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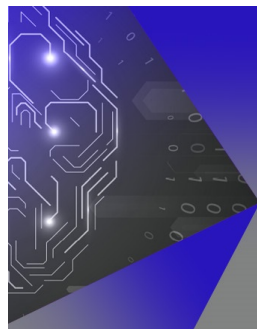
Ban Taha Mohammed and Alaa Aqeel Jasim



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# Evaluation of Nano - Silver and Zinc Particles in Fungi Accompanying Historical Manuscripts at the Hussein Shrine in Karbala

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**Abstract.** This study was conducted in the postgraduate laboratory at the College of Education for Pure Sciences at the University of Kerbala in collaboration with Center for Manuscript Preservation , Restoration and Care of Researchers at the Imam Hussain Holy Shrine in Karbala, for the period from 20-11-2018 to 20-1-2019. In order to preserving manuscripts within the series of isolation and diagnosis of fungi accompanying ancient manuscripts. Controlling fungi that cause damage to the manuscripts using silver and zinc nanoparticles of different concentrations 0,5,10,15, 20 and 25 mg/L to inhibit the growth of *Alternaria atra* , *Aspergillus ustus*, *Cladosporium exasperatum*, *Chaetomium globosum* *Microdochium nivale* , *Penicillium tardochrysogenum -1*, *Penicillium tardochrysogenum -2*. The fungi were registered in pervious study in the International Genbank and had accession numbers : MK503427 و MK503428, MK504425, MK504424, MK503439, MK504426, MK504427 respectively [1]. The results were obtained as the diameter of colony on the PDA medium after 14 days of incubation as well as studying the shape of the fungal colonies on the culture media as well as prepare microscopic slides. The manuscripts damaged by fungi were treated with these two nanomaterials in the dough prepared for the purpose of restoring and preserving the manuscripts. Silver and zinc nanoparticles, showed a significant effect on the mean of colony diameter and the growth morphology that tends towards to decrease colony diameter with increase in the concentration of the nanoparticles . The *Alternaria atra* and *Penicillium tardochrysogenum-1*, gave the highest mean value of inhibition represented by the average fungal colony diameter of 10 mm when treated with Nano zinc at a concentration of 25 mg / L from the PDA culture medium , which were not significantly different from both *Microdochium nivale* and *Penicillium tardochrysogenum- 1*. The *Alternaria atra* and *Penicillium tardochrysogenum -2* showed the highest inhibition value represented by the average fungal colony diameter of 13.33 mm when treated with silver nanoparticles at a concentration of 25 mg / L on the PDA culture medium. The microscopic examinations of the fungal hypha showed that it was affected by the nanoparticles with an increase in the concentration, and this effect ranged between the complete destruction of the fungal hypha and its explosion, especially in the high concentrations, and the aggregation of the protoplasm of the fungal cell, especially in the medium concentrations of the nanoparticles , or a deviation in the course of the fungal flow and its attempt avoidance of the toxic substance by agglomeration in a specific place in the microscopic space under examination, especially in low concentrations, as well as the reduction of the conidia, its small size or its deformation depending on the type of fungus, the concentration of the nanomaterial and its type. The culture tests of samples taken by swabs from ancient manuscripts after being treated with the treated material in the presence of a high concentration of 25 mg /L of paste prepared for the purpose of restoration were for each of the nano silver and zinc nanoparticles separately by reducing the number of fungi compared to the manuscript prior to restoration.

**Keyword:** Nano – Silver, Zinc Particles, Fungi

## INTRODUCTION

The fungi that cause damage to the manuscripts have met the interest of researchers, and many fungi have been isolated, the most frequent of them were *Aspergillus flavus*, *A. fumigatus*, and *A. niger* had the highest rate of occurrence compared to other fungi, and had high enzymatic efficacy in analyzing the components of the manuscripts [2]. Also, in another study at the University of Al-Qadisiyah in Iraq, fungi were isolated from the university's libraries, and the most dominant fungi was the *Aspergillus* species[3] .

The ability of Nano silver to interact with sulfur and phosphorous prevents it from reaching the DNA necessary for the vital processes of the cell [4]. The study presented by Mehdi and his group [5] demonstrated that effect of silver nanoparticles on the fungal hypha of the *Sclerotinia sclerotiorum*, showed an effect represented by deformations of the fungal hypha, the separation of the plasma membrane from the cell wall, the occurrence of a collection of protoplasm and the shrinkage of the fungal cell, as well as the deviation of the flow of nutrients of the

fungus and the acceleration. In the formation of sclerotia primordia . Nano zinc showed a change in the form *Fusarium graminearum* hypha , where they appeared thinner and tended to clump together, in which the wall was shattered, more gaps and cytoplasmic fluidity appeared, and there is an inhibition of fungal growth compared to normal zinc oxide, although both of them liberated the same levels of soluble zinc, which confirms that the toxicity of zinc depends on the particle size, the smaller it is, the more effective and inhibitory, which is represented by nanostructured zinc oxide [6]. He and his group [7] suggested that zinc oxide nanocomposites can be used as a fungicide for plant pathogenic fungi. They studied its effect on the *Botrytis cinerea* and *Penicillium expansum* found that the concentration of 3 mol / cm was effective in inhibiting the fungi, and the particle size 15-70. nm, inhibiting the growth of *Botrytis cinerea* by affecting cellular functions and deforming the mycelium and *Penicillium expansum* due to lack of development and deformed carriers of conidia, causing the death of fungal hypha . In another study, the effect of zinc and silver nanoparticles on some skin fungi(*Trichophyton rubrum* and *Microsporum canis* ) was shown that the treated with the combination between mineral zinc or nano zinc with fluconazole antifungal to reduce the pathogenic fungal growth and deformation of conidia formation [8] .

## MATERIAL AND METHODS

**Fungi used in the study:** Seven fungal isolates used in this study namely : *Alternaria atra* , *Aspergillus ustus*, *Cladosporium exasperatum*, *Chaetomium globosum* *Microdochium nivale* , *Penicillium tardochrysogenum* -1, *Penicillium tardochrysogenum* -2. The fungi registered in a previous study in the International Genbank were obtained by Prof. Ban Taha Mohammad from the Postgraduate Laboratory at the College of Education for Pure Sciences at the University of Kerbala . All isolates were diagnosed and recorded in Global GenBank under serial numbers MK503427 , MK503428 , MK504425 , MK504424 , MK503439 , MK504426 , MK504427 respectively in the previous study [1] . Isolates were activated and cultured on PDA medium, and their phenotypic and microbiological properties were studied before and after subsequent treatments .

**Potato dextrose agar (PDA):** Prepare the (PDA) medium by dissolving 39g of the medium powder in 1000ml of distilled water, according to the manufacturer's instructions.

### Specifications of nanomaterials:

**Nanoparticles (Ag) :** Appearance: Black powder, Stock: A110, particles size: 20nm, purity:%99.99 , morphology: spherical (c6h9oN)n Surfactant, Product Origin: China, HONGWUEWMA TERIAL, [hwnano@xuzhounano.com](mailto:hwnano@xuzhounano.com)

**Nanoparticles (Zn) :** Appearance: Grey powder, Stock: NS630-01-194 CAS:7440-66-6, particles size: (metal Basis), purity: 99.99%, BET specific surface area: / g /30-50 m<sup>2</sup>, Product Origin: USA, NANOSHET, [WWW.nanoshel.com,sales@nanoshel.com](http://WWW.nanoshel.com,sales@nanoshel.com).

**Preparation of silver and nano-zinc concentrations:** After carrying out a number of preliminary experiments in preparing concentrations of nanomaterials, the method of adding the solvent DMSO Dimethyl sulfoxide ( CH<sub>3</sub>)<sub>2</sub>SO was chosen to the PDA culture as followings :-

1. Add 10 ml of dimethyl sulfoxide (DMSO) to 2.5 mg of nanoparticles and placed on the thermal heater and magnetic stirrer for 120 minutes. The stock solution was considered as nanoparticles. The control was prepared without adding the nanoparticles
2. Different concentrations of silver and zinc nanoparticles were prepared from stock solution: 5, 10, 15, 20 and 25 mg / L as well as a control treatment.
3. Before the medium solidified, it was poured into Petri dishes for the purpose of studying the effect of nanoparticles.

**Effect of Zinc and Silver Nanoparticles on Isolated Fungi:** Potato Dextrose Agar medium was prepared, substitution of distilled water with the specific concentration of nanomaterials (silver and zinc nanoparticles separately) (MIC) in addition to a treatment without nanomaterials (PDA) as a control treatment poured into plastic petri dishes. The plates were inoculated with 5 mm disc of the pure colony growing at the age of 3 days at a temperature of 26 ± 2 ° C with three replications. After complete growth the plates were incubated with control. All cultures were examined and the following traits were recorded:-

1- The effect on the average diameter of fungal colonies (percentage of inhibition): -

The inhibition percentage was calculated according to the formula provided in Abbott, (1925), That add in [1]as:  
Inhibition ratio% =  $\frac{\text{Fungal growth rate at control} - \text{Fungal growth rate per treatment}}{\text{Fungal growth rate in control}} \times 100$

2- Study the phenotypic shape of the developing colonies with the presence of the nanomaterial compared with the control

**Test of inhibitory concentration of nanomaterial with old material in manuscript restoration:** After knowing the concentration in which the fungi were inactivated, a substance used in manuscript restoration was prepared from methyl hydroxyethyl cellulose MH6000, the trade name Tylose, was prepared at 1%. Parchment paper was coated with this prepared material. After 3 days, a swab was taken from the manuscript using sterile cotton swabs and then cultivated in the PDA recorded data were either depending on the appearance and growth of the fungus or not.

**Statistical analysis** Completely Randomized Design (C.R.D) was used. GenStat was used to analyze the data and compare the averages with the least significant difference (L.S.D.) at 0.05p level.

## RESULTS AND DISCUSSION

### Effect of Zinc and Silver Nanoparticles on the Average Diameter of Fungal Colonies (Percentage of Inhibition)

Table 1, indicates that there are significant differences in the amount of fungi growth, as the fungus was most inhibited by nanostructured zinc was the fungus *Alternaria atra*, with the mean of 17.56, while the least effect of nano zinc was the fungus *Cladosporium exasperatum* with a rate of 45.84. The concentration 25 mg / L was more effective on fungi as it reached 18.90 and the concentration 5 mg / L was the least conc. able to inhibit fungi, reaching 34.05 compared to the control treatment. As for the relationship between fungi and concentrations, the concentration 25 mg / L was more inhibitory for the fungus *Penicillium tardochrysogenum-2* by 10.00, while the concentration of 5 mg / L was less effective on the *Aspergillus ustus* by 56.67. This was in agreement with previous studies of nanocomposites that play a role in disrupting transport systems and this was reflected by the cellular representation, respiration and interaction between organelles in addition to that zinc and nano silver ions are known to produce free radicals that destroy proteins, fats and nucleic acids [8, 9], and these results were consistent with findings by Mehdi and his group[11], when they used different nanoparticles including zinc oxide at different concentrations that affected the growth of the fungus *Sclerotinia sclerotiorum* (as the pathogen of the plant).

**TABLE 1.** The different concentrations of nanoparticles zinc in the average diameter of the fungi isolated from the manuscripts (percentage of inhibition(mm)) growing on PDF medium at a temperature of  $26 \pm 2$  ° C for 5-7 days.

No.	Fungi	(Nano Zinc Concentration (mg / L))*						Average of Fungi diameter (mm)
		Control	5	10	15	20	25	
1	<i>Alternaria atra</i>	36.67	23.67	11.00	11.67	12.33	10.00	17.56
2	<i>Aspergillus ustus</i>	55.00	56.67	31.67	53.33	19.67	23.33	39.94
3	<i>Chaetomium globosum</i>	43.33	41.67	38.33	32.33	36.67	20.00	35.39
4	<i>Cladosporium exasperatum</i>	55.00	45.00	41.67	50.00	38.33	45.00	45.83
5	<i>Microdochium nivale</i>	30.00	21.67	11.67	19.33	15.67	11.67	18.33
6	<i>Penicilliu tardochrysogenum 1</i>	25.00	26.33	15.00	21.67	12.00	10.00	18.33
7	<i>Penicillium tardochrysogenum 2</i>	27.00	23.33	19.67	19.00	15.00	12.33	19.39
	Average of the nano zinc concentrations	38.86	34.05	24.14	29.62	21.38	18.90	

L.S.D.<sub>0.05</sub> Fungi = 2.90      Concentrations= 3.16      Interaction =7.75

\*= Each number in the table represents the average of three replicates

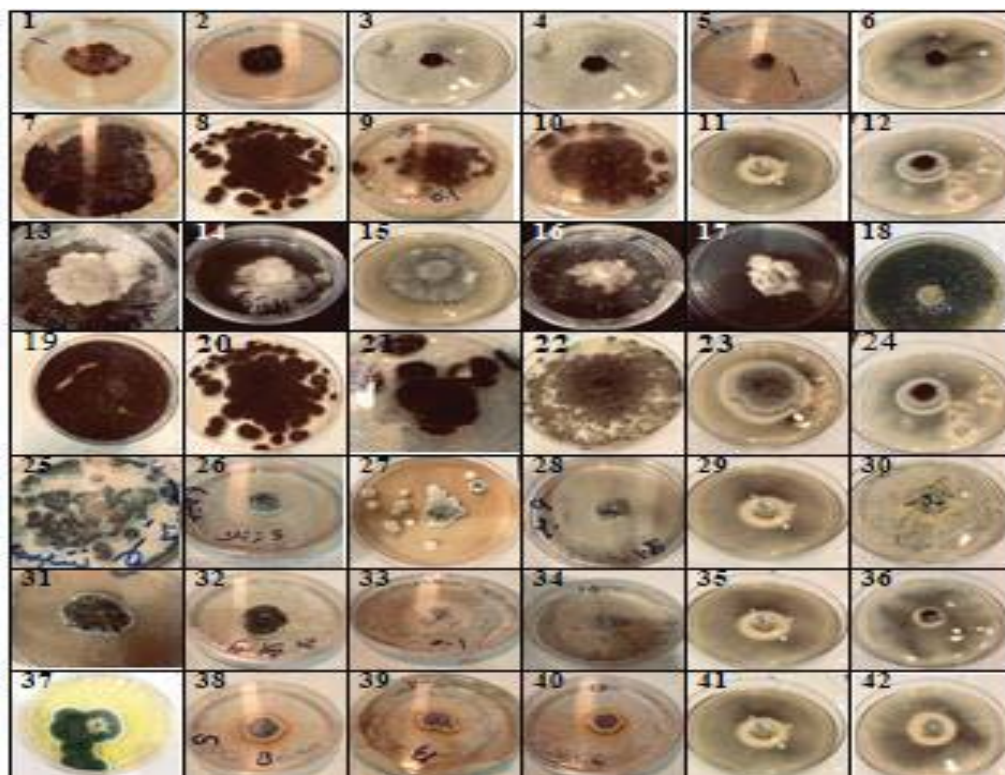
**TABLE 2.** Different concentrations of silver nanoparticles in fungi isolated from manuscripts (mm) (percentage of inhibition) and growing on PDF medium at a temperature of  $2 \pm 26^\circ \text{C}$  for 5-7 days.

No.	Fungi	5	10	15	20	25
1	<i>Alternaria atra</i>	40%	68.5%	66 %	66%	97%
2	<i>Aspergillus ustus</i>	5%	36%	3.6%	60%	54%
3	<i>globosum Chaetomium</i>	24%	50%	8.5%	10%	42%
4	<i>Cladosporium exasperatum</i>	18%	24%	9.0%	31%	18%
5	<i>Microdochium nivale</i>	27%	60%	36.6%	47%	60%
6	<i>Penicilliu Itardochrysogenum</i>	17%	25%	12%	60%	67%
7	<i>Penicillium Itardochrysogenum</i>	8%	23%	26.9%	50%	60%

Table (2) indicates that *Alternaria atra* was more affected by silver nanoparticles, while *Aspergillus ustus* was less affected with significant differences. Concerning silver nanoparticles, the concentration of 25 mg / L was more effective in inhibiting the growth of isolated fungi, while the concentration of 5 mg / L was less effective compared to the control treatment. It was more effective while the concentration of 15.5 mg / L with *Aspergillus ustus* was less effective. The reason for the decrease in the diameter of the colony as a result of the increase in the concentration of the nanoparticles was due to the increase in the saturation and absorption of the nanoparticles by the fungal hypha [12]. It is also to its react with sulfur and thus inhibit protein expression processes by ribosomes units and enzymes necessary for energy production [12and 13]. The nanoparticles acted as a poison to the cells on the membrane permeability leading to cell death [15].

### **Effect on the Phenotype of Colonies Developing by the Presence of the Nanomaterial**

It is evident from figure (1), that the fungi varied in the type of inhibition response due to the action of nano-zinc, and this response was represented by a deformation in growth compared to the control, as the fungal culture tended away from the toxic effect of nanoparticles and preferred to grow vertically for some fungal species according to the concentration. Thus, some fungal cultures treated with nano-zinc tended to rise towards the plate cover. The other change was the production of some dyes that changed the natural color of the culture medium, as happened to the *Penicillium tardochrysogenum*2, for example it is added a black substance, likely melanin, and this happened with other fungi, and this result is close to what was described by Mehdi and his group [11], although the fungi were different. There was also a change in the color of the culture medium in some fungi to pink, dark pink and orange, as it is shown in the same figure.

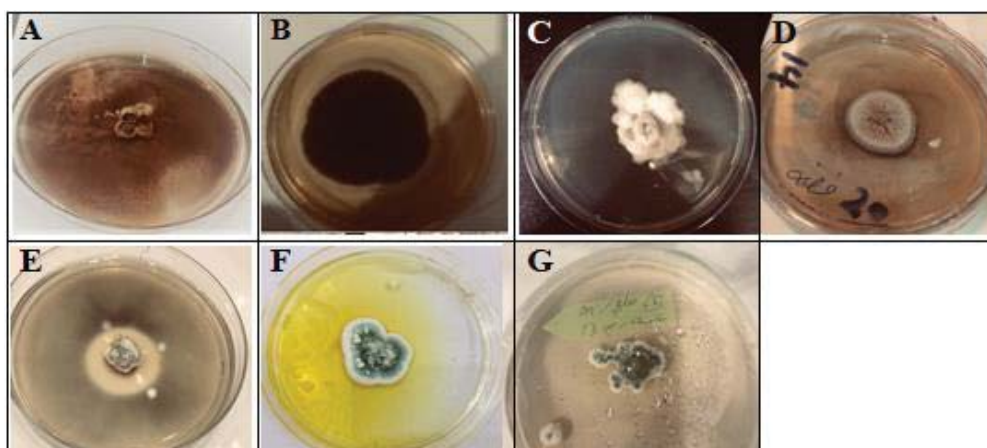


**FIGURE 1.** Different concentrations of zinc nanoparticles in growth for fungal colonies on PDA medium at temperature  $26 \pm 2$  °C, for a period of 5-7 days.

- |  |   |
|--|---|
| 1=Control <i>Alternaria atra</i>                         | 22=Add 15 mg nanozinc to <i>Cladosporium exasperatum</i>        |
| 2=Add 5 mg nanozinc to <i>Alternaria atra</i>            | 23=Add 20 mg nanozinc to <i>Cladosporium exasperatum</i>        |
| 3 =Add 10 mg nanozinc to <i>Alternaria atra</i>          | 24=Add 25 mg nanozinc to <i>Cladosporium exasperatum</i>        |
| 4=Add 15 mg nanozinc to <i>Alternaria atra</i>           | 25=Control <i>Microdochium nivale</i>                           |
| 5=Add 20 mg nanozinc to <i>Alternaria atra</i>           | 26=Add 5 mg nanozinc to <i>Microdochium nivale</i>              |
| 6=Add 25 mg nanozinc to <i>Alternaria atra</i>           | 27=Add 10 mg nanozinc to <i>Microdochium nivale</i>             |
| 7= Control <i>Aspergillus ustus</i>                      | 28=Add 15 mg nanozinc to <i>Microdochium nivale</i>             |
| 8=Add 5 mg nanozinc to <i>Aspergillus ustus</i>          | 29=Add 20 mg nanozinc to <i>Microdochium nivale</i>             |
| 9 =Add 10 mg nanozinc to <i>Aspergillus ustus</i>        | 30=Add 25 mg nanozinc to <i>Microdochium nivale</i>             |
| 10=Add 15 mg nanozinc to <i>Aspergillus ustus</i>        | 31=Control <i>Penicillium tardo-chrysogenum</i> 1               |
| 11=Add 20 mg nanozinc to <i>Aspergillus ustus</i>        | 32=Add 5 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 1  |
| 12=Add 25 mg nanozinc to <i>Aspergillus ustus</i>        | 33=Add 10 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 1 |
| 13=Control <i>Chaetomium globosum</i>                    | 34=Add 15 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 1 |
| 14=Add 5 mg nanozinc to <i>Chaetomium globosum</i>       | 35=Add 20 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 1 |
| 15=Add 10 mg nanozinc to <i>Chaetomium globosum</i>      | 36=Add 25 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 1 |
| 16=Add 15 mg nanozinc to <i>Chaetomium globosum</i>      | 37=Control <i>Penicillium tardo-chrysogenum</i> 2               |
| 17=Add 20 mg nanozinc to <i>Chaetomium globosum</i>      | 38=Add 5 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 2  |
| 18=Add 25 mg nanozinc to <i>Chaetomium globosum</i>      | 39=Add 10 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 2 |
| 19=Control <i>Cladosporium exasperatum</i>               | 40=Add 15 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 2 |
| 20=Add 5 mg nanozinc to <i>Cladosporium exasperatum</i>  | 41=Add 20 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 2 |
| 21=Add 10 mg nanozinc to <i>Cladosporium exasperatum</i> | 42=Add 25 mg nanozinc to <i>Penicillium tardo-chrysogenum</i> 2 |

The effects of silver nanoparticles on the phenotype of the fungal cultures were directed in the same direction and the cases were described in the effect of zinc nanoparticles. Figure (2) represents a model for some of these

effects. Figure2- A, it is noticed that the addition of 25 mg of silver nanoparticles to *Alternaria atra* reduces the size of growth and the direction of growth of the fungus upward with the coloration of the culture medium in a dark brown color. Either in shape B, adding 15 mg of silver nanoparticles to *Aspergillus ustus* notes a decrease in the growth of the fungus compared to the control, with no growth abnormalities or stains added to the culture medium by the fungus. In C, the addition of 15 mg silver nanoparticles to the *Chaetomium globosum* notes a decrease in the growth of the fungus compared to the control, with the presence of deformations in the growth and the upward direction and the non-pigmentation of the culture medium by the fungus compared to control, with the slow formation of spores that stain the surface of the culture in a dark brown to black color, and the culture medium was not stained by the fungi. E also shows the addition of 20 mg of silver nanoparticles to the *Microdochium nivale*. There was a decrease in the growth of the fungus compared to the control and the height of the culture due to the growth of the fungus upward, with the slow formation of spores that colored the surface of the culture in dark turquoise to dark brown, and the culture medium was not stained by the fungus. F when adding 20 mg silver nanoparticles to *Penicillium tardochrysogenum -1*. There was a decrease in the growth of the fungus compared to the control and the height of the culture due to the growth of the fungus towards the top irregularly, and the yellowing of the culture medium by the fungus. Finally, the addition of 15 mg Nano silver to the *Penicillium tardochrysogenum- 2* reduced the growth of the fungus compared to the control, the growth was not deformed, and the culture medium was not stained by the fungus.



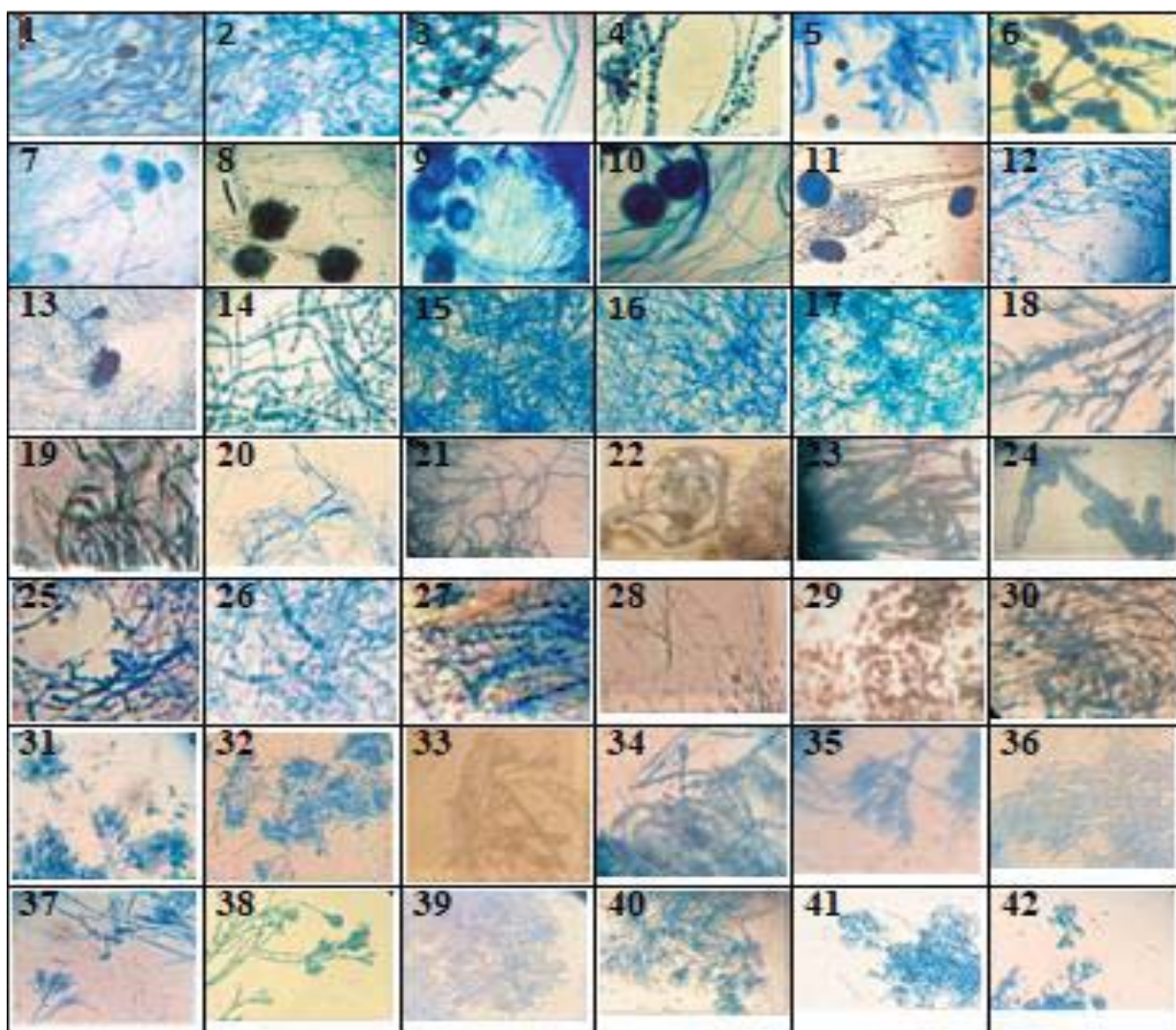
**FIGURE 2.** Different concentrations of silver nanoparticles in growth for fungal colonies on PDA medium at temperature of  $26 \pm 2$  °C for a period of 5-7 days.

- A = Addition of 25 mg silver nanoparticles to *Alternaria atra*
- B = Addition of 15 mg nanoparticles to *Aspergillus ustus*
- C = Addition of 15 mg nanoparticles to *Chaetomium globosum*
- D = Addition of 20 mg nanoparticles to *Cladosporium exasperatum*
- E = Addition of 20 mg nanoparticles to *Microdochium nivale*
- F = Addition of 20 mg silver nanoparticles to *Penicillium tardochrysogenum -1*
- G = Addition of 15 mg nanoparticles to *Penicillium tardochrysogenum -2*

### **Influence on the Microscopic Properties of Fungi**

The results in figures (3 and 4) showed that, silver and zinc nanoparticles had an effect on the growth and integrity of fungal cells, and the occurrence of this depended on the concentration of the nanoparticles and the period of exposure of the fungus to the materials until it reaches the states of its destruction and the appearance of deformations in the fungal hypha and their effect on the cell walls [16]. The microscopic examinations of the fungal hypha showed that it was affected by the nanoparticles with an increase in the concentration of the nanoparticles, and this effect ranged between the complete destruction of the fungal hypha and its explosion, especially in the high concentrations of the nanoparticles. The accumulation of the protoplasm of the fungal cell, especially in the high concentrations of the nanoparticles, or a deviation in the flow of the fungus and its attempt to move away from the

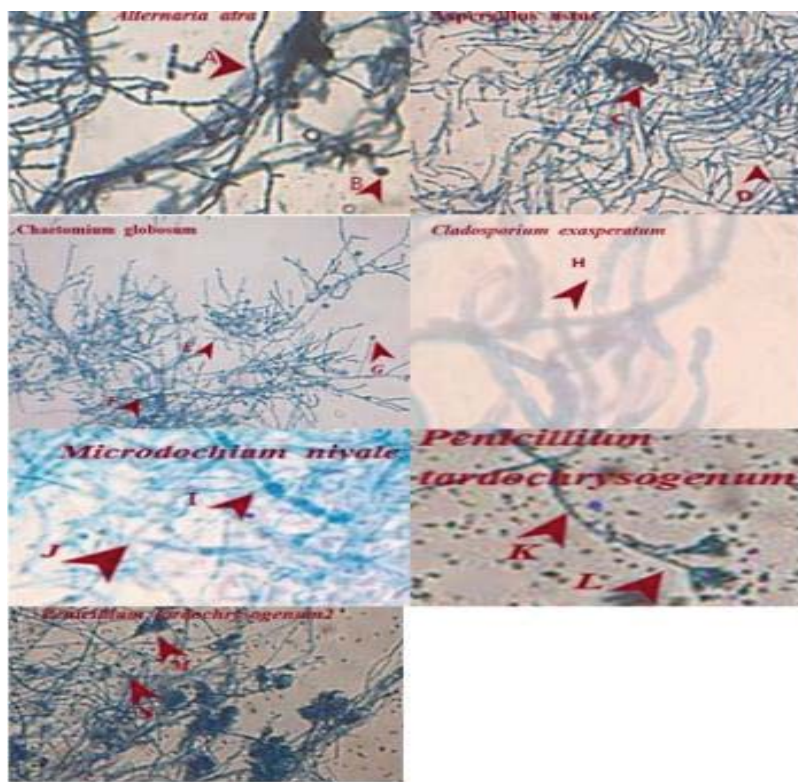
toxic substance by clumping in a specific place in the microscopic space under examination, especially at low concentrations, as well as the reduction of conidia. Its small size or deformation was depending on the type of fungus, the concentration of the nanomaterial and its type. This result was similar to what Mehdi and his group mentioned [4 and 10], cell wall separation from the plasma membrane of *Alternaria atra* treated with 10 mg / L silver nanoparticles may occur or deformation and atrophy of conidia occurs with silver nanoparticles at a concentration of 10 mg / L. The conidial head and the hypha of the *Aspergillus ustus*, with a concentration of Nano silver 10 mg / L, also showed deformation while , the conidiophore was deformed, and the fungal hypha of *Chaetomium globosum* gets cut with losing its shape at a concentration of 10 mg / L . Note the emergence of terminal chlamyospore and the occurrence of swelling of mycelium without presence of conidia at a concentration of 10 mg / L, in addition to the aggregation of protoplasmic material in one of the fungal hypha at a concentration of 10 mg / L accompanied by discharging of the other hypha from the protoplasm as well as a deformed conidiophore with loss of the fungal hypha and its damage at a concentration of 10 mg / L. The occurrence of a deformed conidial head with a concentration of 10 mg / L was deformed at concentration 10 mg / L . In N with a denatured conidia carrier with fungal hypha loss and damage at concentration of 10 mg / L for the latter fungus (Fig. 4).



**FIGURE 3.** The effect of different concentrations of zinc nanoparticles in the microscopic shape of fungal cultures on PDA medium at a temperature of  $25 \pm 2$  0 C. for a period of 5-7 days. With 40X magnification



- 1=Control *Alternaria atra*  
 2=Add 5 mg nanozinc to *Alternaria atra*  
 3 =Add 10 mg nanozinc to *Alternaria atra*  
 4=Add 15 mg nanozinc to *Alternaria atra*  
 5=Add 20 mg nanozinc to *Alternaria atra*  
 6=Add 25 mg nanozinc to *Alternaria atra*  
 7= Control *Aspergillus ustus*  
 8=Add 5 mg nanozinc to *Aspergillus ustus*  
 9 =Add 10 mg nanozinc to *Aspergillus ustus*  
 10=Add 15 mg nanozinc to *Aspergillus ustus*  
 11=Add 20 mg nanozinc to *Aspergillus ustus*  
 12=Add 25 mg nanozinc to *Aspergillus ustus*  
 13=Control *Chaetomium globosum*  
 14=Add 5 mg nanozinc to *Chaetomium globosum*  
 15=Add 10 mg nanozinc to *Chaetomium globosum*  
 16=Add 15 mg nanozinc to *Chaetomium globosum*  
 17=Add 20 mg nanozinc to *Chaetomium globosum*  
 18=Add 25 mg nanozinc to *Chaetomium globosum*  
 19=Control *Cladosporium exasperatum*  
 20=Add 5 mg nanozinc to *Cladosporium exasperatum*  
 21=Add 10 mg nanozinc to *Cladosporium exasperatum*  
 22=Add 15 mg nanozinc to *Cladosporium exasperatum*  
 23=Add 20 mg nanozinc to *Cladosporium exasperatum*  
 24=Add 25 mg nanozinc to *Cladosporium exasperatum*  
 25=Control *Microdochium nivale*  
 26=Add 5 mg nanozinc to *Microdochium nivale*  
 27=Add 10 mg nanozinc to *Microdochium nivale*  
 28=Add 15 mg nanozinc to *Microdochium nivale*  
 29=Add 20 mg nanozinc to *Microdochium nivale*  
 30=Add 25 mg nanozinc to *Microdochium nivale*  
 31=Control *Penicillium tardochrysogenum*1  
 32=Add 5 mg nanozinc to *Penicillium tardochrysogenum*1  
 33=Add 10 mg nanozinc to *Penicillium tardochrysogenum*1  
 34=Add 15 mg nanozinc to *Penicillium tardochrysogenum*1  
 35=Add 20 mg nanozinc to *Penicillium tardochrysogenum*1  
 36=Add 25 mg nanozinc to *Penicillium tardochrysogenum*1  
 37=Control *Penicillium tardochrysogenum* 2  
 38=Add 5 mg nanozinc to *Penicillium tardochrysogenum* 2  
 39=Add 10 mg nanozinc to *Penicillium tardochrysogenum*2  
 40=Add 15 mg nanozinc to *Penicillium tardochrysogenum* 2  
 41=Add 20 mg nanozinc to *Penicillium tardochrysogenum*2  
 42=Add 25 mg nanozinc to *Penicillium tardochrysogenum*2



**FIGURE4.** The effect of different concentrations of nanoparticles in the microscopic shape of the fungal cultures on the PDA medium at a temperature of  $25 \pm 2$  0 C. for a period of 5-7 days. With 40X magnification  
 A=separation of the cell wall from the plasma membrane of *Alternaria atra* with silver nanoparticles of 10 mg/ L.  
 B = deformation and atrophy of the conidia with silver nanoparticles of 10 mg / L.

C = the conidial head of the *Aspergillus ustus* at a concentration of 10 mg / L silver nanoparticles  
 D = cut the fungal hypha, with the deformation of the conidia carriers  
 E = fungal hypha of *Chaetomium globosum* with losing its shape at a concentration of 10 mg / L  
 F = The fungus could not produce perithecium fruiting bodies  
 G = terminal chlamydospore appearance.  
 H = mycelial hypha swelling and no conidia present at a concentration of 10 mg / ml  
 I = a grouping of protoplasmic material in one of the fungal hypha at a concentration of 10 mg / L  
 J = discharging the other Hypha from protoplasm at a concentration of 10 mg / L  
 K = a deformed conidial carrier with the loss of the fungal yarn and its damage at a concentration of 10 mg / L  
 L = a deformed conidial head at a concentration of 10 mg / ml  
 M = Conidial head deformed at concentration 10 mg / L  
 N = denatured conidia carrier with fungal hypha loss and degradation at concentration of 10 mg / L.

## Test of Inhibitory Concentration of Nanomaterial with Old Material in the Manuscript Restoration

The combination of individual nanoparticles to the restoration material (1% methyl hydroxyethyl cellulose MH6000) had a good efficacy in inhibiting the growth of fungi on manuscript, as the number of fungi was reduced compared to the manuscript before treatment . The emergence of fungi of different genera, in very small numbers and at very cheap prices, which indicates the effectiveness of nanoparticles in controlling their neighboring areas[12].

## CONCLUSION

Zinc Nanoparticles had proved its ability to inhibit the growth of fungi more than silver nanoparticles, especially at a concentration of 25 mg / L. The presence of Zinc nanoparticles or silver nanoparticles in the manuscript conservation paste contributes to Inhibiting the fungi that cause spoilage and speeding up manuscript obsolescence.

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