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The Microbial Impact of Zinc Oxide (ZnO) and Silver (Ag) Nanoparticles on Fungi Isolated from a Paper Manuscript

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ABSTRACT

Samples were taken from a manuscript dating back to 1370 AD, and fungi were isolated from different parts of the manuscript as well as from the storage environment. Seven types of fungi were identified, including *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus tamarii*, *Penicillium chrysogenum*, *Trichoderma viride*, *and Gliocladium fimbriatum*. Zinc oxide nanoparticles at concentrations of 0.3%, 0.6%, and 0.9%, along with silver nanoparticles at concentrations of 50, 30, and 20 parts per million, were used. A concentration of 0.9% of isopropyl alcohol in zinc oxide nanoparticles and a concentration of 50 parts per million in silver nanoparticles showed an inhibition rate of 90%, reaching 100% for *A. niger*. These concentrations were applied to the infected samples, which were examined using scanning electron microscopy. The infected samples showed a high density of fungi, especially *A. niger and A. terreus*, with clear penetration of hyphae and microorganisms between the fibers. There was a general weakness in the mechanical properties of the paper, such as tensile strength and elongation. The selected concentrations were applied to paper surfaces, and their mechanical properties were studied. It was found that samples treated with zinc oxide nanoparticles and silver nanoparticles showed an increase in tensile strength and elongation, along with some improvement in the properties of severely damaged samples, especially those infected with *A. niger and A. terreus*.

1. Introduction

The manuscripts are considered portable cultural heritage, representing an important element of written heritage. They are known for their ease of production, transportation, and preservation compared to other archaeological artifacts. Manuscripts are an essential component of written heritage, originating in China in 105 AD by Ts'ai Lun [1]. The invention of paper dates back to the era of Emperor Ho Ti of Han. Paper was made from mulberry, hemp, jute, rice straw, banana, and hemp fibers, as well as scraps of old clothes [2]. The tradition of papermaking spread to Islamic countries in 751 AD, when a group of Chinese people was captured after the Battle of Talas [3]. Muslims began producing paper and gained the name "Kaghazi" after Muslim papermakers. The word "Kagaz" is derived from the Urdu word "Kavas." Manuscripts consist of various components, including supports based on cellulose, inks, dyes, pigments, and fillers [4]. These components share common factors of deterioration while exhibiting different phenomena.

Manuscripts are susceptible to external damage, which becomes evident with the appearance of spots and the spread of foul odors. These signs indicate the presence of a destructive agent that attacks cellulose, colors, and inks, affecting the mechanical properties of the paper along with the spread of colored spots. Foxing spots can be classified into two types: bull's-eye, which have a metallic appearance and do not fluoresce under ultraviolet light, and snowflake, which are associated with fungal and spores [5]. Hydrolytic degradation of cellulose in paper primarily occurs through acid-catalyzed hydrolysis, which can lead to random cleavage of glycosidic bonds [6, 7]. This deterioration can be further exacerbated by the growth of fungi such as *Alternaria spp., Aspergillus spp., Penicillium spp., Cladosporium spp*., and *Botrytis spp*. in museum and library environments, causing skin inflammation, pulmonary infections, and anemia in individuals exposed to polluted air [8].

Various chemical substances are used, such as alcohols, azole antifungals, volatile oils, phenolic derivatives, photosensitizers, quaternary ammonium compounds, salts, and acidic esters, in preserving documents, manuscripts, and printed materials damaged by fungi. On the other hand, physical methods aim to prevent fungal growth and are used in many museums, libraries, and restoration centers. These methods include document drying, gamma irradiation, high-frequency currents, ultraviolet radiation, plasma techniques, X-rays, extreme temperatures, and low-oxygen environments with electron beam irradiation. These methods have proven effective in eliminating fungal growth and inhibiting its activity [9,10], but

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they often come with high costs and technical challenges due to the required equipment. Therefore, attention has been directed towards a new method that is easy to implement and exhibits unique properties, such as ease of application and effectiveness at very low concentrations, especially in hard-to-reach areas [11].

In this study, zinc oxide and silver nanoparticles will be utilized on fungi isolated from ink-written paper and other substrates containing pigments, as well as on the storage environment in the National Library and Archives in Cairo. The focus will be on investigating the effects of these materials on the mechanical properties of paper and any potential color changes that may occur. Samples will be comprehensively examined using scanning electron microscopy.

2. Materials and Methods

2.1. Materials

2.1.1. Isolation source

The historical book named '139 Mustalah al-Hadith Taimur'' deposited in the National Library and Archives in Cairo and dated to the year 1370 CE,Egypt, Swabs, and Fungal Cultivation Sterile cotton swabs were used to collect isolates from different sites in the manuscript139 Mustalah al-Hadith Taimur." Samples were obtained by lightly and momentarily touching the affected areas, including the pigments,inks, covers, margins, and paper pages of the manuscript. Three Petri dishes with PDA medium were placed open in the manuscript's storage environment, as depicted in Fig.1 and Table 1.

Fig.1. Swabs from the edges (139B) and the pigments (139m I, 139II, 139III,139IV, 139V).

Table 1: Names and Locations of swabs.

Episode	Sample	Location
1	139m I	From colored pages
2	139II	From colored pages
3	139III	From colored pages
4	139IV	Blue pigment
5	139V	Red pigment
6	139A	Spots and edges of pages
7	139B	Spine
8	139G	Binding
9	139D	Outer cover

The swabs were inoculated onto Petri dishes containing Potato Dextrose Agar (PDA) medium that had been sterilized in a sterile isolation room at 25°C [12]. The dishes were then incubated for 6 days. During the incubation period, various fungal colonies appeared with different sizes and colors, indicating the presence of different fungal species. Fungal colonies were also found in the Petri dishes placed in the storage environment. The fungal isolates were diagnosed based on morphological and cultural characteristics, as well as microscopic examination of fruiting bodies and spores using standard keys for *Aspergillus spp*, [13,14] *Trichoderma spp*., and *Gliocladium spp. [15, 16*] *Penicillium spp*. [17,18].

2.1.2. Paper model used

The bleached sugarcane pulp was used in manufacturing paper samples supplied by Qena Paper and Pulp Company, Qena, Egypt. The pulp consists of the following composition: 21% hemicellulose, 65% alpha-cellulose, 1.3% ash, and 0.2% lignin [19]. 75 milliliters of the pulp solution were poured into a Büchner funnel after lining it with filter paper moistened with distilled water. The sample (average diameter 8 cm, thickness 1 mm) was then subjected to drying, pressing, and sterilization in the autoclave, as illustrated in Fig. 2.

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Fig.2.Production of paper samples.

2.1.3. Infected Paper Samples

Samples of paper measuring 1×7 cm were prepared from the pre-manufactured pulp to study the effect of test materials on the mechanical properties of the paper. Samples measuring 1×1 cm were also prepared for examination under scanning electron microscopy to observe the spread of fungi and nanomaterials between the fibers and the paper surface and their effect on cellulose, as depicted in Fig.3. Additionally, samples measuring 8×8 cm were prepared to study potential color changes. All paper samples were sterilized using an autoclave. Suspensions of isolated fungi were used, and the paper samples were inoculated with fungal suspensions for an extended period following the method described by El Bergadi (2014), Mansour (2015), Salem (2015), Nofhyutta et al. (2016), Hassan (2016),and Mansour (2017) which was applied for several months. We left them for 10 months (300 days) to evaluate long-term fungal growth [20, 21, 22, 23]. Imaging and observation were conducted at regular intervals.

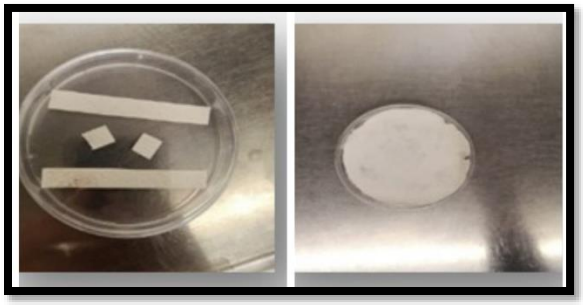

Fig. 3. The samples used in the experiment.

2.1.4. Nanoparticles used

Nanoscale zinc oxide (30±5 nm) was prepared by the Arts Conservation Agency using hydrolysis and condensation of zinc acetate dihydrate with potassium hydroxide in alcohol at low temperature. This process led to the deposition of nanocrystalline zinc oxide particles at the bottom, and the excess was removed from the solution. The deposited material was washed with methanol, then dispersed in a mixture of methanol and chloroform, as illustrated in Fig.4, which shows the morphology of the nanoparticles under scanning electron microscopy.

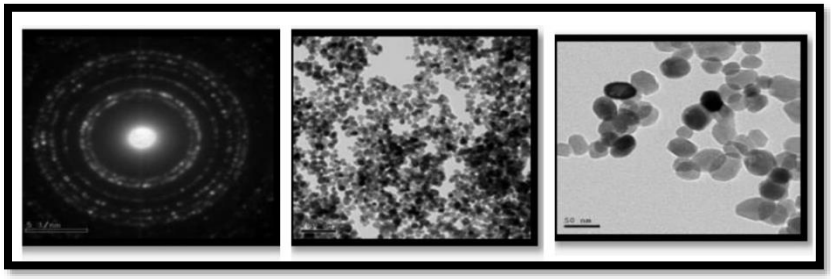

Fig. 4. TEM image of ZnO Nps.

Nanoscale silver particles (less than 25 nm) Nanoscale silver particles (less than 25 nm) were prepared by the Arts Conservation Agency using a chemical reduction method. A solution containing dimethyl sulfoxide (DMSO) and silver nitrate (AgNO3) as a source of Ag+1 ions was used. Polyvinylpyrrolidone (PVP) was used as a stabilizing agent, and sodium borohydride was used as a mild reducing agent. The solution slowly turned a grayish-yellow color, indicating the reduction of Ag+1 ions into nanoscale Ag particles. Fig.5 illustrates the shape of the nanoscale particles under a scanning electron microscope.

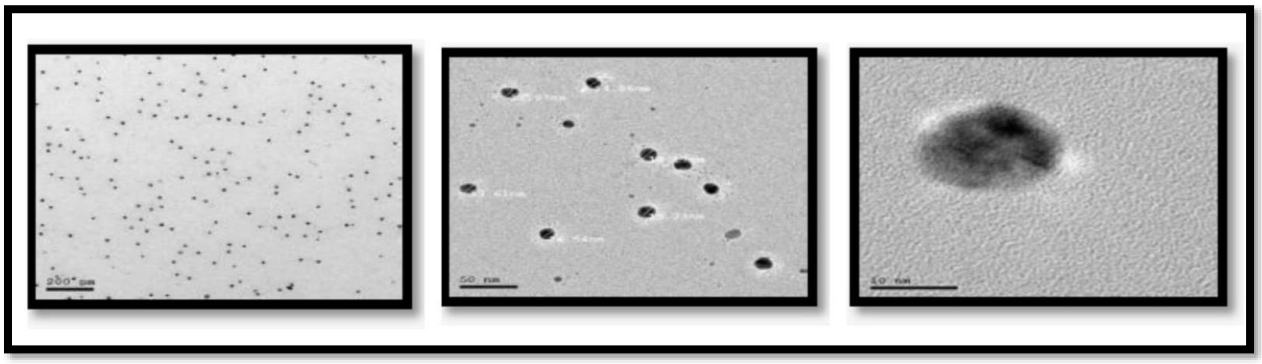

Fig. 5. TEM image of Ag NPs.

Isopropyl alcohol was used as a solvent for both materials. Three different weights of nanozinc oxide (0.3–0.6–0.9 g) were dissolved in 100 milliliters of alcohol [24]. Additionally, the silver nanoparticles were divided into three concentrations (20–50 ppm). Subsequently, the six concentrations were exposed to ultrasound waves using the ultrasound bath at the Faculty of Agriculture, Fayoum University, for 30 minutes.

The efficiency of the nanomaterials was measured using the following equation, which calculates the inhibition rate based on colony volume.

$$\text{Percentage of Inhibition (PI)} = \frac{Dc - Dt}{Dc} \times 100$$

Dc = average diameter of fungal growth (cm) under control conditions. Dt = average diameter of fungal growth (cm) in the treatment. PI = inhibition rate [25].

2.2. Testing

2.2.1. Scanning Electron Microscope (SEM)

The examination was conducted using the scanning electron microscope (SEM) at Assiut University, JEOL JSM-5400LV model. Standard, infected, and treated paper samples were coated with gold and examined at a size of 1×1 cm.

2.2.2. Mechanical properties

Mechanical testing was carried out on untreated, infected, and treated paper samples at the National Research Center in Dokki, Cairo, Egypt. The tests were conducted using the LLOYD-LR10K instrument (code 1-353) on samples measuring 1 × 7 cm, in accordance with the TAPPI T494 standard method, maintaining a constant traverse speed of 6.25 cm/minute.

2.2.3. Optical properties

Optical properties tests were conducted on standard, infected, and treated samples at the Faculty of Archaeology, Fayoum University, using the Swiss Exact X-Rite device. The tests involved measuring the values of B, A, and L according to the CIEL 2004 color system. Color changes were calculated using the following equation, where ΔL, Δa*, and Δb* represent differences in color coordinates.:

:

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad [26]$$

3. Results

3.1. Fungi

Seven types of fungi were identified based on morphological characteristics at the laboratory of Archaeological Research and Conservation at the Ministry of Antiquities in Egypt (*Aspergillus terreus, A. niger, A. flavus, Penicillium chrysogenum, A. tamarii, Trichoderma viride, and Gliocladium fimbriatum*). Genera such as *Trichoderma, Penicillium,* and *Aspergillus* are known for their wide distribution in museums and libraries [27]. The majority of the swabs contained *A. niger*, *A. flavus, P. chrysogenum, T. viride,* and *A. terreus*. The same fungal species were also found in the storage environment.

3.2. Nanomaterial Concentrations

Based on previous studies and the selected concentrations in Fig.6, the highest concentration of Ag NPs achieved 100% inhibition rates for *A. niger* and *T. viride*, with no presence of any germs or mycelium after 21 days of incubation at a concentration of 50 parts per million. The inhibition rate for *A. flavus* was 74%, and for *A. terreus*, it was 53%. However, *P. chrysogenum* showed high resistance at a rate of 26% at the same concentration.

In contrast, the lowest concentration of zinc oxide nanoparticles (ZnO NPs) showed lower effectiveness. The highest concentration of the nanoparticle metal achieved inhibition rates of 79.85% for *T. viride*, with a decrease in germs and pigmentation after 21 days of incubation. For *A. flavus*, the inhibition

rate was 73.5%; for *A. terreus*, it was 69.67%; and for *P. chrysogenum*, it was 68%. However, *A. niger* showed high resistance at 65.5% at the same concentration.

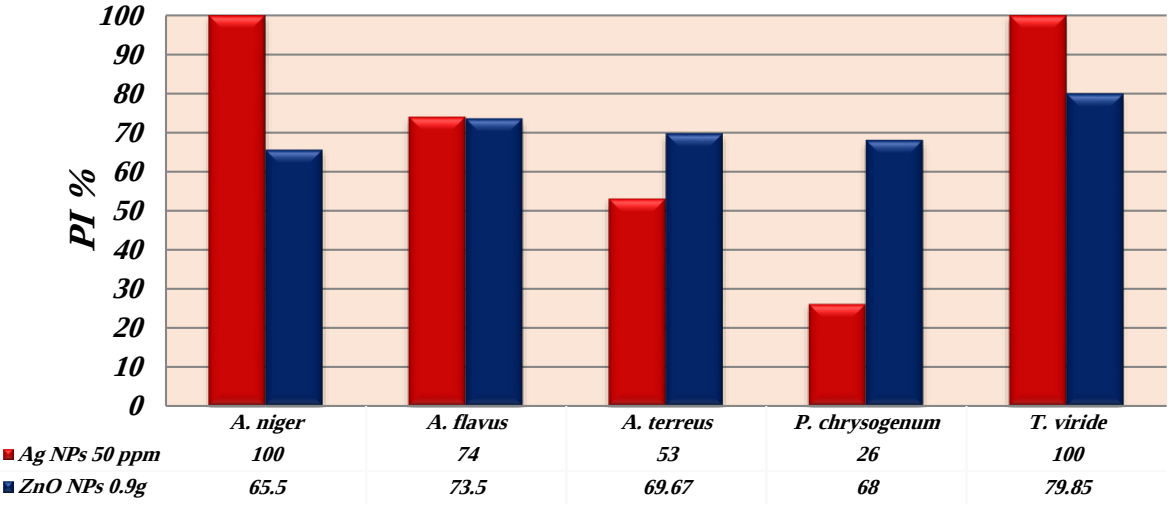

Fig. 6. Fungal inhibition rates.

3.3. Scanning Electron Microscopy (SEM)

Scanning electron microscopy of the standard sample showed its freedom from any fungal infection, and the fiber condition was very good. However, samples infected with fungi exhibited scattered fungal colonies among the fibers, with general weakness in samples infected with the fungi *A. niger* and *A. terreus*. In Fig. 7, Image (a) illustrates the examination of the standard sample at a magnification of 10 micrometers, showing no fungal growth. In contrast, image (b) clearly shows the presence of *A. niger* colonies. Image (c) demonstrates decreased microbial activity in the sample infected with *A. flavus*, as does image (d) for samples of *P. chrysogenum*. However, image (e) reveals the evident spread of *A. terreus* colonies, and image (f) depicts the presence of fungal.

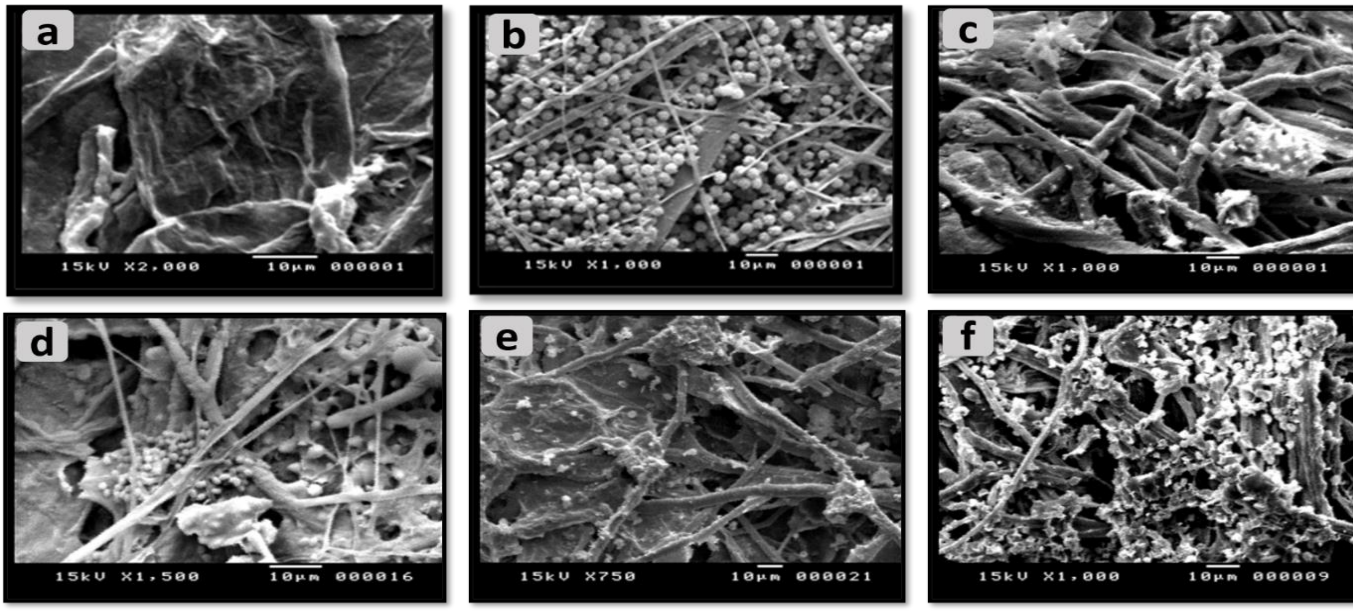

Fig. 7. SEM image of various paper samples (standard- infected).

3.4. Results of Nanomaterials

Applying 0.9 grams of ZnO NPs by spraying on the fungal-infected samples and then incubating them for 15–21 days resulted in the complete absence of microbial growth and the elimination of any germs or mycelium. Additionally, there was the deposition of nanoparticles on the paper fibers. Fig. 8. illustrates the surface condition of the treated paper when examined. Image (a) represents the sample infected with *A. niger* and treated with zinc oxide nanoparticles, while image (b) shows the sample infected with *A. flavus*. Image (c) demonstrates the presence of metal oxide nanoparticles on the

surface of the paper for the treated sample infected with *A. terreus*. Furthermore, image (d) depicts the condition of fungus-free fibers after treatment with *P. chrysogenum*, and image (e) represents the absence of fungal growth on the paper surface.

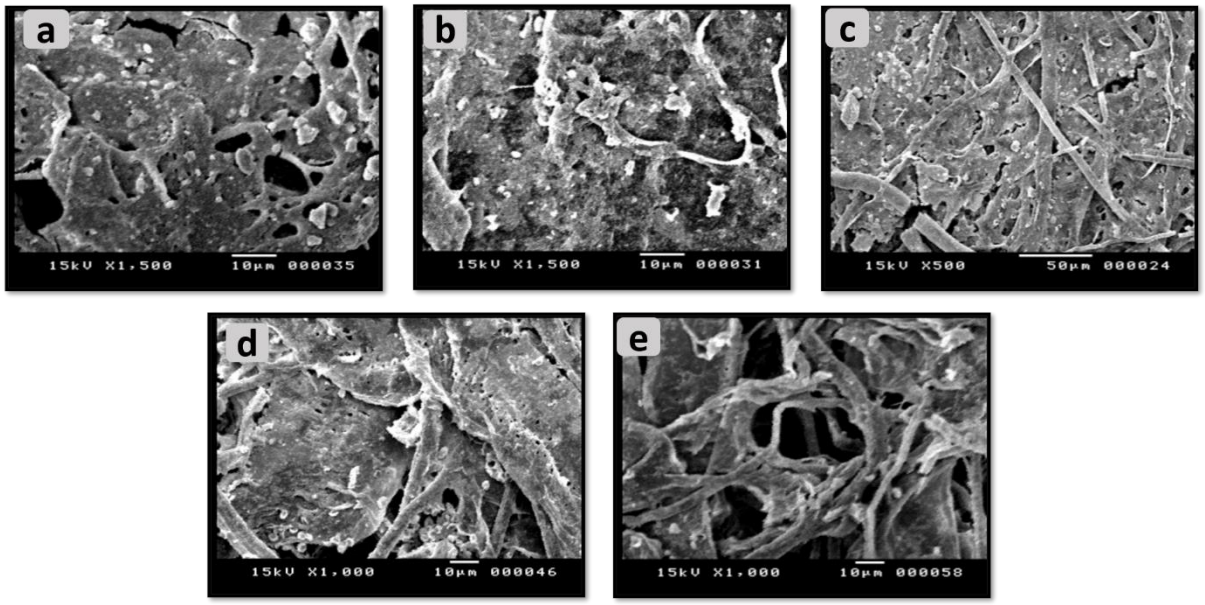

Fig. 8. SEM image of samples treated with ZnO NPs.

Applying 50 ppm of Ag NPs led to the complete absence of microbial growth and the deposition of silver nanoparticles on the paper fibers. Fig.9 illustrates the surface condition of the treated paper upon examination. Image (a) represents silver nanoparticles for the sample infected with *A. niger* and treated with silver nanoparticles, while image (b) shows a sample infected with *A. flavus*. Image (c) demonstrates the paper surface of the treated sample infected with *A. terreus*. Furthermore, image (d) depicts the condition of fungus-free fibers after treatment with *P. chrysogenum* and the deposition of silver nanoparticles. Finally, image (e) represents the absence of fungal growth on the paper surface.

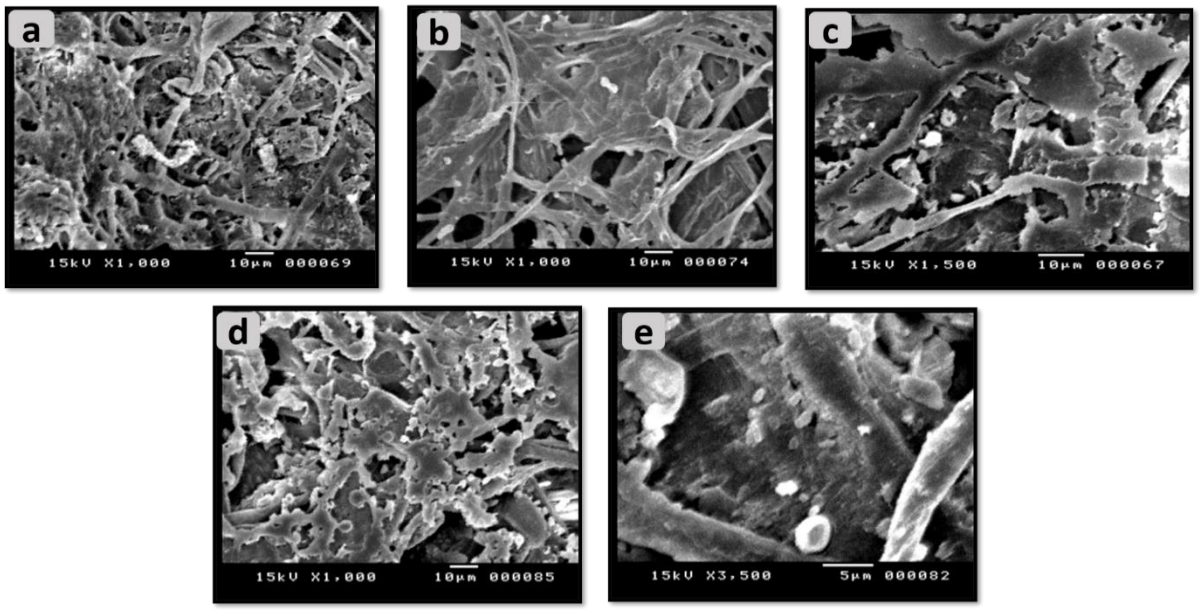

Fig. 9. SEM image of samples treated with Ag NPs.

3.5. Mechanical properties

Mechanical property tests were conducted on untreated, infected, and nanoparticle-treated paper samples using a mechanical property measurement device model (LLOYD-LR10K).

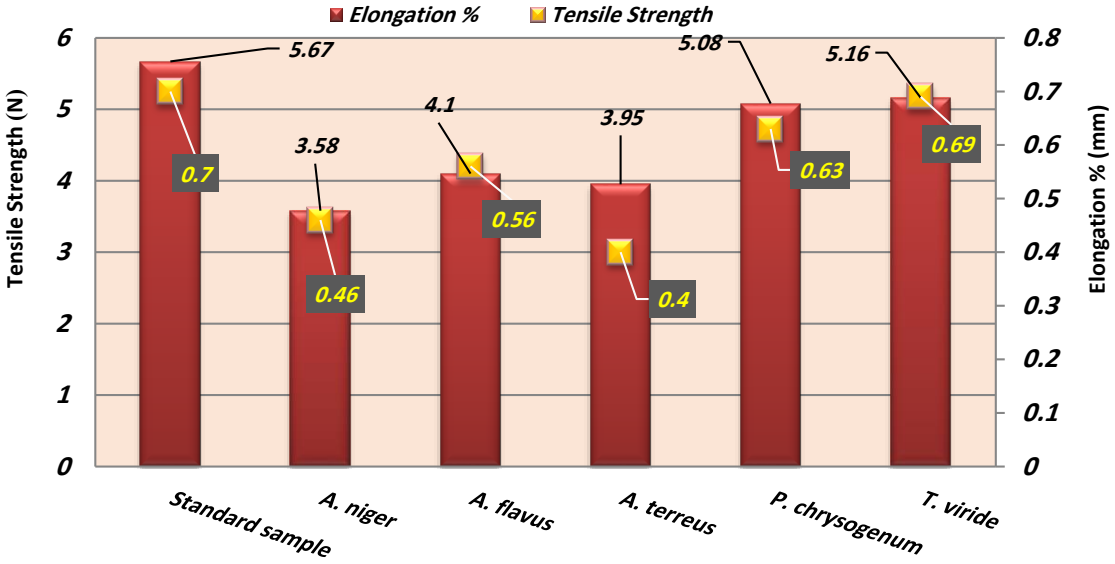

Fig. 10. Tensile strength of the standard and infected samples.

From Fig. 10, it is evident that the average tensile strength significantly decreased for samples infected with *A. terreus* and *A. niger* (3.58N, 3.95N) compared to the standard sample (5.67N). This decrease in tensile strength was visually confirmed due to the severe damage and color change in the samples caused by fungal growth. On the other hand, the average tensile strength showed a relatively smaller decrease with *A. flavus* (4.1N). Similarly, *P. chrysogenum* and *T. viride* showed a slight decrease in average tensile strength (5.16N, 5.08N) compared to the standard sample, as observed visually during sample examination.

Likewise, the average elongation ratio decreased significantly for samples infected with *A. terreus* and *A. niger* (0.46 mm, 0.4 mm) compared to the standard sample (0.7 mm). Visually, severe damage and color changes in the samples due to fungal growth were confirmed. *A.* flavus showed a relatively smaller decrease in average elongation ratio (0.56 mm). Similarly, *P. chrysogenum* and *T. viride* showed a slight decrease in average elongation ratio (0.69 mm, 0.63 mm) compared to the standard sample, as observed visually during sample examination.

While the tensile strength improved for samples treated with ZnO NPs, as shown in Fig.11, reaching 6.44N for the sample infected with the fungus *A. flavus* and reaching 6.66N for the sample treated with a concentration of 0.9% of *T. viride* fungus, Samples infected with *A. terreus* showed less improvement in their mechanical properties, measuring 4.21N due to severe damage. The nanoparticle material did not prove effective in improving its mechanical properties. Similarly, stress ratio measurements for the same samples treated with the same concentration showed a close average stress ratio of 0.62 mm, 0.61 mm, and 0.62 mm for *P. chrysogenum*, *T. viride*, and *A. flavus* fungi, respectively, compared to the standard sample of 0.7 mm. However, the material did not contribute to an increase in stress ratio for *A. terreus* and *A. niger* fungi, measuring 0.3 mm and 0.48 mm, respectively. Therefore, the use of zinc oxide nanoparticles is recommended as an antifungal agent and enhancer of mechanical properties in paper samples.

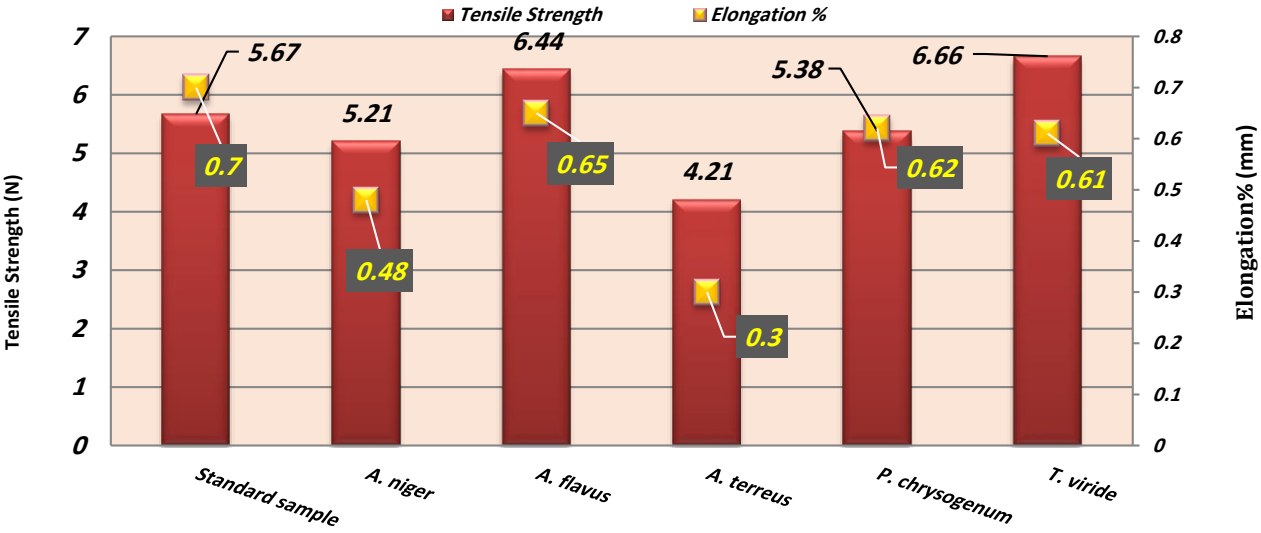

Fig.11. Tensile strength of samples treated with ZnO NPs.

When comparing the concentration of silver nanoparticles (50 ppm) with zinc oxide nanoparticles, as shown in Fig. 12, despite their antifungal activity against isolated fungi, they failed to improve the mechanical properties of the infected samples. The average tensile strength for samples infected with *A. terreus* and *A. flavus* was 2N and 3.68N, respectively. However, samples infected with *A. niger* suffered severe damage, preventing the measurement of their mechanical properties due to the severe infection, leading to extensive sample corrosion and hindering the measurement process.

On the other hand, samples infected and treated with *T. viride* showed improvements in their mechanical properties. The average tensile strength increased from 5.67 N (the standard sample) to 6.56 N. Additionally, the relaxation elongation for the sample treated with *T. viride* (0.78 mm) was close to the standard sample (0.7 mm).

In contrast, the elongation at break significantly decreased for samples treated with *A. terreus* (0.4 mm) and slightly for *A. flavus* and *P. chrysogenum* compared to the standard sample, with a decrease of 0.2 mm.

In summary, when comparing the concentration of silver nanoparticles at 50 ppm with zinc oxide nanoparticles in Fig. 11, despite their successful antifungal activity, they did not enhance the mechanical properties of the infected samples. However, treatment with *T. viride* led to improved mechanical properties of the infected samples, with an increase in tensile strength and similar elongation at break compared to the standard sample. Conversely, treatments *with A. terreus, A. flavus,* and *P. chrysogenum* showed varied effects on relaxation and elongation compared to the standard sample.

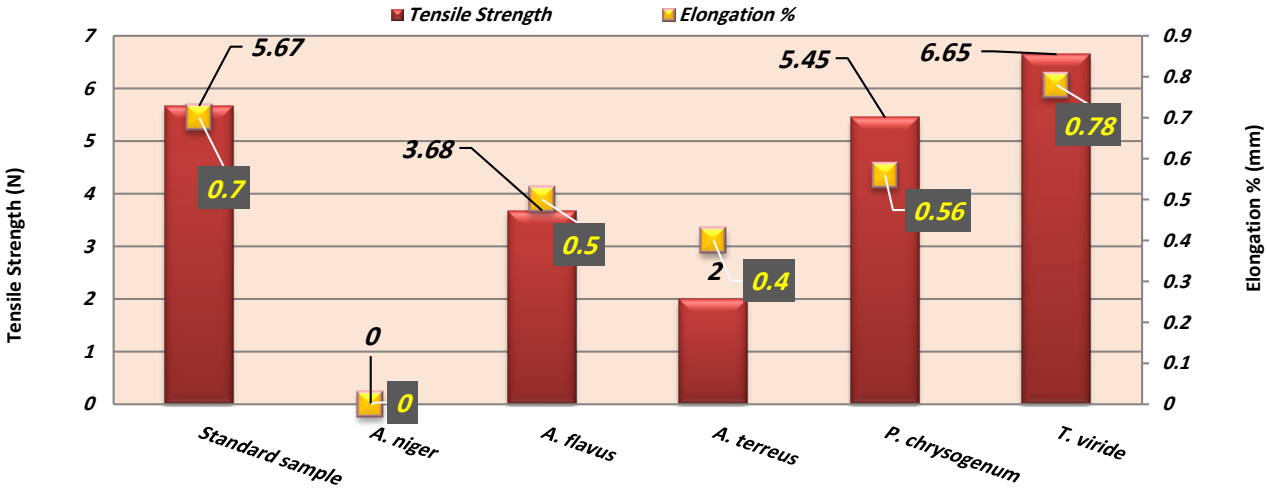

Fig. 12. Tensile strength of samples treated with Ag NPs.

3.6. Optical properties

The difference between using ZnO NPs and Ag NPs on paper samples infected with selected fungi is evident. In Table.2, the recorded color change values varied slightly, with the standard sample showing a color change value of (1.40=ΔE*), while the sample infected with *A. niger* showed the highest value (11.11=ΔE*), and the sample infected with *A. terreus* showed the lowest color change value (2.03=ΔE*). However, when treating the paper samples with ZnO NPs, Table.3, indicated the highest color change value (3.64=ΔE*) for the sample treated with *A. terreus* at a concentration of 0.9 grams , while the sample treated with *A. flavus* showed the lowest color change value (1.29=ΔE*). Similarly, in Table.4, the paper sample infected with *A. flavus* and treated with Ag NPs at a concentration of 50 ppm showed the highest color change value (3.28=ΔE*), while the sample treated with *T. viride* showed the lowest color change value (0.62=ΔE*).

Therefore, the color change values for the treated samples were relatively better compared to the infected samples, especially those treated with silver nanoparticles, as shown in Fig.13.

Table. 2. Color changes were measured according to the CIE L*a*b* system for the standard and infected samples.

Samples	L*	a*	b*	ΔE*
Standard Sample	90.14	0.84	5.82	1.40
A. niger	64.26	3.39	10.62	11.11
A. flavus	86.31	2.41	9.17	3.79
A. terreus	66.34	6.91	19.93	2.03
P. chrysogenum	90.91	0.42	5.35	2.24
T. viride	86.64	1.1	7.15	2.10

Table. 3. Color changes were measured according to the CIE L*a*b* system for the standard and ZnO NPs-treated samples.

Samples treated with ZnO NPs	L*	a*	b*	ΔE*
Standard Sample	90.14	0.84	5.82	1.40
A. niger	68.08	3.03	11.77	3.28
A. flavus	89.09	1.19	7.3	1.29
A. terreus	76.88	4.22	14.03	3.64
P. chrysogenum	91.54	0.79	5.25	1.51
T. viride	73.01	3.45	12.49	1.51

Table. 4. Color changes were measured according to the CIE L*a*b* system for the standard and Ag NPs-treated samples.

Samples treated with Ag NPs	L*	a*	b*	ΔE*
Standard Sample	90.14	0.84	5.82	1.40
A. niger	70.46	2.87	8.74	2.76
A. flavus	74.73	4.46	16.27	3.28
A. terreus	63.64	9.13	20.60	1.54
P. chrysogenum	88.17	1.39	7.63	1.54
T. viride	87.62	0.94	6.61	0.62

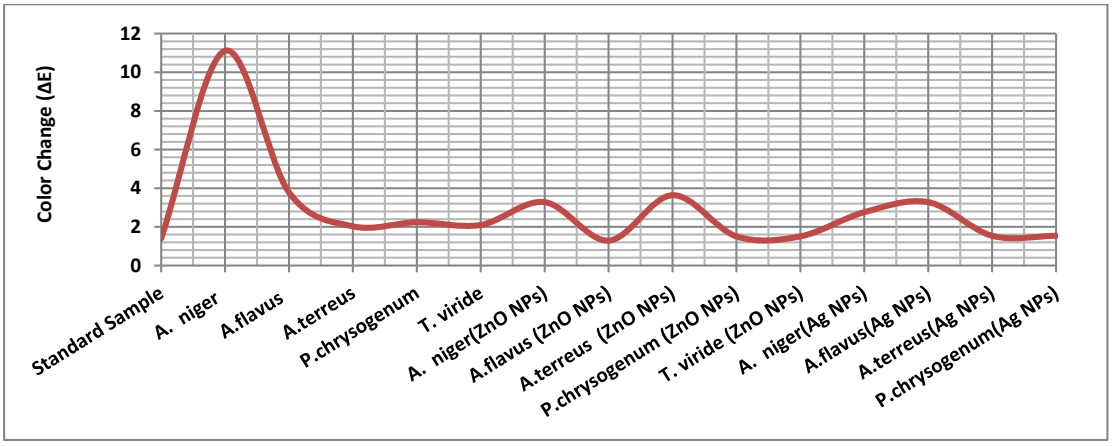

Fig. 13. Color Change Measurements.

4. Discussion

Previous studies have focused on the inhibitory effects of ZnO NPs and Ag NPs on microbial growth. It was found that blending zinc oxide nanoparticles with titanium dioxide nanoparticles (ZnO-TiO2) was more effective than using them individually. A concentration of 300 micrograms/mL of the nanoparticle blend showed complete inhibition (100%) against *A. flavus*, while a concentration of 37.5 micrograms/mL showed inhibition rates of 72.5%. Fouda et al. [28] conducted a study on the impact of different quantities of nanomaterials, such as ZnO NPs and Ag NPs, on the growth *of Penicillium chrysogenum* and *A. niger*. They found that a concentration of 1 milliliter of silver nanoparticles inhibited 59.9% of *P. chrysogenum* after 21 days of incubation. Increasing the concentration to 2 milliliters led to complete inhibition (100%). On the other hand, a concentration of 1 milliliter of zinc oxide nanoparticles inhibited *P. chrysogenum* by 51.9% after 21 days, and increasing the concentration to 2 milliliters resulted in a higher inhibition rate of 89.7% for the same fungus [29]. For *A. niger*, a concentration of 1 milliliter of silver nanoparticles led to an inhibition rate of approximately 53.5% after 21 days, while increasing the concentration by 2 milliliters resulted in a higher inhibition rate of 97.1%. However, a concentration of 1 milliliter of zinc oxide nanoparticles led to a lower inhibition rate of 36.3% after 21 days, and increasing the concentration to 2 milliliters caused a higher inhibition rate of 98.2% for the same fungus [30]. Deshluk et al. [31] succeeded in reducing the growth of *A. niger, A. terreus,* and *Trichoderma viride* by 2-3 times using ZnO NPs (2–7 nm) at a concentration of 0.25%. In another study conducted by Mohammed et al. (2020), nanoscale particles of zinc oxide ranging in size from 9 to 35 nanometers and at a concentration of 10 milligrams were used against the fungus *A. terreus*, resulting in a reduction of the growth diameter in the nutrient medium to 13 millimeters. Jambin et al. [33] found that increasing the concentration of ZnO NPs had an inhibitory effect on the growth of fungi *A. niger* and *P. chrysogenum*. A concentration of 0.125% led to a growth inhibition of 36% and 13% for *P. chrysogenum* and *A. niger*, respectively, after 10 days of incubation. Increasing the concentration to 0.25% resulted in a growth inhibition of 39% and 20% for both fungi, respectively. Jia et al. (2019) indicated that immersing paper samples in a solution containing a mixture of 0.2 grams of ZnO NPs and varying concentrations of cellulose nanocrystals (CNC) and isopropyl alcohol reduced the colony size *of A. niger* by 7.8% compared to control samples after one month of incubation. Samples treated with larger-sized cellulose nanocrystals and ZnO NPs showed a greater reduction of 75.6%. [34]. Furthermore, Zajac *et al.* [35] found that using Ag NPs with nanoscale sizes (10–80 nanometers) at a concentration of 90 ppm reduced the growth rate by 51.73–88.5%.

The impact of fungi on paper samples becomes evident over time, as fungal colonization on the paper surface leads to the secretion of destructive acids that weaken the bonds between cellulose units, resulting in a decrease in the mechanical properties of the paper. Upon examination of the infected samples, fungi spread among the fibers, leading to a decrease in tensile strength for samples infected with *A. niger* and *A. terreus* (3.58N, 3.95N) compared to the standard sample (5.67N). Likewise, the average elongation decreased to 0.46 mm (0.4 mm) for the infected samples compared to the standard sample (0.7 mm).

The use of Ag NPs had an inhibitory effect on fungal growth, with an inhibition rate of 100% for *A. niger* and *T. viride* fungi at a concentration of 50 ppm in isopropyl alcohol. Conversely, the lower concentration of zinc oxide (0.9 g) was less effective, with higher concentrations of zinc oxide nanoparticles resulting in inhibition rates of up to 79.85% for *T. viride*. High concentrations of nanoparticles contributed to improving the tensile strength of the samples. The use of ZnO NPs led to an increase in tensile strength to 6.44N for the sample infected with *A. flavus*, while it reached 6.66N for the sample treated with *T. viride*. Similarly, the average tensile strength increased to 6.56N for the infected sample treated with silver nanoparticles. Only the sample infected with *A. niger* showed significant color changes, with the highest color change ratio recorded at 11.11=ΔE*. In contrast, the sample infected with *A. terreus* showed the lowest color change ratio at 2.03=ΔE*. Regarding samples treated with zinc oxide nanoparticles, the highest

color change value (3.64) was recorded for the sample treated with *A. terreus*, while the sample treated with *A. flavus* showed the lowest color change value at 1.29=ΔE*. The sample infected with *A. flavus* and treated with silver nanoparticles showed the highest color change value at 3.28=ΔE*, while the sample treated with *T. viride* showed the lowest color change value at 0.62=ΔE*. Therefore, the color change values for treated samples were relatively better compared to infected samples, especially those treated with silver nanoparticles.

5. Conclusions

The current study discovered that historical paper documents are colonized by a variety of fungi, *including Aspergillus terreus, A. niger, A. flavus, Penicillium chrysogenum, Trichoderma viride, A. tamarii,* and *Gliocladium fimbriatum*. While most of these isolates are recognized as common paper contaminants, some have been previously observed on library materials. Furthermore, it has been confirmed that these isolates alter the aesthetic appearance and cause significant losses in industrially contaminated paper. The presence of these fungi within paper materials, coupled with their ability to degrade cellulose, indicates their potential role in the deterioration of historical paper. This report enhances understanding regarding the microbial communities inhabiting ancient paper materials in libraries and their association with paper degradation and the decline in paper properties. Based on the positive results obtained from silver nanoparticles acting as antifungal agents and zinc oxide nanoparticles improving mechanical and optical paper effects, concentrations of 0.3%, 0.6%, and 0.9% zinc oxide nanoparticles, as well as concentrations of 50, 30, and 20 ppm silver nanoparticles, were utilized. A concentration of 0.9% isopropyl alcohol in zinc oxide nanoparticles and a concentration of 50 ppm in silver nanoparticles exhibited inhibition rates reaching 90% and 100% for *A. niger*, respectively. These concentrations were applied to infected samples, which were examined using scanning electron microscopy. The infected samples displayed high fungal density, especially *A. niger* and *A. terreus*, with clear penetration of threads and microorganisms between the fibers. There was a general weakening in the mechanical properties of the paper, such as tensile strength and elongation. We anticipate that future studies will focus on preparing a mixture of these two substances and conducting tests on experimental samples as an initial step before implementing them in papers.

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