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# THE IMPACT OF NANOMATERS ON THE MICROBIAL INFECTION ON A WOODEN COFFIN COVERED WITH A LAYER OF BLACK RESIN AND COLOURED MATERIALS

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***Abstract***

*The goal of this work is to use novel mixed additives of Nano Chitosan combined with nanoparticles and other substances that could inhibit the microbial growth of the wooden coffin. The wooden coffin, covered with a layer of black resin and coloured materials, was found in bad condition, and covered with a thick layer of dust. Several ties were used to preserve the sides of the coffin in the previous restoration. Cellulose agar was used to cultivate fungi, and nutrient agar was used to cultivate bacteria. The fungi were Aspergillus flavus, Cladosporium herbarum, and Aspergillus niger, while the bacteria were G+ve Bacillus sp., Bacillus megatrium, and Bacillus jeotgali. composite of ZnO NP, Ag NP, p-chloro-m-crysol (PCMC), Shim plant (Sh) with Nano Chitosan with 1, 2, and 3% concentrations were chosen for the purpose of treating microbial infections and determining which materials would work best to prevent microbial growth. Nano-chitosan + Ag NP and ZnO NP (1%) gave the best inhibition for Aspergillus niger (30mm). Nano-chitosan + PCMC (1%) gave the best inhibition for Aspergillus flavus (25mm), Cladosporium herbarum (25mm), and Bacillus jeotgali (40mm). The same effect was found for Nano-chitosan + PCMC (1%) and Nano-chitosan for Bacillus megatrium and G+ve Bacillus sp. (40mm).*

***Keywords****: Wooden coffin; Black resin; Fungi; Bacteria; Nano materials; Ag NPs; ZnO NPs*

## Introduction

Wood is a natural material that can be attacked in service by several biological pathogens, such as wood-destroying fungi and molds [1, 2]. Biodeterioration takes place when living organisms change or alter the appearance of material objects chemically or physically [3]. Wood deterioration is a complex biological process involving a wide variety of microorganisms, such as fungi and bacteria. It is influenced by changing environmental conditions [4, 5]. The coffin, covered from the outside by a layer of black resin, was used for the first time in the New Kingdom for religious purposes to cover the funerary wooden artefacts [6 - 8]. Wood exposed to a wide variety of factors (including organic and inorganic pollutants, climatic factors, or microbial colonisation) can lose its aesthetic characteristics as well as functional and mechanical properties [9-11]. Molds can grow on the surfaces of wood, utilise the wood carbohydrates as a nutrient source, and have colourless or pale-coloured mycelium

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and coloured spores over the wood and wood products surfaces, causing discoloration by their pigmented spores and mycelia [12].

Chitosan derivatives are polysaccharides, which have similar molecular structures to cellulose, the main component of wood. They have similar chemical, physical, and mechanical properties and are thus compatible [13]. Nano-chitosan-ZnO hybrid coatings cause better inhibition of bacterial growth in comparison to chitosan surfaces alone [14]. Silver particles showed solid antibacterial and antifungal properties when utilized at the nanoscale [15, 16]. The Shim plant (sh) is used to make a plant extract that inhibits bacteria and fungi [17, 18]. Moreover, p-chloro-m-cresol (PCMC), also known as chlorocresol, is used as an external germicide and bactericide agent. It has both bactericidal and antifungal activity [19]. This paper tries to highlight the importance of using nano-chitosan with different materials to prevent the formation of microorganisms on a coffin covered with coloured materials and black resin.

## Materials and Methods

From various areas of the coffin, samples were collected (Fig. 1). Five samples were utilized: colour, wood, and a black resin layer. For the surrounding air of the coffin, two Petri dishes were used and left for 15 minutes in the storage environment to obtain accurate results for the organisms in the air.

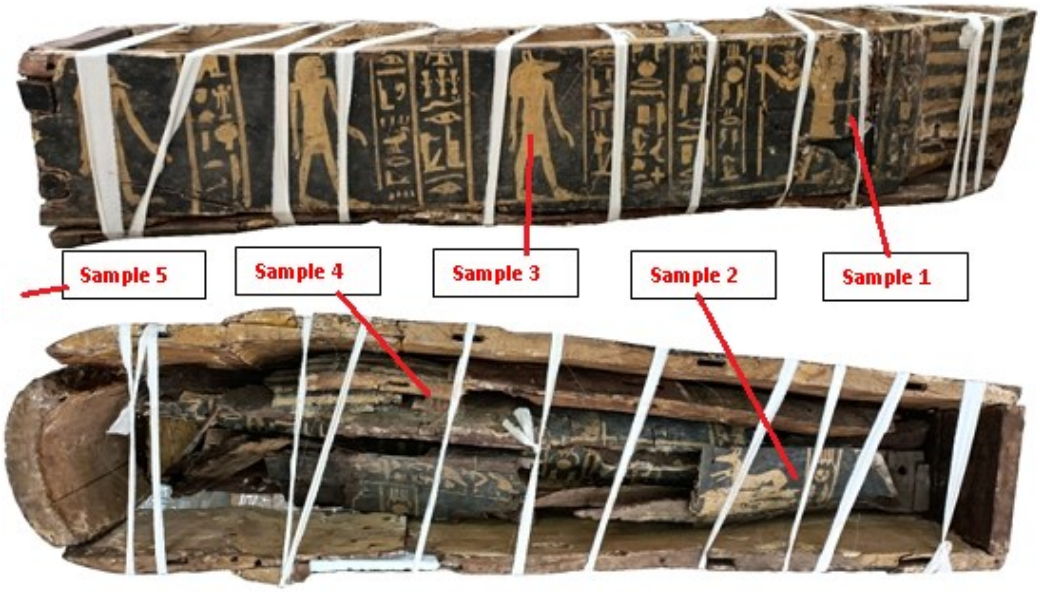


**Fig. 1.** Locations of microbial swabs: 1) Black resin base;2) Black resin lid;
3) Yellow colour; 4) Wood; 5) Surrounding air of the coffin

### *Cultivation of Swabs*

The swabs were cultured on the microbial growth media (nutrient agar and cellulose agar) and incubated at 30–28 °C for 3–21 days. The media was then produced. After that, the microbial growth was investigated to wrap up the lab tests.

### *Preparation of Nanocomposites*

To determine their MIC and examine their effect on the contained microorganisms, five microcides were used (Table 1). Nano-chitosan + Ag Np, Nano-chitosan + ZnO Np, Nano-

chitosan + p-chloro-m-cresol (PCMC), Nano-chitosan + a plant concentration of Shim plant (Sh), and Nano-chitosan, Nano-chitosan helps to improve the properties of other materials.

**Table 1.** Materials for microbiological infection resistance experiments

| No | Treatment |
|---|---|
| 1 | Ag Np + Nano Chitosan 1% |
| 2 | Ag Np + Nano Chitosan 2% |
| 3 | Ag Np + Nano Chitosan 3% |
| 4 | ZnO Np +Nano Chitosan 1% |
| 5 | ZnO Np +Nano Chitosan 2% |
| 6 | ZnO Np +Nano Chitosan 3% |
| 7 | p-chloro-m-crysol (PCMC) + Nano Chitosan 1% |
| 8 | p-chloro-m-crysol (PCMC) + Nano Chitosan 2% |
| 9 | p-chloro-m-crysol (PCMC) + Nano Chitosan 3% |
| 10 | Shim plant (Sh)+ Nano Chitosan 1% |
| 11 | Shim plant (Sh)+ Nano Chitosan 2% |
| 12 | Shim plant (Sh)+ Nano Chitosan 3% |
| 13 | Nano Chitosan |

A 2g chitosan powder was dissolved in 2% 100mL (v/v) of glacial acetic acid to make the chitosan solution, which was then used to create nano-chitosan nanoparticles [20]. An ionic crosslinker called sodium tripolyphosphate (TPP) (1%w/v) was employed. By expanding 1 millilitre of TPP to 10 millilitres of chitosan solution and sonicating the mixture for an hour at room temperature with 350 watts of power, chitosan nanoparticles were produced. The chitosan nanocomposite solution was made by combining 0.09g of ceratophyllum demersum, 0.06g of Ag, 0.03g of NPs ZnO, and p-chloro-m-cresol separately with 2% acetic acid. For roughly ten minutes, each solution was combined. Then, 2.0g of chitosan nanoparticle powder was added and blended enthusiastically. The mixture was sonicated for 15 minutes at 350-watt sonication dosages with a recurrence of 2MHz (focal recurrence of 0.7MHz and transmission capacity of 1.4MHz). The accuracy of the estimations was ±10m/s using an oscilloscope (60MHz time-base oscilloscope, Philips, Eindhoven, Netherlands).

Nanoparticles have sizes ranging from 1 to 100nm [21]. Using nanomaterials with pesticides can penetrate deeper into the wood and improve the durability of treated woods against biodegradation agents [22].

***Morphological Analysis of the Prepared Nanocomposites***

The morphological examinations of the prepared nanocomposites were performed using Transmission Electron Microscopy (TEM), and the images were acquired using a JEM-1230 electron magnifying instrument at 60kV (JEOL Ltd., Tokyo, Japan). Before taking a TEM image, the sample was diluted by water multiple times. A drop of well scattered weakened sample was placed onto a copper gird (200 mesh and coated with a carbon layer) and dried at room temperature [23] for the morphological characterization (size, shape, and structure) of zinc oxide nanoparticles [20]. The TEM images Figure 2A and B show the prepared nanomaterials, where the accumulation of Ag to ZnO NPs increased on the surface of chitosan [24, 25].

On one nutritional agar plate, one millilitre of the bacterial suspension was disseminated, and on a PDA plate, one millilitre of the infectious spore suspension was spread. It was decided to let the plates dry. Next, three pores were created in each plate using a stopper cleaner. 100µL of each combination of the tested microcides was added to a single plate's pore. In contrast to control plates (nano-chitosan), plates were hatched at 30°C for one to three days. Using A.H. Brantner's approach [26], the inhabition zone was measured in order to determine the MIC [27]. The Carl Zeiss Axio Vision request Ks300/4000 for light microscopy Delivery: 4.72 with the computerized camera Cam MRc5 and inspection unit used to identify the bacteria.

## Results and Discussion

These isolates were molecularly characterized by PCR using the 16S rRNA region as the basis [28- 30], showed the *Aspergillus flavus* in the black resin base sample and *Bacillus jeotgali* in the black resin lid sample. The yellow colour materials were *Bacillus megatrium* and *Cladosporium herbarum*, whereas the air samples were *Bacillus megatrium, G+ve Bacillus sp, Aspergillus flavus, and Cladosporium herbarum* (Table 2 and figures 2 and 3).

**Table 2.** Identification results of microbiological isolates

| | Swab number | Media used | |
|---|---|---|---|
| | | Nutrient agar | Cellulose agar |
| **1** | Black resin base | *-ve* | *Aspergillus flavus* |
| **2** | Black resin lid | *Bacillus jeotgali* | *-ve* |
| **3** | Yellow color | *Bacillus megatrium* | *Cladosporium herbarum* |
| 4 | Wood | *Bacillus jeotgali, G+ve Bacillus sp.* | *Asp. niger* |
| 5 | Air | *Bacillus megatrium*, *G+ve Bacillus sp.* | *Aspergillus flavus, Cladosporium herbarum* |

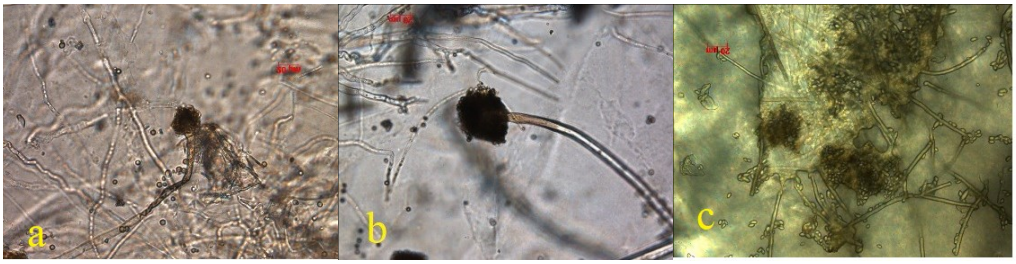


**Fig. 2**. Isolated fungi from the wooden coffin: *a) Asp flavus; b) Asp niger; c) Clado herbarum*

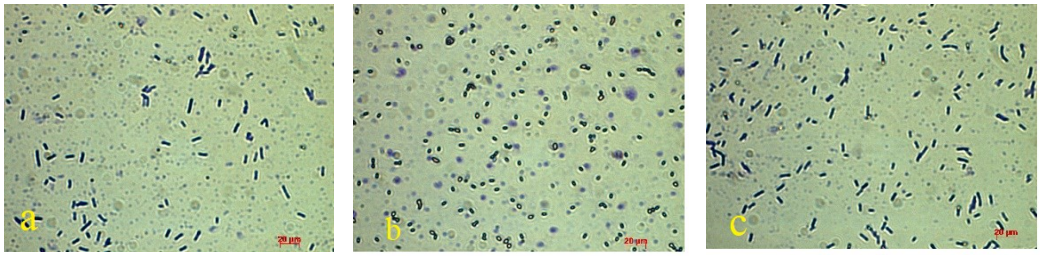


**Fig. 3.** Isolated bacteria from the wooden coffin: a*) Bacillus megatrium; b) Bacillus jeotgali; c) G+ve Bacillus sp.*

There was only one kind of fungus present in the black resin base sample, *Aspergillus flavus*, which was also present in the coffin's surrounding air. There were no bacterial growths present in this sample. The black resin lid sample contained only one species of bacteria, *Bacillus jeotgali*, and no fungi (Fig. 4b), which was also found on the wood sample. In contrast, the black resin material had antifungal and bacterial substances [31, 32].

The microorganism found on the black resin layer may be the result of the same species found on the wood sample and the surrounding air of the coffin, the thick layer of dust found on the coffin, the previous restoration material used in the coffin, or the presence of these fungi and bacteria. The sample of the yellow colour had *Bacillus megatrium* bacteria and *Cladosporium herbarum* that was also found in the surrounding air of the coffin. The wood *Bacillus jeotgali, G+ve Bacillus sp. And Asp. Niger* may be because of the dust.

Dust is hygroscopic and a site for mold growth [33]. Also, insects feed on dust. Humidity 50–55%RH, keeps daily variation at ±5%. Humidity over 65–70% supports mold growth. Additionally, the types of building materials within the indoor environment play a significant influence on the kinds of microorganisms and their growth potential [34].

On the chitosan surface, nanoparticles (NPs) were uniformly distributed and did not clump together (Fig. 2a and b). The NPs were addressed by the dark regions, and the chitosan surface was addressed by the bright portions. Round forms were seen in the pre-arranged nanocomposites, with molecule sizes ranging from 23 nm for Ag NPs to 34 nm for ZnO NPs (Fig. 4).

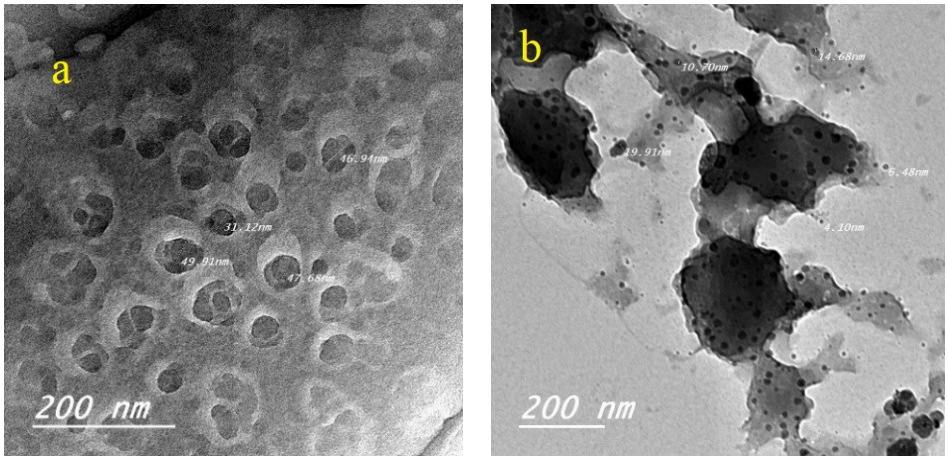


**Fig. 4.** TEM images of the nanomaterials that have been prepared:
(a) Chitosan plus Ag NP; (b)Chitosan plus ZnO NP

Nano-chitosan is an antifungal substance [35] with ZnO for the preservation of wood surfaces applied by spraying on the wood surfaces pre-treated with a suspension of ZnO in isopropyl alcohol [36]. Ag NPs represent a broad-spectrum antimicrobial activity resulting from the attack on multiple microbial cellular processes: Ag NPs increase oxidative stress via ROS formation, interfere with nutrient transport processes in the cytoplasmatic membranes, and disrupt metabolic processes. This activity is boosted by the release of Ag+ ions, which impede DNA replication and inhibit enzymes and peptides that eventually lead to the microorganism's death [37]. ZnO nanoparticles cause the degradation of the cell wall and the plasmatic membrane of the bacteria [23]. and the disruption of DNA replication [38].

Figures 5-9 show the effect of nano-chitosan microcode mixed with the antifungal additives studied.

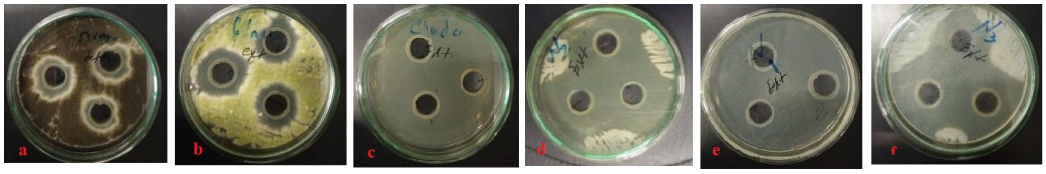


**Fig. 5.** The effect of microcode nano-**c**hitosan + Sh on the fungi growth: a*) Asp.niger; b) Asp. flavus; c) Cladosporium herbarum; d) Bacillus megatrium; e) Bacillus jeotgali; f) G+ve Bacillus sp.*

**Fig. 6.** The effect of microcode nano-chitosan + PCMC to the fungi growth: a*) Asp.niger; b) Asp. flavus; c) Cladosporium herbarum; d) Bacillus megatrium; e) Bacillus jeotgali; f) G+ve Bacillus sp.*

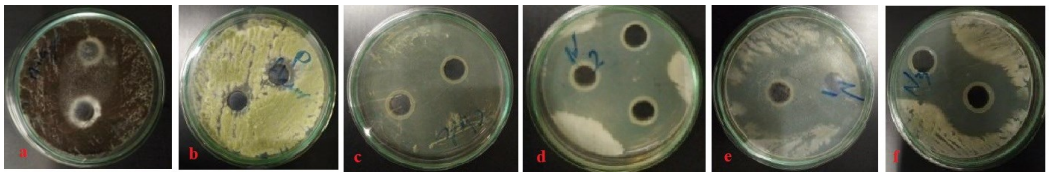

**Fig. 7.** The effect of microcode nano-chitosan to the fungi growth: a) *Asp.niger; b) Asp. flavus; c) Cladosporium herbarum; d) Bacillus megatrium; e) Bacillus jeotgali; f) G+ve Bacillus sp.*

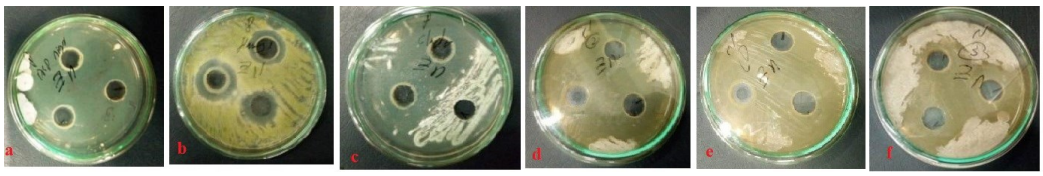

**Fig. 8.** The effect of microcode nano-chitosan + ZnO Np to the fungi growth: *a) Asp.niger b) Asp. flavus; c) Cladosporium herbarum; d) Bacillus megatrium; e) Bacillus jeotgali; f) G+ve Bacillus sp.*

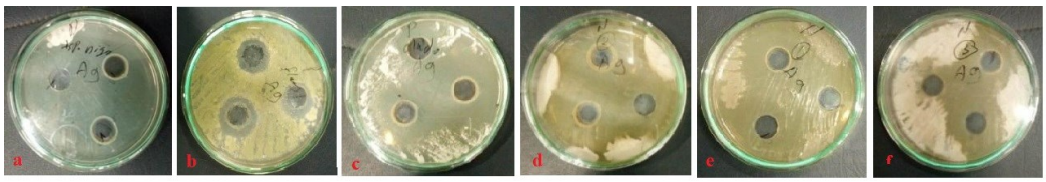

**Fig. 9.** The effect of microcode nano-chitosan + Ag Np to the fungi growth: a*) Asp.niger b) Asp. flavus; c) Cladosporium herbarum; d) Bacillus megatrium; e) Bacillus jeotgali; f) G+ve Bacillus sp.*

A subset of the test materials demonstrated strong antibacterial and antifungal activity against the microbial strains under consideration, as indicated by the MIC values presented in Table 3.

**Table 3.** Comparing the efficacy of different biocide-containing nanoparticles against wood decay fungi

| | Diameter of clearing zone (mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nano-chitosan + PCMC (mL) | | | Nano-chitosan + Sh (mL) | | | Nano-chitosan (mL) | Nano-chitosan + Ag Np (mL) | | | Nano-chitosan + ZnO Np (mL) | | |
| **Name of Microorganism** | **1 m/L** | **2 m/L** | **3 m/L** | **1 m/L** | **2 m/L** | **3 m/L** | **2 m/L** | **1 m/L** | **2 m/L** | **3 m/L** | **1 m/L** | **2 m/L** | **3 m/L** |
| ***Asp. niger*** | 21 | 26 | 31 | 19 | 23 | 25 | 21 | 30 | 40 | 50 | 30 | 40 | 50 |
| ***Asp. flavus*** | 25 | 29 | 32 | 20 | 24 | 26 | 19 | 20 | 23 | 27 | 22 | 24 | 30 |
| ***Cladosporium herbarum*** | 43 | 46 | 50 | 40 | 49 | 50 | 35 | 20 | 30 | 35 | 0 | 30 | 35 |
| ***Bacillus megatrium*** | 40 | 45 | 50 | 38 | 42 | 45 | 40 | 30 | 35 | 40 | 35 | 40 | 45 |
| ***Bacillus jeotgali*** | 40 | 46 | 50 | 37 | 44 | 50 | 30 | 25 | 28 | 33 | 30 | 40 | 50 |
| ***G+ve Bacillus sp.*** | 40 | 43 | 50 | 35 | 40 | 45 | 40 | 0 | 25 | 35 | 28 | 35 | 40 |

Depending on the treatment components, the fungi had varying impacts. For instance, the items utilized to treat the microbial infestation had an impact on *Asp. niger* (Fig. 5a). The results indicated that nano-chitosan + ZnO Np and nano-chitosan + Ag Np (30mm) had the greatest effect at 1%, followed by nano-chitosan and nano-chitosan + PCMC (21mm). The combination of nano-chitosan + PCMC (25mm), nano-chitosan + ZnO Np (22mm), nano-chitosan + Sh, and nano-chitosan + Ag Np (20mm) had the greatest impact on the *Asp. Flavus* treated materials. The effects of nano-chitosan + PCMC (43mm) were greater than those of nano-chitosan + Sh (40mm), and then nano-chitosan (35mm) on *Cladosporium herbarum*. ZnO Np + Nano-chitosan showed no impact (Table 3).

While bacteria had different effects on treatment materials. *Bacillus megatrium* had an equal effect on nano-chitosan + PCMC and nano-chitosan 40mm followed by nano-chitosan + Sh (38mm) and then nano-chitosan + ZnO Np (35mm). The least effect was for nano-chitosan + Ag Np 30mm. *Bacillus jeotgali* had a greater effect on nano-chitosan + PCMC (40mm) followed by nano-chitosan + Sh 37mm and the effect of nano-chitosan and nano-chitosan + ZnO Np (30mm). It was followed by nano-chitosan + Ag Np (25mm). G+ve *Bacillus sp.* had an effect on nano-chitosan + PCMC and nano-chitosan (40mm). The effect of nano-chitosan + Sh 35mm was followed by nano-chitosan + ZnO Np (28mm). nano-chitosan + Ag Np had no effect on the G+ve *Bacillus sp*. (Fig. 10).

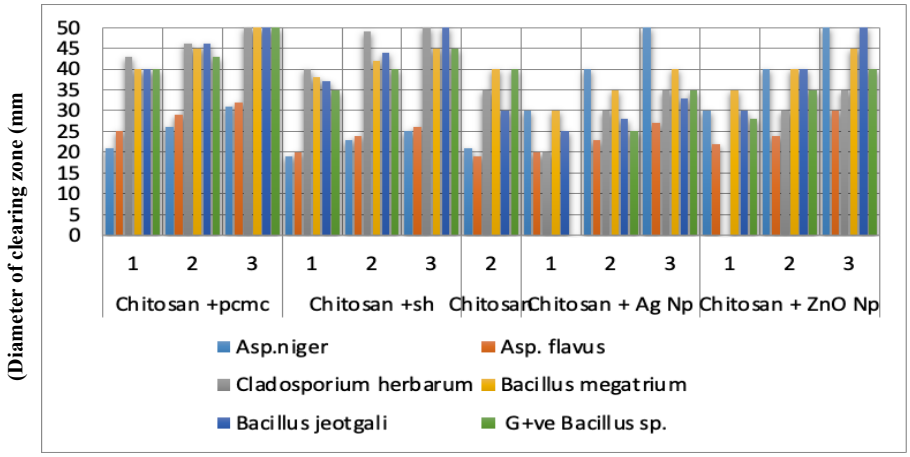


**Fig. 10.** A brief statistical analysis of the materials' efficacy in treating the coffin's microbial infection

## Conclusions

The main goal of the current study was to determine the antimicrobial properties of some materials tested on the isolated fungi and bacteria from the wooden coffin covered with the black resin layer. The fungi were *Aspergillus flavus, Cladosporium herbarum,* and *Aspergillus niger,* while the bacteria were *Bacillus megatrium, G+ve Bacillus sp.* and *Bacillus jeotgali*. These microcides were nano-chitosan + ZnO Np, Ag Np, a plant extract of Shim plant (Sh), and p-chloro-m-cresol (PCMC). They were used at a concentration of 1 - 3%. As per the MIC for the tested materials, the lowest concentration of 1% from nano-chitosan + ZnO Np showed the greatest suppression (30mm) of *Asp. niger mycelia* growth. With nano-chitosan + PCMC, the greatest inhibition of Asp. Flavus (25mm) was seen. The most effective material for *Cladosporium herbarum* was nano-chitosan + PCMC (43mm), and the most effective material for *Bacillus megatrium* was nano-chitosan + PCMC and nano-chitosan (40mm). Additionally, the most effective material for *Bacillus jeotgali* was nano-chitosan + PCMC (40mm), while the

bacteria *G+ve Bacillus sp*. had the same effect with nano-chitosan + PCMC and nano-chitosan (40mm).

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