in Europe. **Forests**, 14(2), 2023, 283, Doi.Org/10.3390/F14020283.

[9] A.M. Abdelmoniem, N. Mahmoud, M.A. Abdel-Fatah, S. Mohamed, N.M. Badr, *Multi analytical techniques of anthropoid wooden Coffin from Egypt late period*, **International Journal of Conservation Science**, **13(**4), 2022, pp. 1087-1100.

[10] A.M. Abdelmoniem, N. Mahmoud, W.S. Mohamed, A.Y. Ewais, A. Abdrabou, *Archaeometric study of a polychrome wooden coffin from 26th dynasty-Egypt*, **Mediterranean Archaeology and Archaeometry**, **20**(1), 2020, DOI: 10.5281/zenodo.3364825.

[11] J. Harwood, W. Tribaldos, S. Lobo, W. Wcislo, *Wood Identification of The Altarpiece and a Sculpture of The Cathedral Basilica Santa María La Antigua*, **Europrean Journal of Science and Theology**, **17**, 2021, pp. 137-149.

[12] A.M. Abdelmoniem, N. Mahmoud, M. Abd El Fatah, W.S. Mohamed, A.M. Omar, *Wooden Coffin Biodeterioration and How to Restore It with Different Antimicrobial Substances*, **International Journal of Conservation Science**, **13**(1), 2022, pp. 73-84.

[13] * * *, https://microbiologyinfo.com/potato-dextrose-agar-pda-principle-uses-composition-procedure-and-colony-characteristics/

[14] * * *, https://sharebiology.com/potato-dextrose-agar-pda/

[15] W.D. Black, *A comparison of several media types and basic techniques used to assess outdoor airborne fungi in Melbourne, Australia*, **PLoS One**, **15**(12), 2020, Article Number: e0238901. DOI: 10.1371/journal.pone.0238901.

[16] G. Lemańczyk, H. Kwaśna, *Effects of sharp eyespot (Rhizoctonia cerealis) on yield and grain quality of winter wheat*, **European Journal of Plant Pathology**, **135**, 2013, pp. 187-200. ,DOI: 10.1007/s10658-012-0077-3.

[17] A.M. Omar, A.M. Abdelmoniem, F. EL Wekeel, A.S. Taha, *Spectroscopic and Molecular Investigation of Cheops Wooden Boat for Microbial Degradation Applying Proper Microbicides and Methods*, **Scientific Culture, 8**(1), 2022, pp. 115-127. DOI: 10.5281/zenodo.5717174.

[18] A.M. Abdelmoniem, N. Mahmoud, W.S. Mohamed, *Isolation and Characterization of Microbial Infection of Polychrome Wooden Coffin from the 26th Dynasty, Egypt*, **Scientific Culture**, **6**(3), 2020, pp. 1-6. DOI: 10.5281/zenodo.3956802.

[19] * * *, https://www.swedishwood.ae/wood-facts/choosing-wood/from-log-to-plank/properties-of-softwood.

[20] F. Bulian, J. Graystone, **Wood Coatings: Theory and Practice**, Elsevier, 2009.

[21] M.R.M. Farahani, *Decay resistance of modified wood*, **PhD Thesis**, Bangor University (United Kingdom), 2003

[22] A. Khalil, M. Mir, S. Nabi, M.M. Mir, U. Iqbal, N. Nazir, S.A. Banday, R. Bhat, S.Q. Khan, T.F. Wani, **Canopy Management**, In Temperate Nuts, Springer Nature Singapore, 2023, pp. 209-245.

[23] K. Vahabi, G.A. Mansoori, S. Karimi, *Biosynthesis of silver nanoparticles by fungus Trichoderma reesei (a route for large-scale production of AgNPs)*, **Insciences Journal, 1**(1), 2011, pp. 65-79. DOI:10.5640/insc.010165.

[24] E.B. El Domany, T.M. Essam, A.E. Ahmed, A.A. Farghali, *Biosynthesis physico-chemical optimization of gold nanoparticles as anti-cancer and synergetic antimicrobial activity using Pleurotus ostreatus fungus.* **Journal of Applied Pharmaceutical Science, 8**(5), 2018, pp. 119-128. DOI: 10.7324/JAPS.2018.8516.

[25] A.M. Abdelmoniem, N. Mahmoud, S. Mohamed, Mostafa Abdel-fatah, M. Mohammad, N. Badr, *Multidisciplinary approach for documentation of an anthropoid wooden coffin from the late period in Egypt*, **Advanced Research in Conservation Science, 3**, 2022, pp. 15-26. DOI: 10.21608/arcs.2022.136413.1026.

[26] M. Sakthivel, N. Karthikeyan, R. Jayaveny, P. Palani, *Optimization of culture conditions for the production of extracellular cellulase from Corynebacterium lipophiloflavum*, **Journal of Ecobiotechnology, 2**(9), 2010, pp. 6-13.

[27] P. Gupta, K. Samant, A. Sahu, *Isolation of Cellulose-Degrading Bacteria Determination of Their Cellulolytic Potential*, **International Journal of Microbiology**, 2012, pp. 1-5. DOI: 10.1155/2012/578925.

[28] D.E. Eveleigh, M. Mandels, R. Andreotti, C. Roche, *Measurement of saccharifying celluase*, **Biotechnology for Biofuels and Bioproducts**, **2**, 2009, Article number: 21. DOI: 10.1186/1754-6834-2-21.

[29] G.L. Miller, *Use of dinitrosalicylic acid reagent for determination of reducing sugar*. **Analytical Chemistry, 31**(3), 1959, pp. 426-428. DOI: 10.1021/ac60147a030.

[30] U. Jagadeesh, R. Muthura, *Isolation and Screening of Potential Lignocellulolytic Microorganisms from Different Ecosystems*, **Mysore Journal of Agricultural Sciences, 56**(4), 2022, pp. 201-208.

[31] B.T. Shawky, T.I. Ragab, H.M. Hussein, M.K. Zahran, *Optimization of Cellulolytic enzymes production from local potent fungi using full factorial design for bioethanol production from MFEX-treated rice straw using Klebsiella oxytoca P2*, **Bioscience Research, 15**(1), 2018, pp. 250-261.

[32] A.R. Shah, D. Madamwar, *Xylanase production by a newly isolated Aspergillus foetidus strain and its characterization*, **Process Biochemistry**, **40**(5), 2005, pp. 1763-1771. DOI: :10.1016/j.procbio.2004.06.041.

[33] X. Jiang, A. Geng, N. He, Q. Li, *New isolate of Trichoderma viride strain for enhanced cellulolytic enzyme complex production*, **Journal of Bioscience and Bioengineering**, **111**(2), 2011, pp. 121-127. DOI: 10.1016/j.jbiosc.2010.09.004.

[34] M. Goyal, K.L. Kalra, V.K. Sareen, G. Soni, *Xylanase production with xylan rich lignocellulosic wastes by a local soil isolate of Trichoderma viride*, **Brazilian Journal of Microbiology**, **39**(3), 2008, pp. 535-541. DOI: 10.1590/S1517-83822008000300025.

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