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NANOMATERIALS FOR THE INHIBITION OF MICROBIAL GROWTH ON ANCIENT EGYPTIAN FUNERAL MASKS

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ABSTRACT

Funeral masks were manufactured in ancient Egypt since several periods ago. They consist of one or more material such as wood, textile, gypsum, faience, silver and gold. They were exposed to microbiological infections from the surrounding environmental conditions such as fungi and bacteria, which caused various deterioration aspects : stains, disintegration, discoloration, cracking, and may promote the decay of funeral masks. In the last few years, nanoparticles have widely been used in treatment and conservation of artifacts. In this paper, the antimicrobial activities of nanomaterials silver, titanium dioxide and copper II oxide, were evaluated against the fungal strain of Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus and the bacterial strain of Bacillus alvei, Gthe short Bacilli, and Gthe Bacilli spore former. Transmission electron microscope TEM, and scanning electron microscope SEM attached with energy dispersive X-ray spectrometer EDX unit were utilized for characterization of nanoparticles. The data showed that silver nanoparticles are the best effective one for inhibition the growth of both isolated fungi and bacteria. It was applied for treatment and conservation of three ancient Egyptian funeral masks in saqqara, Egypt.

KEYWORDS: Nanomaterials, Funeral masks, Biodeterioration, microbial inhibition, Fungi, Bacteria.

INTRODUCTION

Ancient Egyptians manufactured funeral masks since the first intermediate period (2160-2025 B.C) , become very essential by New kingdom (1567-1085 B.C), still used to the late period (1085-332B.C) and in the Greco Roman period (332B.C-395A.D), (Redford D.B.,2001), (Walker,S., et al., 1997).

They used them for protection heads of their mummies and to facilitate identification the souls on their bodies in the second world. Funeral masks consist essentially of organic materials such as wood, textile, in addition to inorganic materials such as gypsum, faience beads, silver and gold. (Ikram, S. and Dodson, D., 1998), (Walker, S., et al., 1997).

Those funeral masks suffered from several microbiological, chemical, and mechanical deterioration factors, either in tombs or at museums. The infection by fungi and bacteria is still the most dangerous one due to secretion of organic and inorganic acids & enzymes, led to chemical and mechanical effects. (Johnson, C., et al., 1997). Fungi play a serious role in the disintegration of artifact materials due to their enormous enzymatic activity. Because of the high melanization of the fungal cell wall, their colonies appear spotty or filmy. Furthermore fungi cannot be easily killed by biocides or other antimicrobial treatments due to their thick walls (Sterflinger, K., 2010). On the other hand, bacteria are widely involved in the deterioration of objects. Several bacterial strains have been isolated from different artifacts .The potential deteriorating activity of bacteria occurred through the production of acids and surfactants. The removal of microorganism growth is a very delicate process. One must taking in consideration protection of artifacts as well as getting rid of these harmful microbes. Interference against microbial growth could be mechanical, physical, and/ or chemical removal of biodeteriogens. Many organic and inorganic compounds have been used as biocide agents, but they have several harmful side effects on artworks.

In the recent years, nano-materials were used for the inhibition of microorganisms in the archaeological field. Preparation of nano-sized metals and metal oxides silver Ag, titanium dioxide TiO_2, Zinc oxide ZnO, and copper II oxide CuO has allowed the development of a new generation of biocides. The advantages of using nanoparticles are referred to their unique chemical electrical, optical, and physical properties and biological activity. These properties are corresponding to ultra-small size, large surfaces to mass ratio and characteristic reactivity with microorganisms (Wang, Z., et al., 2012), (Zhang, X., et al., 2011), (Allaker, R.P., et al., 2012), (Maliszewska, I., 2011), (Altavilla, C., et al., 2011). Several researches confirmed that nanoparticles cause damage of the fungi and bacteria cells showing formation of pits in the cell walls and accumulation of their membranes. This exhibits a significant increase in permeability, result a death of the cell. (Egorova, E.M., 2010), (Sintubin, L., et al., 2012), (Sharma, V.K., et al., 2009), (Li, W., et al., 2010), (Chauhan, R., et al., 2013).

The aim of the present work is to evaluate silver, titanium dioxide and copper II oxide nanoparticles for inhibition the growth of fungi and bacteria to apply the best one for treatment and conservation three selected funeral masks in Saqqara, Egypt.

MATERIALS AND METHODS

1. Nanomaterials

Ag , TiO_2 and CuO nanoparticles NPs were used for evaluation inhibition of microbial growth. The first nanomaterial was prepared at chemistry department, Faculty of science, Fayoum University, Egypt. The other two nano-materials were supplied by Aldrich chemical company, Germany number 637254, 544868. Three concentrations had been applied 5, 10, 15 µg/ml for each one (C,B,A) respectively.

1.1 Transmission electron microscope TEM

TEM model tecnai G20, FEI company, Netherlands was utilized for determining the grain size of the previous nano-materials. The operating conditions were as follows: 200 KV, magnification range up to 1 million X, gun type lanthanum hexaboride LaB6.

1.2 Scanning electron microscope SEM attached with energy dispersive x-ray analysis EDX unit

SEM model Quanta 250, FEI company, Netherlands was used for characterization of nanomaterials and their degree of purification. Operating conditions were 30 KV, magnification from 14X reaches to million X.

2. Funeral masks

Three funeral masks as shown in Fig. 1 from Saqqara were selected for isolating fungi and bacteria using swabs techniques.

Second intermediate period (2160-2025 B.C) | 21 Dynasty (1085-945B.C) | 28-29 Dynasty (404-380 B.C)

Fig. 1 Selected funeral masks, Saqqara, Egypt.

3. Isolation and identification of fungi and bacteria

3.1 Media used

Two media were utilized for cultivation of isolated fungi and bacteria. For fungi cellulose agar medium was prepared by using cellulose 10gm, agar 20gm, $NaNO_3$ 2gm, KH_2PO_4 1gm, KCl 0.5 gm, $MgSO_4.7H_2O$ 0.5 gm, in 1000 ml distilled water. (Difco, 1984) For bacteria, nutrient agar was formed by employing meat extraction 3 gm, pepton 5 gm in 1000 ml distilled water.

3.2 Cultivation method

The media were putted in the petri dishes. Each bacteria or fungi was mixed with a broth liquid in an incubated test tube and putted in a shaker for 15 minutes. One ml of each bacterial isolation culture was spread onto nutrient agar dish. One ml of spore suspension of fungi was spread in Agar cellulose medium. They were incubated at 30 C for 3-7 days for cultivating bacteria and fungi.

3.3 Optical microscope OM

Identification of isolated fungi and bacteria was carried out using OM Siemens X-Vision KS 300 model according to the used scientific indexes for this purpose. (Cowan, S. T., and Steels, 1974), (Domsch, K.H., et al., 1980), (Gilman., J.C., 1974).

4. Evaluation of Nano-materials

A cork porer (1cm diameter) was used to make three pores in each petri dish (10cm diameter) containing the suitable medium for bacteria or fungi. Then each concentration of nanomaterial was placed in the three pores (as replicates). Dishes were incubated for fungi at 27 C for seven days and for bacteria at 37 C for three days.. Evaluation of the three concentration of each Nano-material for inhibition the growth of fungi and bacteria was determined by measuring the radius in mm of the inhibition clear zone around the pores according to the method of (Brantner, A.,et al, 1993).

RESULTS

1. Transmission electron microscope TEM

TEM images declared that the grain size of titanium dioxide NPs are less than 25 nanometers (nm), whereas each of copper II oxide NPs and silver NPs are less than 50 nm as given in Fig. 2. They show also that those particles are agglomerated mostly spherical in shape.

2. Scanning electron microscope SEM coupled with EDX unit

SEM micrographs show the small grain size of TiO_2 NPs whereas both of silver NPs and copper II oxide NPs have relatively larger grain size. EDX results show the purity of each nanomaterial as given in Fig. 3.

3. Optical microscope OM

Twenty one fungi and three bacteria species were identified. Three common fungi Asp. niger, Asp.flavus, Asp. fumigatus , and three bacteria Bacillus alvei, Gthe Bacilli spore former were selected for applying nano-materials as given in Figs 4 and 5.

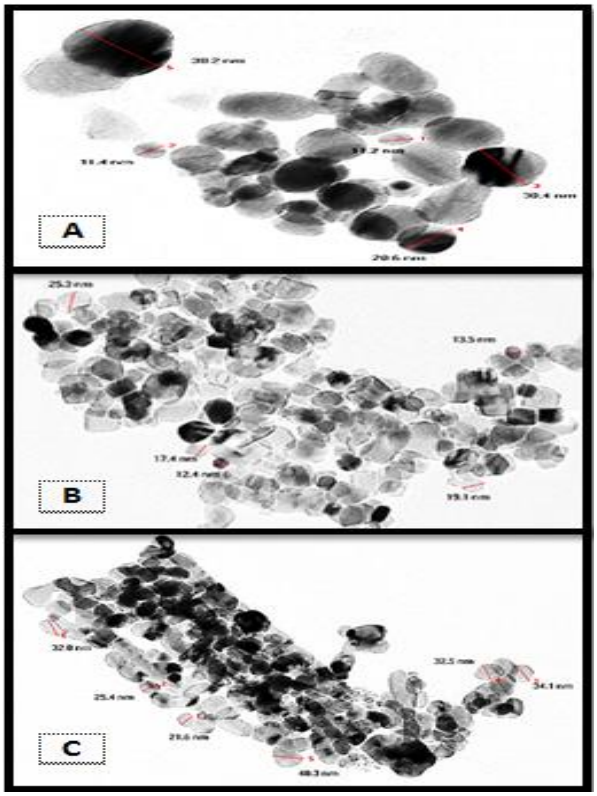

Fig. 2 TEM images of nanoparticles A) Ag , B) TiO_2, C) CuO

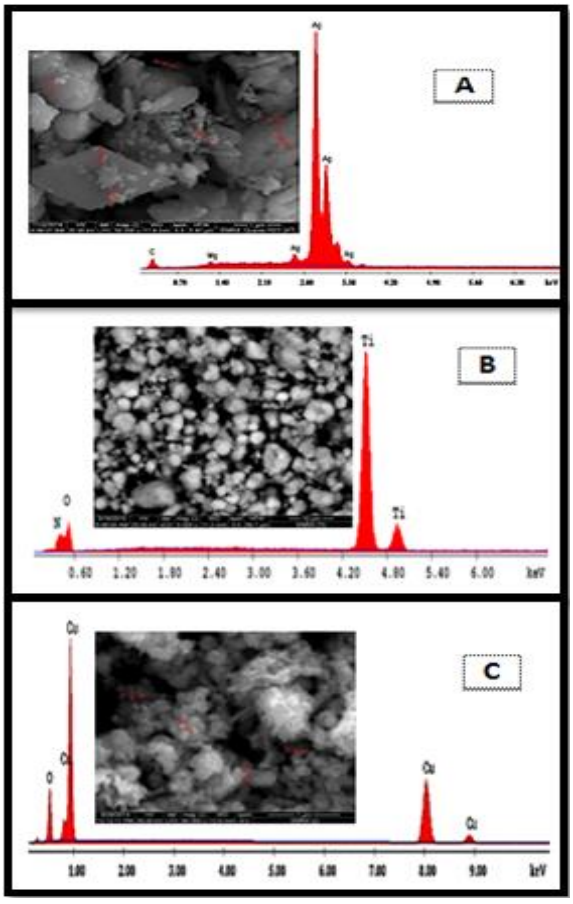

Fig. 3 SEM micrographs and EDX analysis of nano-materials A) Ag, B)TiO_2, C) CuO

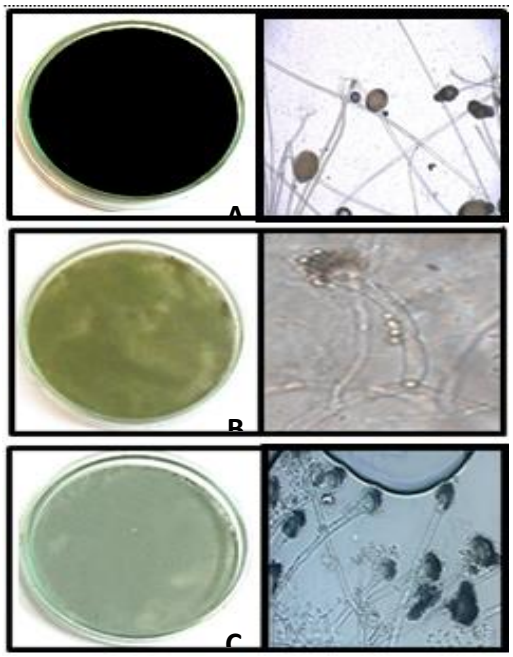

Fig.4 The Petri dishes containing Fungi after incubation and their optical microscope images A) Aspergillus niger, B) Aspergillus flavus, C) Aspergillus fumigatus.

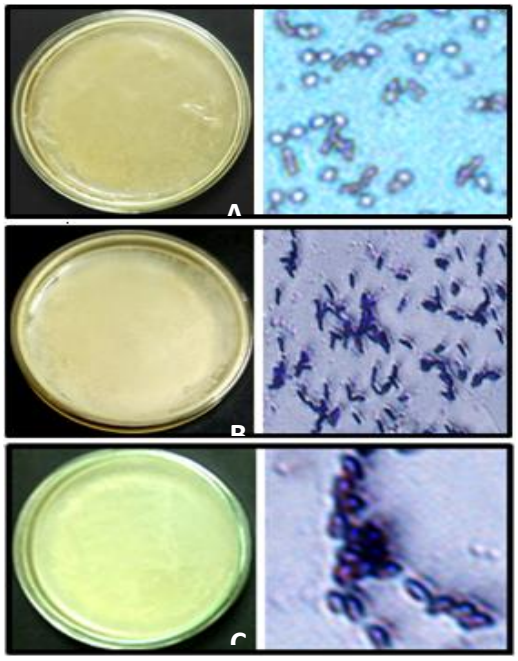

Fig.5 The Petri dishes containing bacteria after incubation and their optical microscope images A) Bacillus Alvei, B) Gthe Short Bacilli, C) Gthe Bacilli Spore Former.

4. Evaluation of Nano-materials

Data declare that silver NPs had mean value radius of inhibition clear zones for fungal strain Asp.niger, Asp. flavus, Asp. fumigatus (17, 19, 20 mm) respectively.For bacterial strain, Bacillus alvei, Gthe short Bacilli, Gthe Bacilli spore former (23.5, 25, 33 mm). Titanium dioxide NPs give inhibition zones for fungal strain(0, 0, 12.5 mm), and that for

bacterial strain (13, 12.3, 12 mm). Copper II oxide NPs give inhibition zones for fungal strain (20, 18.5, 20 mm) and for bacterial strain (18, 18.3, 26 mm). See Figs 6- 11.

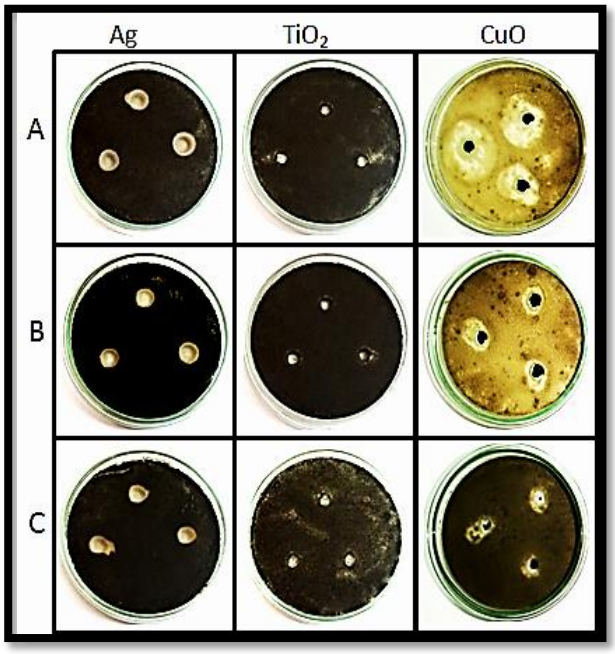

Fig. 6 The petri dishes showing inhibition zones of NPs against fungal strain of Asp. niger

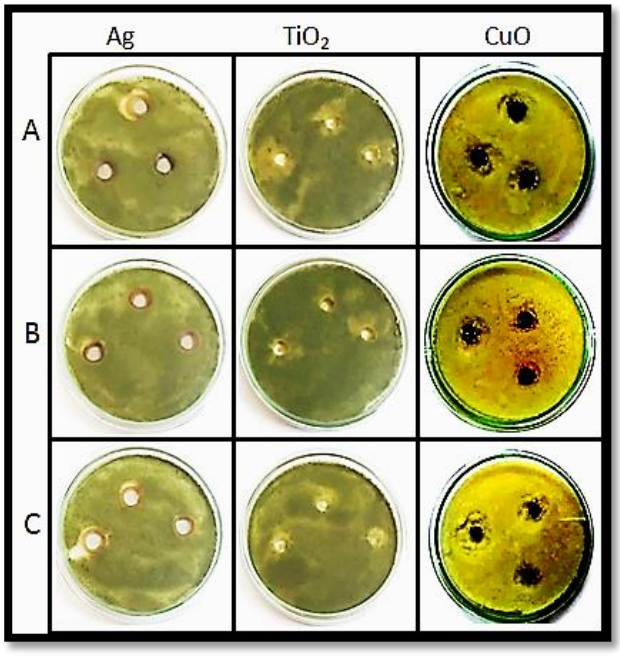

Fig. 7 The petri dishes showing inhibition zones of NPs against fungal strain of Asp. Flavus

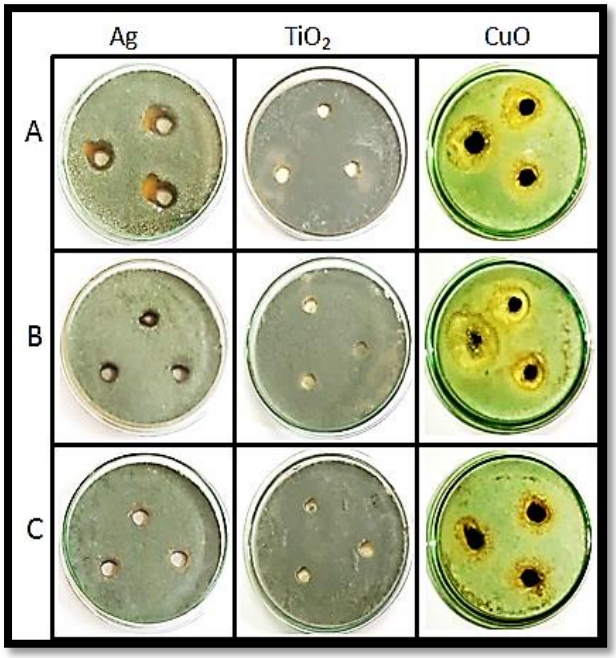

Fig. 8 The petri dishes showing inhibition zones of NPs against fungal strain of Asp. Fumigatus

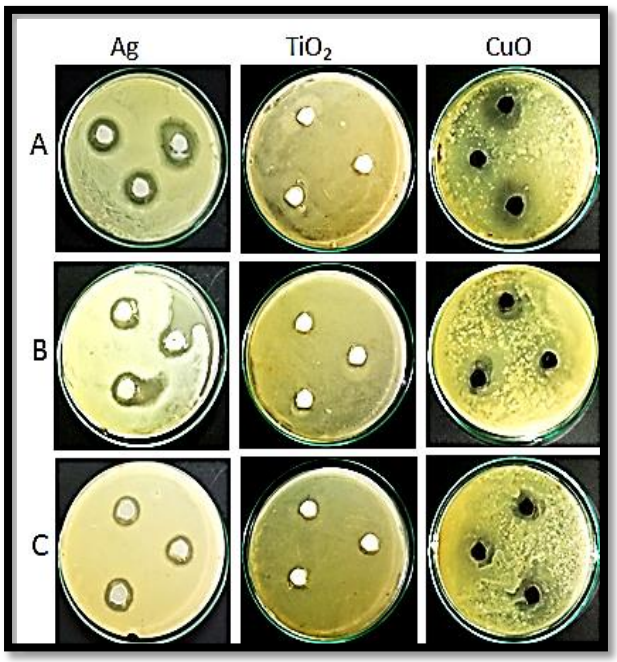

Fig. 9 The petri dishes showing inhibition zones of NPs against bacterial strain of Bacillus alvei

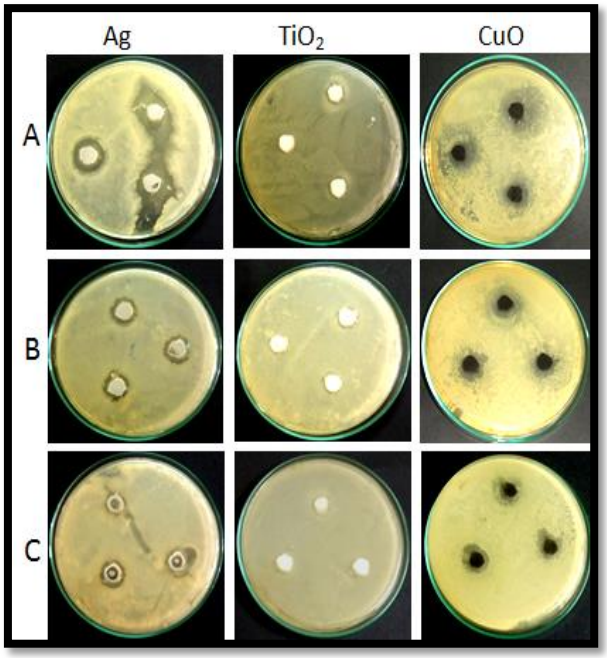

Fig. 10 The petri dishes showing inhibition zones of NPs against bacterial strain of Gthe Short Bacilli

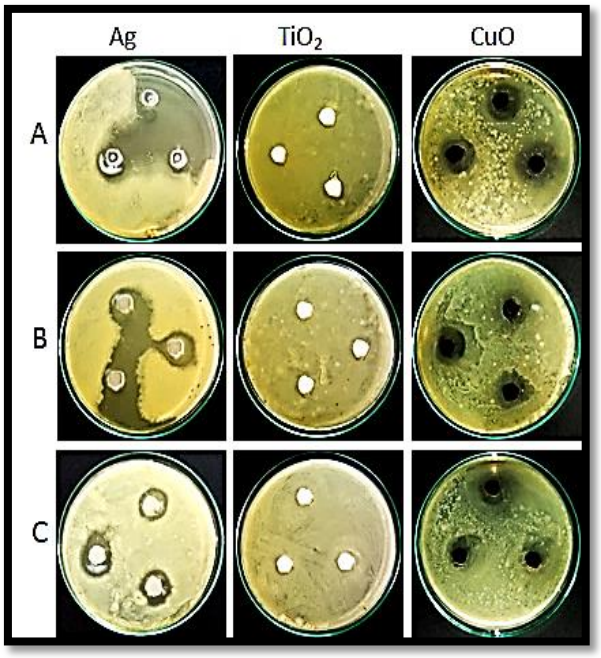

Fig. 11 The petri dishes showing inhibition zones of NPs against bacterial strain of Gthe Bacilli Spore Former.

DISCUSSION AND CONCLUSION

In the present study, three nano-materials silver, titanium dioxide and copper II oxide with three concentrations 5, 10, 15 µg /ml for each one, were evaluated for inhibition the growth of selected fungi and bacteria. Three fungal strain Asp.niger, Asp.flavus, Asp.fumigatus , and bacterial strain Bacillus alvei, Gthe Short Bacilli, Gthe Bacilli Spore Former are isolated from three ancient Egyptian funeral masks. It was found that the inhibition degree for both fungi and bacteria was increased with increasing concentration of each nanomaterial

Silver NPs gives good results for inhibition growth of isolated fungi . It exhibits activity against Asp.niger, Asp.flavus, Asp.fumigatus with mean value inhibition zones 17, 19, 20 mm respectively for 15 µg/ml concentration. It is found that Asp.niger is the more resistant strain of the three examined fungi. It may result from the activity of chemical components (such as ascorbic acid), which are produced by Asp.niger. This agrees with results carried out by (Pullit, J. et al., 2013), who stated that using nanosilver suspension at the concentration of 50 ppm inhibited the growth of cladosporium cladosporoides and Asp.niger by 90% and 70% respectively. Previous studies showed that the effectiveness of nanoparticles did not depend solely on its formulation, but also on the type of fungus (Saraniya Devi, J., et al, 2014), (Ing, L.Y., et al., 2012).

Silver NPs reactivity with bacteria was higher than that with fungi. Its inhibition zone mean values are 25.5, 25, 33 mm against bacterial strain Bacillus alvei, Gthe short Bacilli, Gthe Bacilli spore former respectively. This may be referred to the simplicity of bacterial cell and less thickness of cell wall. On the another hand, the enormous enzymatic activity of fungi, high melanization, and their thick cell wall (Sterflinger, K., 2010).

Titanium dioxide NPs gives nearly no inhibition zones against Asp. niger and Asp. flavus with concentrations 5, 10 µg/ml, and 12.5 mm with concentration 15 µg/ml. This assures the high resistance of Asp. niger against nanoparticles.TiO_2 NPs reactivity is also less than that of Ag NPs against Asp. Fumigatus. Moreover, it gives the lowest inhibition zones against bacterial strain Bacillus alvei, Gthe short Bacilli, Gthe Bacilli spore former 13, 12.3, 12 mm respectively.

Copper II oxide NPs shows high inhibition zones against fungal strains Asp.niger, Asp.flavus, Asp.fumigatus 20, 18.5, 20 mm respectively. Its reactivity against Asp.niger is higher than that of both silver NPs and titanium dioxide NPs. On the other hand CuO NPs shows medium inhibition zones against bacterial strain Bacillus alvei, Gthe short Bacilli, Gthe Bacilli spore former 18, 18.3, 26 mm respectively. Its reactivity is less than that of Ag NPs, but higher than that of TiO_2 NPs.

The mechanism of interaction of nanoparticles with fungi and bacteria includes diffusion into fungal or bacterial cells, hence disrupt the synthesis of DNA as well as RNA, causing a direct cell death (Ing, L.Y., et al., 2012), (Damm, c.,et. Al., 2008). SEM analysis confirms the interaction of silver NPs and the membrane structure of fungal species shows significant changes to their membranes led to formation

of pits in their surfaces and finally result in the formation of pores and cell death Narollahi, A. et al., 2011), (Martinez – gutierrez, F., 2010).

Titanium dioxide NPs has photocatalytic property which generate strong oxidizing power when illuminated to U.V. light wavelength of less than 38nm. Interaction of TiO_2 NPs with fungi and bacteria includes cell reaction with TiO_2- photocatalysis. Evidents are given, exhibit rapid cell inactivation, strong decrease of co-enzyme independent respiratory chains, lower ability to assimilate & transport Fe, P, and low capacity for biosynthesis (Kuback, A., et al., 2014).

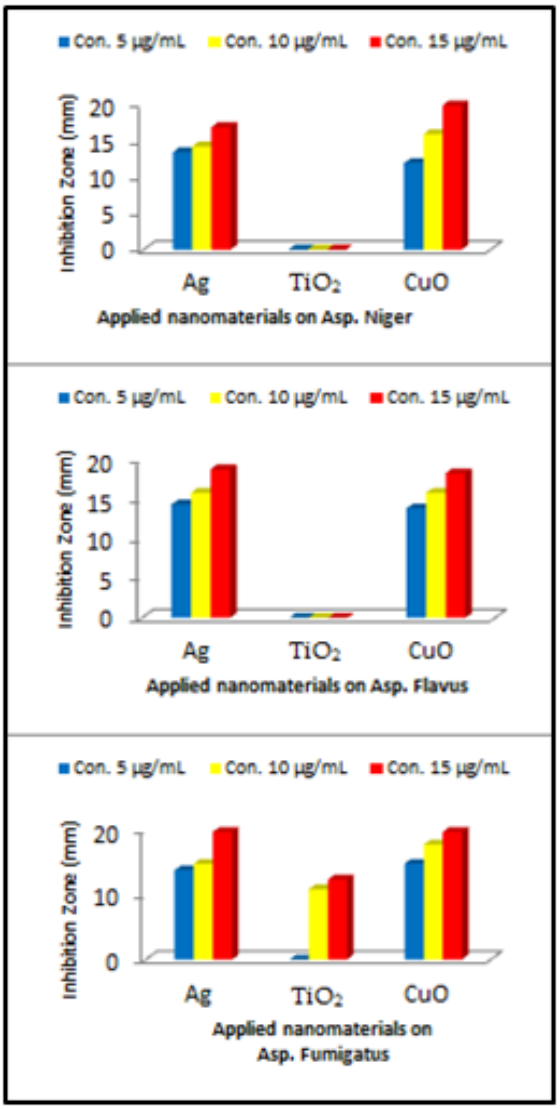

Fig. 12 Antimicrobial activity of NPs against fungal strain

Copper II oxide NPs adhered to the cell wall of bacteria and penetrated through the cell membrane. This resulted into inhibition of bacterial cell growth and multiplication which finally led to cell lysis (Kumar, H., 2014). Results also demonstrate that CuO NPs mediated dissipation of cell membrane potential which was the probable reason for the formation of cell filaments. It was found that CuO NPs cause multiple toxic effects such as generation of reactive species, lipid, peroxidation, protein oxidation and DNA degradation in bacterial cells (Kumar, A., et al., 2014).

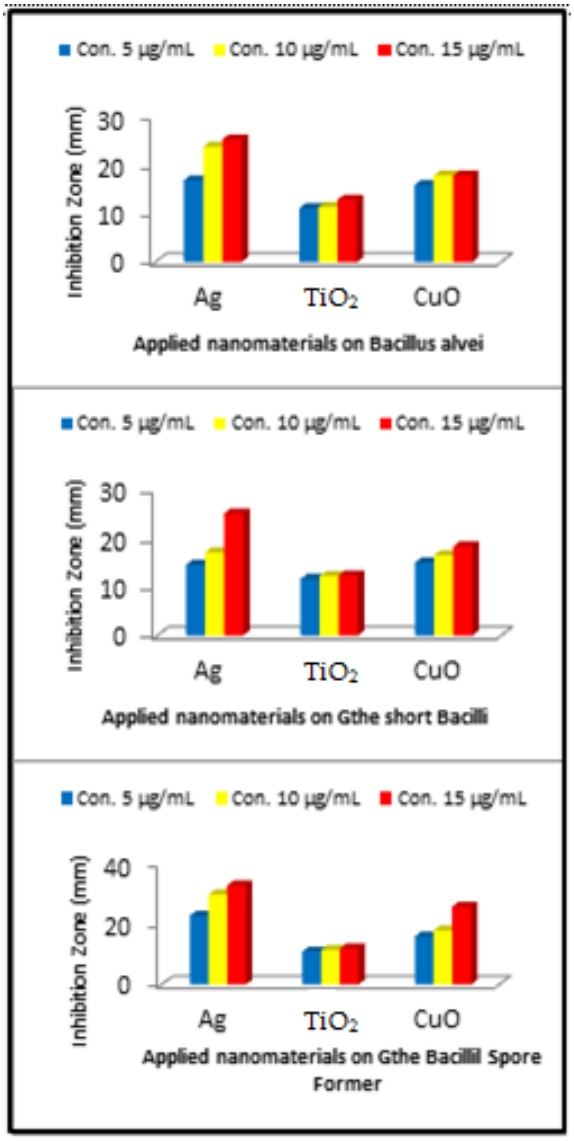

Fig. 13 Antimicrobial activity of NPs against bacterial strain

Silver NPs achieved better results than that of titanium dioxide NPs for inhibition the growth of the isolated fungi and bacteria. Inspite of good results of copper II oxide NPs it may be refused for application in the archaeological field due to the probability of changing colors of artifacts. The use of silver NPs for treatment and conservation of ancient Egyptian funeral masks is recommended. It will inhibit the growth of both fungi and bacteria safely without any effect on their colors. In the same time it excludes the disadvantages of using traditional chemical methods.

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