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Comparative Inhibition Study by Nanomaterial, Plant Extract and Chemical Microcide on the Screaming Mummy in Egyptian Museum Store

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Abstract: Mummies in museums are exposed to different deterioration factors like microorganisms, especially unwrapped mummies, such as the screaming mummy. This screaming mummy in the store of the Egyptian museum is suffering from stains due to microbial infection. There are three trends of materials to inhibit microbial growth: nano materials, plant extraction and chemical materials. This research compares three materials representing the three trends such as nano zinc oxide (ZnO-NPs), *Ceratophyllum demersum* and 4-chloro-m-cresol, respectively. Microorganisms, isolated from the degraded mummy, were identified with an optical microscope and ribosomal ribonucleic acid (rRNA) analysis to guarantee identification accuracy. Results indicated that the bacteria in the mummy are *Bacillus jeotgali*, *Kocuria turfanensis*, *Microbacterium imperial*, *Micrococcus luteus* and *Bacillus megaterium*. Fungi are *Monascus pallens* and *Rhizopus oryzae*. The results of minimum inhibitory concentration (MIC) illustrated that the best concentrations for the bio treatment of isolated microorganisms is plant extract (*Ceratophyllum demersum*) at 600 ppm/100 mL, followed by 4-chloro-m-cresol at 600 ppm/100 mL and finally nano zinc oxide at 700 ppm/100 mL.

Keywords: nano zinc oxide; *Ceratophyllum demersum*; 4-chloro-m-cresol; bacteria; fungi

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1. Introduction

Mummification is a process that was done to preserve dead body in ancient Egypt by embalming and wrapped with linen bandages [1]. The ancient Egyptians did this process due to religious concepts, such as the afterlife [2]. Mummification includes the treatment of the body with oils, resin and wax wrapped with linen and placing it in a sealed coffin to ensure the preservation of the body [3]. Screaming (Glaring) mummies do not have good embalming. There are two types of screaming mummies: the first and most famous ones were murdered because of committing a crime, so they do not have good mummification or linen wrapping. The second type died as a result of a disease and all the usual embalming steps are done for them [4].

The mummy suffers from several factors that cause deterioration in museums, for example microorganisms [5], especially fungi more than bacteria. These organisms produce enzymes that cause staining and a decayed mummy [6]. The damage percentage by microorganisms depends on species, the surrounding climate (humidity, temperature) and the quality of the embalming [7,8]. Screaming mummies of the first type are more exposed to deterioration by fungi and bacteria because of bad mummification especially because they were not wrapped in linen strips.

The main concern of the study is the mummy of an unknown woman (Figure 1) from the store of the Egyptian Museum in Cairo, Egypt. The mummy's number is CIT 8; it was discovered in an unknown tomb by Winlock's excavations at ThT71. Winlock's written

note cards say: S.A.K. (1935–1936) Servants Cemetery burial No. 2 (SENMUT) of the 18th Dynasty. The height of the mummy is 158 cm (height from the heel to the crown of the head), and the age was estimated in the elderly.

Figure 1. The screaming mummy from the 18th dynasty in the Egyptian museum store.

Lately, nano materials are used as an antimicrobial agent [9]. Nanomaterials (1–100 nm) have a lot of properties: reducing UV effects, consolidation, cleaning, deacidification and inhibition of microbial growth [10–13]. Nano zinc oxide (ZnO-NPs) is known as a good antimicrobial Aaent, [14] especially for leather [15]—thus, it was chosen for the current study.

Ancient Egyptians used plants that have antimicrobial activities (due to contents of phenols and esters) and essential oils for mummification [16]. Currently, plant extracts and essential oils are used as antimicrobial agents in different ways for archaeological objects. A plant extract is prepared byusing different parts of the plant (often the leaves) soaked in 100 mL of water and 100 mL of ethanol, then evaporated to obtain a substance dissolved in 200 mL of sterile distilled water. Essential oils are more effective for inhibiting microbial growth, but they are not suitable for every archaeological materials. They can cause darkening in color [17]. Scientific studies have mentioned that camphor and thyme are more effective than other essential oils [18,19]. Another study proved that plant extract and nano materials did not change or produce a reaction with archaeological objects' color [20]. Thus, it was chosen for the current study.

Some of hydrophytes in the Nile River have antimicrobial activity, including *Ceratophyllum demersum* [21]. *Ceratophyllum demersum* is a submerged aquatic plant from Saluga and Ghazal island [22]; they are protected areas in Aswan, Egypt [23].

There are a lot of chemical materials used as antimicrobials and pesticides. Some of them could be used in the treatment of archaeological objects. It is preferable to use safe antimicrobials for objects as a restorer, such as 4-chloro-m-cresol [24]. 4-chloro-m-cresol is considered a safe material to be used even in cosmetic products (up to 0.2%). This material is a synonym for p-chloro-m-cresol [25,26].

The current research aims to study microorganisms isolated from the screaming mummy and to study its biological activities. The study also aims to compare the inhibition of three materials: nano zinc oxide, *Ceratophyllum demersum* and 4-chloro-m-cresol

representing, respectively. These three materials are for inhibiting microbial attacks, and are applied to the microorganisms isolated from the screaming mummy.

2. Materials and Methods

2.1. Materials

The nano material used in this study, zinc oxide (ZnO-NPs) < 50 nm particle size (BET), >97%, was purchased from Sigma-Aldrich Company, No: 677450. The plant extract was *Ceratophyllum demersum.* The plant is a dominant species within the Nile River system. The plant was collected from sites located around Isis island (24°04.646′ N; 32°52.701′ E) and Saluga and Ghazal island (24°04.328′ N; 32°52.279′ E). It was prepared by washing the plant, then it was dried and ground into a powder. The powder was then left in methanol. The chemical antimicrobial was 4-chloro-m-cresol, purchased from Loba Chemie Pvt. Ltd., Mumbai, India, No: 02750. The media used for isolation and purification were cellulose, protein and nutrient agar.

2.2. Methods

A collection of swabs was taken from the screaming mummy in the Egyptian Museum store, specifically from skin areas including the head, wig, rib cage, abdominal and pelvic cavity, right leg, left leg and mouth of the mummy as the presence of microbial lesions is clear in the form of brown spots (Figure 2). Microbial swabs were cultured on plates of cellulose, protein and nutrient agar media. Regarding isolation and purification, the growths that presented in the Petri dishes were taken separately and cultured on the same previous media in order to obtain the microorganisms in pure forms to complete the steps of characterization.

Figure 2. Collecting swabs from the mummy.

Microbial colonies that grew on the incubated plates were sub-cultured into separate fresh sterile cellulose agar and nutrient agar plates and then incubated to obtain pure cultures of causative microorganisms. The purified isolates were kept in slants and stored for characterization. Identification of all microbial isolates was carried out at the Laboratory of Microbiology, Grand Egyptian Museum, Ministry of Antiquities with optical microscopy and with sequencing of rRNA genes at Solgent Company, South Korea.

Bacterial identification was performed with a molecular approach. Bacterial isolates were cultured in sterile test tubes containing 10 mL of nutrient broth medium [27]. Cultures were incubated at 28 °C for 48 h. A small amount of bacterial culture was scraped by sterile spatula suspended in 100 µL of sterile distilled water in 2 mL of sterile vials and boiled at 100 °C for 15 min. Bacterial DNA was extracted and isolated using SolGent purification bead. Prior to sequencing, the ribosomal rRNA gene was amplified using the polymerase chain reaction (PCR) technique in which two universal bacterial primers 27F (forward) and 1492R (reverse) and were incorporated in the reaction mixture. Primers used for gene amplification have the following composition: 27F(5′AGAGTTTGATCMTGGCTCAG) and 1492R(5′TACGGYTACCTTGTTACGACTT).

The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1% agarose gel. Then, these bands were eluted and sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Each sample was sequenced in the sense and antisense directions using the same primers [28]. Sequences was further analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of Meg Align (DNA Star) software version 5.05. Identification of bacterial isolates was done by sequencing of rRNA genes at the Solgent Company, South Korea.

Fungal isolates can be identified with different methods (morphological, molecular approach and biochemical), but the molecular approach was used because of its high accuracy compared to the other methods. Fungal isolates were cultivated in Petri plates containing 20 mL of Czapek's yeast extract agar with 20% sucrose (CY20S) medium at 28 °C for 7 days [29]. A small amount of fungal culture was scraped by sterile spatula suspended in 100 µL sterile distilled water in 2ml sterile vials and boiled at 100 °C for 15 min. Fungal DNA was extracted and isolated using SolGent purification bead. Prior to sequencing, the ribosomal rRNA gene was amplified using the polymerase chain reaction (PCR) technique in which two universal fungal primers were sequenced with ITS1 and ITS4 primers [30], which were incorporated in the reaction mixture. Primers used for gene amplification have the following composition: ITS1 (5′-TCCGTAGGTGAACCTGCGG3′) and ITS4 (5′-TCC TCCGCTTATTGATATGC-3′) for fungi.

The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1% agarose gel. The amplicons were sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Sequences were further analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05. Identification of fungal isolates was done by sequencing of rRNA gene at the Solgent Company, South Korea.

In order to determine cellulose (s) and protease activity of the isolated microorganisms we used the following steps:

- Enzyme Production

Production was carried out in 250 mL conical flasks. Each contained 100 mL of the production medium for producing cellulase and protease enzymes and the main source of carbon (sucrose) was replaced with 10g of cellulose and 10 g of gelatin, respectively. Flasks were sterilized at 121 °C for 15 min. After that, cooling inoculated with 2 ml of standard inoculums of each isolate. The inoculated flasks were incubated at 28–30 °C for the proper time. At the end of the incubation period, the liquid cultures were centrifuged at 3000 RPM for 15 min. The supernatant was taken for determination of enzyme (cellulase and protease) activity as described below.

- Enzymes Assay

The cup plate clearing zone technique (CCZ) was used for assaying activities of cellulase and protease enzymes. The procedure was carried out by pouring 20 mL aliquots

of the detection medium [31] into a sterile Petri dish and it was then allowed to solidify. A sterile cork borer (15 mm diameter) was used to make three cups in each plate and 0.1 mL of the supernatant (cell free enzyme) of each isolate was placed into the three cups. Plates were incubated at 30 °C for 24 h after which the plates were flooded with a Lugol's iodine solution to assay cellulose and with an acid mercuric chloride solution for protease assay. Enzyme activities were compared based on the diameter (mm) of clear zone.

2.2.1. Determine Minimal Inhibitory Concentration (MIC) of Antimicrobial Agents against the Isolated Microorganisms

The three substances categories microcides chosen were: nano material, plant extract and chemical antimicrobial (ZnO NPs, *Ceratophyllum demersum* and 4-chloro-m-crysol) prepared to determine their minimal inhibitory concentrations. A stock solution of each microcide was prepared by dissolving 1 g/L ethyl alcohol (95%) in the case of 4-chloro-m-crysol and nano zinc oxide and 1 mL/L of ethyl alcohol (95%) in the case of *Ceratophyllum demersum*. Gradient concentrations of each microcide (400, 500, 600 and 700 ppm) were prepared by diluting the stock solution with alcohol.

One milliliter of spore suspension was spread on nutrient agar and Dox's agar plate. The controls were the solvents used for every extract. Plates were left to be dry. A cork borer was used to make three pores in each plate and 100 µL of the three concentrations was added for each microcide materials. Concentrations start with 400 ppm/100 mL. The zones of growth inhibition around the wells were measured after 18 to 24 h of incubation at 37 °C for bacteria and 48 to 96 h for fungi at 28 °C. The sensitivities of the microorganism species to the test substances were determined by the sizes of inhibition zones (including the diameter of well) on the agar surface around the wells. Values less than 15 mm were considered as not active against microorganisms compared to control plates that used ethyl alcohol (dissolvent solution of microcides) alone.

2.2.2. Evaluate Treatment for Isolated Microorganisms from Mummy

Microbial growth was examined by taking of swabs from each treated plates after 48 h, 3 months and 6 months. The swabs were cultured in Dox's medium for fungi and Nutrient agar for bacteria. A concentration of the best microcide will be used.

3. Results

3.1. Identification of the Microbial Isolates

3.1.1. Optical Microscope Identification

The resulting microbial colonies were subjected to preliminary characterization depending on the type of organism, as mentioned previously. The following genera were identified: *bacillus*, *coccus* and *Rhizopus* (Table 1 and Figures 3 and 4).

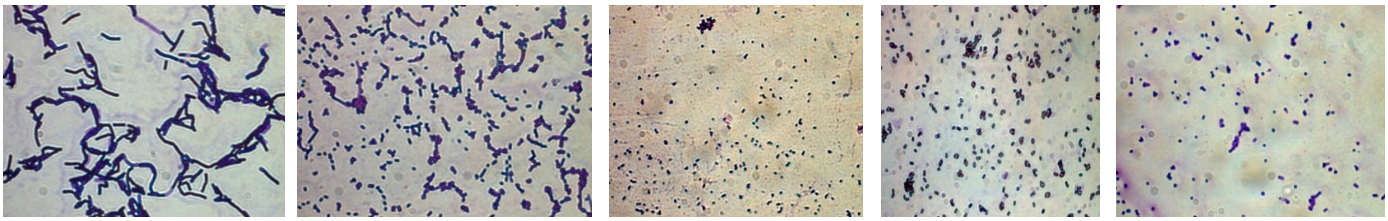
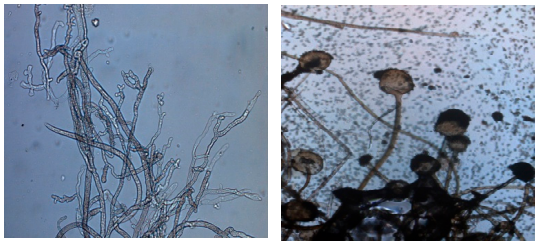


Figure 3. Bacteria isolated from mummy with optical microscope at 1000× magnification.

Based on the results, it can be seen that the genus *bacillus* was the dominant genus in seven sites having 33.3% of the total bacterial isolates and the genus *Rhizopus* was the dominant genus in seven sites having 55.2% of the total fungal isolates.



Monascus pallens *Rhizopus oryzae*

Figure 4. Fungi isolated of mummy with optical microscope at 400× magnification.

Table 1. Isolated microorganisms grow on different media (cellulose, protein and nutrient agar).

Swab Cite	Media Used		
	Cellulose Agar	**Protein Agar**	**Nutrient Agar**
Head	Fungus 1 *Rhizopus oryzae*	G+ve *Shortbacilli* spore former1	G+ve *Shortbacilli* spore former2 *Microbacillus* spp.
Wig	*Microbacillus* spp. Fungus 1	*Microbacillus* spp. G+ve *Shortbacilli* spore former3	G+ve *Shortbacilli* spore former3
Rib cage	*Rhizopus oryzae*	G+ve *Shortbacilli* spore former2	G+ve *Shortbacilli* spore former4 *Microbacillus* spp.
Abdominal and pelvic cavity	Fungus 1	*Microbacillus* spp.	G+ve *Shortbacilli* spore former3 *Micrococus* spp.
Right leg	*Rhizopus oryzae*	*Microbacillus* spp. G+ve *Shortbacilli* spore former1	G+ve *Shortbacilli* spore former3
Left leg	*Rhizopus oryzae* *Microbacillus* spp.	G+ve *Shortbacilli* spore former3 G+ve *Shortbacilli* spore former4	G+ve *Shortbacilli* spore former3 *Microbacillus* spp.
Mouth	*Rhizopus oryzae* Fungus 1	*Micrococus* spp.	G+ve *Shortbacilli* spore former2 *Micrococus* spp.

3.1.2. rRNA Sequencing Identification

Based on the results in Tables 2 and 3, it was found that the bacterial isolates were identified according to thew molecular approach by 16S rRNA sequencing analysis compared with closely related strains accessed from the Gen Bank, such as *Bacillus jeotgali, Kocuria turfanensis, Microbacterium imperial, Micrococcus luteu* and *Bacillus megaterium*. Fungal isolates were identified according to the molecular approach by ITS region of rDNA sequencing analysis and compared with closely related strains accessed from the Gen Bank, such as *Monascus pallens* and *Rhizopus oryzae*.

3.2. Determination of Cellulases and Protease Produced by the Isolated Microorganisms by Cup Plate Technique

Data recorded in Table 4 and illustrated by Figure 5 indicate that the tested microorganisms proved the various abilities to produce the cellulases and protease enzymes. Microorganisms are different in the degree of decomposition of proteins and cellulose. The tabulated data show the highest activity of cellulolytic activity observed in *Bacillus jeotgali* and a highest activity of proteolytic activity observed in *Microbacterium imperial.*

Table 2. Identification of bacterial isolates obtained from deteriorated mummy by 16S rRNA sequencing analysis compared with closely related strains accessed from the Gen Bank.

Swab Cite	Identification	Closely Related Microbial Strains Accessed from Gen Bank		
		Strain No.	Accession No.	Similarity
Head	*Bacillus jeotgali*	YKJ-10	NR025060T	99.93%
	Kocuria turfanensis	HO-9042	NR043899T	88.22%
	Microbacterium imperiale	DSM 20530	NR026161T	99.86%
Wig	*Microbacterium imperiale*	DSM 20530	NR026161T	99.86%
	Bacillus jeotgali	YKJ-10	NR025060T	99.93%
Rib cage	*Microbacterium imperiale*	DSM 20530	NR026161T	99.86%
	Bacillus jeotgali	YKJ-10	NR025060T	99.93%
Abdominal and pelvic cavity	*Microbacterium imperial*	DSM 20530	NR026161T	99.86%
	Micrococcus luteu	MB5	MH450098	99.71%
	Bacillus jeotgali	YKJ-10	NR025060T	99.93%
Right leg	*Bacillus megaterium*	NBRC 15308 = ATCC 14581	NR112636T	100%
	Microbacterium imperial	DSM 20530	NR026161T	99.86%
	Kocuria turfanensis	HO-9042	NR043899T	88.22%
Left leg	*Microbacterium imperial*	DSM 20530	NR026161T	99.86%
	Bacillus jeotgali	YKJ-10	NR025060T	99.93%
	Bacillus megaterium	NBRC 15308 = ATCC 14581	NR112636T	100%
Mouth	*Bacillus jeotgali*	YKJ-10	NR025060T	99.93%
	Micrococcus luteus	MB5	MH450098	99.71%

Table 3. Identification fungal isolates obtained from deteriorated mummy ITS region of rDNA sequencing analysis compared with closely related strains accessed from the Gen Bank.

Scheme	Identification	Closely Related Microbial Strains Accessed from Gen Bank		
		Strain No.	Accession No.	Similarity
Head	*Monascus pallens*	IMI 356820	GU733328	100%
	Rhizopus oryzae	IDI 354840	GU732332	99.88%
Wig	*Microbacterium imperial*	DSM 20530	NR026161T	99.86%
	Monascus pallens	IMI 356820	GU733328	100%
Rib cage	*Rhizopus oryzae*	IDI 354840	2GU732332	99.88%
Abdominal and pelvic cavity	*Monascus pallens*	IMI 356820	GU733328	100%
Right leg	*Rhizopus oryzae*	IDI 354840	GU732332	99.88%
Left leg	*Microbacterium imperial*	DSM 20530	NR026161T	99.86%
	Rhizopus oryzae	IDI 354840	GU732332	99.88%
Mouth	*Monascus pallens*	IMI 356820	GU733328	100%
	Rhizopus oryzae	IDI 354840	GU732332	99.88%

3.3. The MIC of Isolated Micro-Organisms

In this part of the study, the three microcides were applied to the isolated microorganisms to determine the MIC. Nano zinc oxide showed no response at various concentrations (400, 500 and 600 ppm/100 mL) in all isolates, except for at the concentration 700 ppm/100 mL that showed a response to all isolates. Thus, it could be reported that using nano zinc oxide at concentrations of 700 ppm/100 mL gave the diameter of a clear zone that ranged from 18–25 mm.

Table 4. Determine of cellulases and protease produced by the isolated microorganisms using cup plate technique.

Microbial Isolates	Diameter of Clearing Zone (mm)	
	Cellulases	Protease
Monascus pallens	23	22
Rhizopus oryzae	24	21
Bacillus jeotgali	28	27
Kocuria turfanensis	19	20
Microbacterium imperial	26	32
Micrococcus luteus	24	25
Bacillus megaterium	22	21

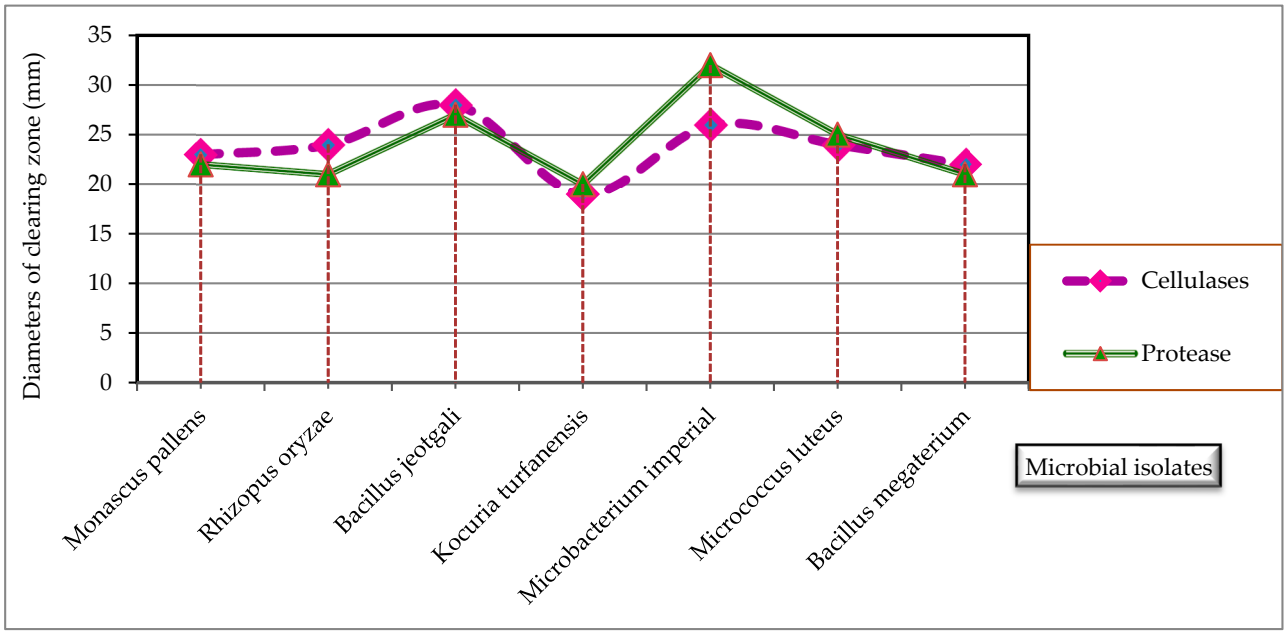


Figure 5. Cellulase(s) and protease produced by isolated microorganisms using cup plate technique.

Natural substance plant extract (*Ceratophyllum demersum*) had a good response at 600 ppm/100 mL concentration, which gave a response in all isolates. Therefore, it could be reported that using a natural substance at 600 ppm/100 mL gave the diameter of a clear zone ranging from 25–48 mm.

In the case of 4-chloro-m-cresol, no response could be detected at 400 ppm/100 mL concentration of all isolated microorganisms. It showed a response at 500 ppm/100 mL with most isolated microorganisms, but the good response was at 600 ppm/100 mL, where the diameter of a clear zone ranged from 24–45 mm.

These data can be used to recommend the best concentrations of a specific microbicide for the microbial treatment of an infected mummy. For instance, a natural substance plant extract (*Ceratophyllum demersum*) at 600 ppm/100 mL is sufficient to inhibit all isolated microorganisms followed by 4-chloro-m-cresol at 600 ppm/100 mL and Nano zinc oxide at 700 ppm/100 mL. These results are listed in Table 5 and shown in Figures 6 and 7.

3.4. Evaluation of Different Methods for Treatment of Deteriorated Mummy

The plant extract was the best to be applied using the fumigation method (30 mL/m^3). A concentration of 600 ppm/100 mL of plant extract was used to inhibit microbial growth on the mummy. Microbial growth was examined by taking of swabs from each treated specimen after 48 h, 3 months and 6 months. The swabs were cultured in Dox's agar medium for fungi and Nutrient agar for bacteria (Table 6).

Table 5. Determine inhibition zone (mm) of microbial species grown on Czapek agar and Nutrient agar as affected by three microcides: nano zinc oxide, natural plant extract (*Ceratophyllum demersum*) and 4-chloro-m-cresol.

Microorganisms	Inhibition Zone by mm at Different Concentrations (ppm/100 mL).									
	Nano Zinc Oxide				Plant Extract			4-chloro-m-cresol		
	400	500	600	700	400	500	600	400	500	600
Monascus pallens	0	0	0	18	0	20	37	0	25	35
Rhizopus oryzae	0	0	0	25	0	30	48	0	30	45
Bacillus jeotgali	0	0	0	18	0	0	28	0	0	27
Kocuria turfanensis	0	0	0	22	0	27	40	0	25	28
Microbacterium imperial	0	0	0	21	0	19	25	0	20	24
Micrococcus luteus	0	0	0	22	0	33	43	0	25	32
Bacillus megaterium	0	0	0	21	0	25	36	0	25	33

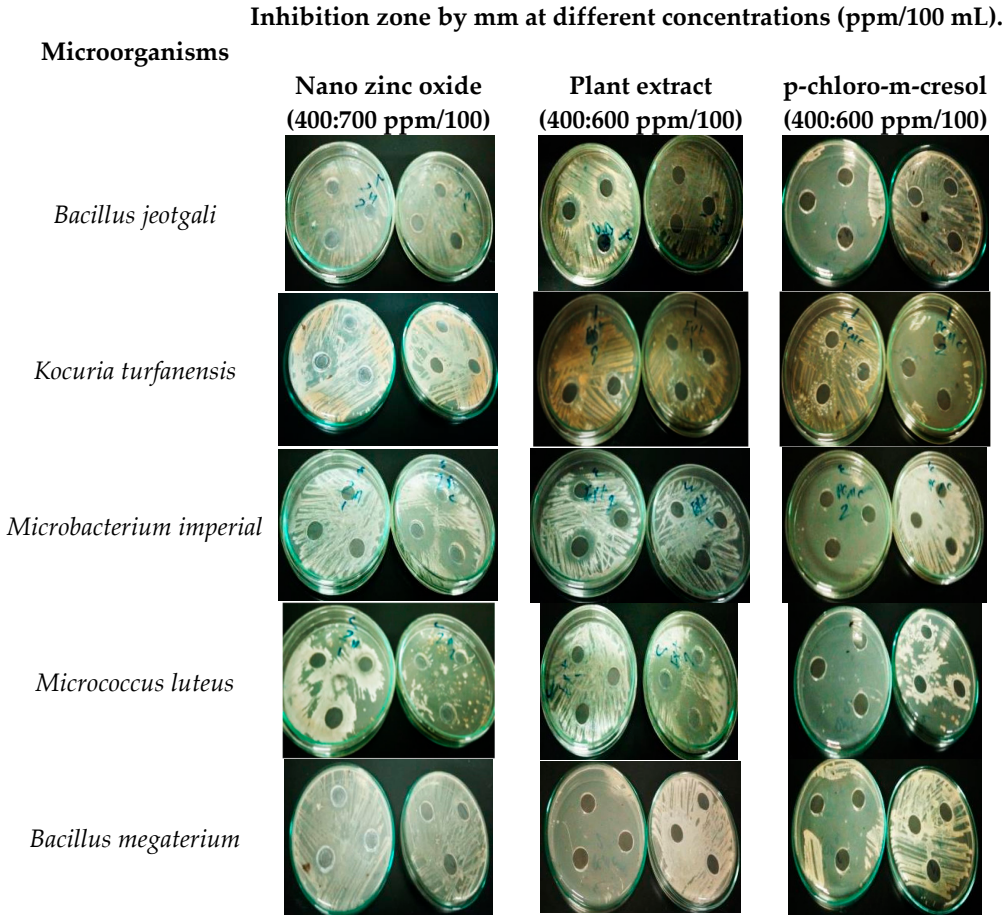


Figure 6. Effect of the three microcides: nano zinc oxide, natural plant extract (*Ceratophyllum demersum*) and 4-chloro-m-cresol on bacteria isolated from the mummy.

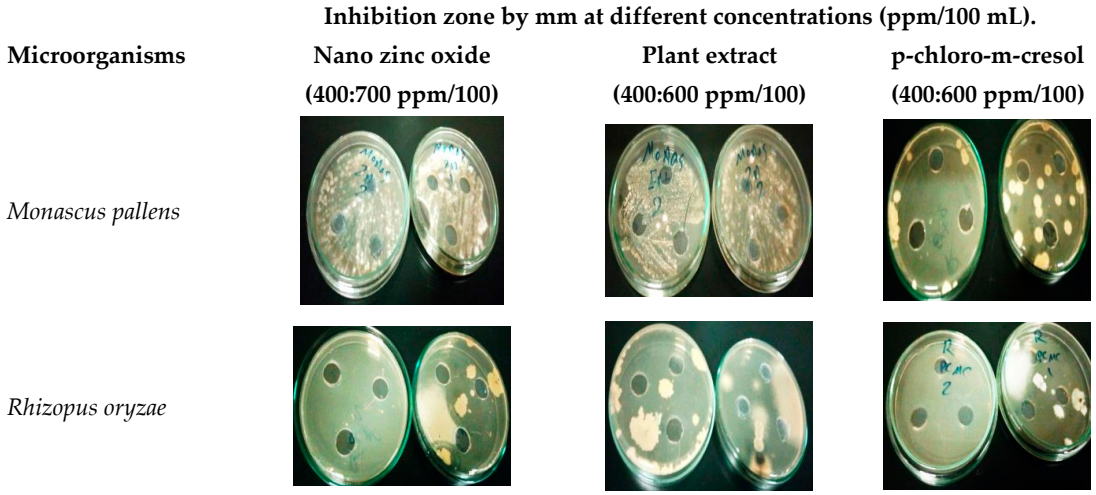


Figure 7. Effect of the three microcides: nano zinc oxide, natural plant extract (*Ceratophyllum demersum*) and 4-chloro-m-cresol on fungi isolated from the mummy.

Table 6. The effect of applying *Ceratophyllum demersum* at (600 ppm/100 mL) on microbial growth isolated from mummy.

Growth Detection Time	Growth of the Infectious Isolates after Treatment with (*Ceratophyllum demersum* L.) at 600 ppm/100 mL
48 h	No Growth
3 Month	No Growth
6 Month	No Growth

4. Discussion

Results show that the concentration 500 ppm/100 mL of nano zinc oxide does not have any effect, but the plant extract had the highest inhibition zone with most isolated microorganisms. In the concentration of 600 ppm/100 mL, there is no response for nano zinc oxide as well, although the plant extract and chemical microcide have a good inhibition zone. Therefore, nano zinc oxide in the concentration 700 ppm/100 mL was compared to a concentration of 600 ppm/100 mL of the for natural Plant extract (*Ceratophyllum demersum*) and chemical microcide, 4-chloro-m-cresol (Figures 8 and 9).

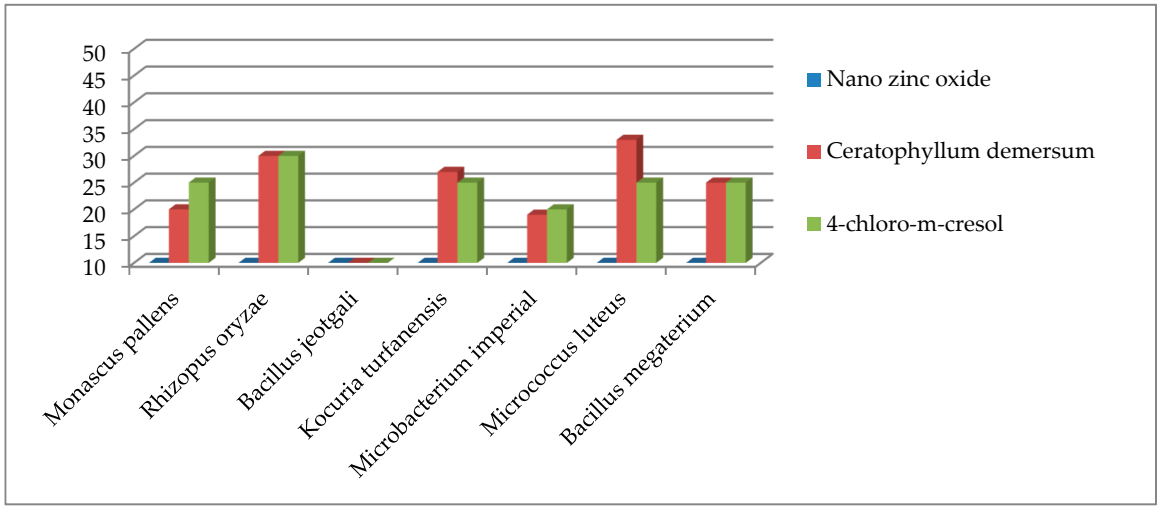


Figure 8. Comparison of the inhibition zone (mm) of microbial species grown on Czapek agar and Nutrient agar as affected by the 500 ppm/100 mL concentration of three microcides: nano zinc oxide, *Ceratophyllum demersum* and 4-chloro-m-cresol.

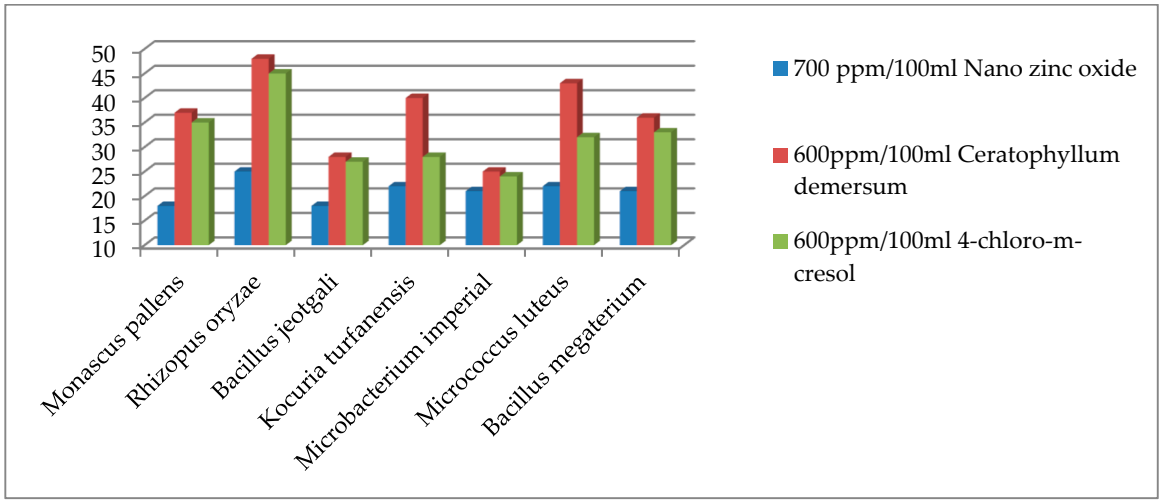


Figure 9. Comparison of the inhibition zone (mm) of microbial species grown on Czapek agar and Nutrient agar as affected by the 700 ppm/100 mL nano zinc oxide and 600 ppm/100 mL concentration of *Ceratophyllum demersum* and 4-chloro-m-cresol.

The chemical microcide (4-chloro-m-cresol), applied by brush or spray, could be concentrated in places and forgotten in others. This material could have an effect on the restorer's health if they do not wear a mask. For that reason, the chemical microcide (4-chloro-m-cresol) was not used. The nano material (ZnO NPs) is safer than 4-chloro-m-cresol for the restorer, but could be also concentrated in some places and cause a whitening of the mummy's appearance, especially if the mummy does not have wrapped linen.

Natural plant extracts (*Ceratophyllum demersum*) are used by fumigation in closed areas. A tent from polyethylene (plastic) was made and the mummy was put inside to be treated. Then, determining the volume of the tent in cubic meters and the corresponding volume of the plant extract is put into a Petri dish at the ratio of 30 mL/m^3. After that, the tent was sealed and left for 48 h. Microbial growth was examined by taking swabs from each treated specimen after 48 h, 3 months and 6 months. The swabs were cultured in Dox's medium for fungi and Nutrient agar for bacteria. This fumigation method is the best for archaeological objects that have many details, such as the screaming mummy. This mummy does not have wrapped linen strips. In addition, the *Ceratophyllum demersum* does not cause any change to the archaeological material's color and does not harm the restorer's health. This result is similar to the treatment of rare books [32].

5. Conclusions

The ancient mummies preserved in the Egyptian Museum store deteriorated due to the poor level of preservation conditions. Microorganisms isolated from the screaming mummy were identified based on ribosomal RNA analysis. Results show the presence of bacteria: *Bacillus jeotgali*, *Kocuria turfanensis*, *Microbacterium imperial*, *Micrococcus luteus* and *Bacillus megaterium*. The fungi are *Monascus pallens* and *Rhizopus oryzae*.

The isolated microorganisms produced activity in cellulose and protease enzymes that can cause biodeterioration of ancient mummies. The comparison between the three types of microcides material showed that the plant extract, *Ceratophyllum demersum*, at a concentration of 600 ppm/100 mL is the best. It is sufficient to inhibit all isolated microorganisms.

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