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A Comparative Study in Antifungal Activity Between Zinc Oxide and Titanium Dioxide Nanoparticles Against Aspergillus Niger in Tanned Leather

Elsayda-Nafesa EL-SHAMY
Faculty of Archaeology & Tourism Guidance, Misr University of Science & Technology, Egypt
alsaydanafesa@hotmail.com

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ABSTRACT

Tanned leather artifacts are susceptible to microbiological growth, causing deterioration when suitable temperature and humidity conditions are found due to leather containing a nutrient medium of protein and lipids. This study aims to compare the antifungal activity between Zinc Oxide (ZnONPs) and Titanium Dioxide (TiO2 NPs) nanoparticles against Aspergillus niger on the infected leather samples through microbiological ageing. Also the author applied two different techniques of applying nanomaterials via soaking and spraying. Color changes; Mechanical properties (tensile strength – elongation) and pH value have been measured to estimate the stability of leather against Aspergillus niger. The results revealed that ZnONPs can prevent the hyphal growth of Aspergillus in comparison to untreated samples because of the high antifungal activity in ZnONPs. Compared with the control sample in which the inhibition growth percentage was 0%, the inhibition of growth ZnONPs against Aspergillus in tanned leather, was treated by the soaking technique was 78%. Also, the inhibition of the growth of TiO2 NPs against Aspergillus in the tanned leather sample treated by the same soaking method was 22.2%. However, in the case of spraying, the results were not satisfying as the inhibition of growth in the case of ZnONPs was 32.4% but the inhibition of growth in the case of TiO2 NPs was 18.6%. The results proved a decrease in the color change for both Zinc and Titanium nanoparticles compared with the control samples. The author found ZnONPs are better than TiO2 NPs in results of lightness (L value), red-green color (a value) and yellow-blue color (b value). The results showed a difference between the ZnONPs and TiO2 NPs results as ZnONPs is better than TiO2 NPs in Elongation and Tensile Strength. Also, the soaking technique results are better than the spraying technique. pH value proved the effectiveness of the ZnONPs more than TiO2 NPs in controlling Aspergillus due to the enzymes produced by the fungi which affected and damaged the leather samples. So, the author recommended the ZnONPs in controlling the Aspergillus niger and preferred the soaking technique rather than spraying for applying the materials.

INTRODUCTION:

Tanned leather artifacts represent a complex composition. However, the international standards of conservation are not applied in places where their preservation is expected to occur e.g. museums, libraries, etc. in Egypt where leather artifacts are widespread (Fatma M. Helmia, 2021, pp. pp.40-52.).

Leather is very susceptible material to microbiological degradation. It exposed to fungi attack during manufacture and finishing process as the skin has a nutrient source, which comprises fats, proteins and carbohydrates that serve as substrates for the growth of microorganisms. Therefore, storing leather under varying unsuitable environmental conditions resulted in different types of biological degradation as an essential factor for impairing aesthetic, functional and other properties. The author chose *Aspergillus Niger* because it is most common in affecting leathers (furfuracea & Zopf EXTRACTS, 2013, p. p.179; Rodica Roxana Constantinescu, Comparative Study Regarding Leather Biodegradability, 2015, pp. 15-2). These microorganisms produce enzymes that analyze macromolecules into smaller parts which can be absorbed through cell membranes as nutrients for an energy source. The fungal contamination appears as colored stains on the leather. Hence, fungus prevention becomes a critical processes in conservation (Giovanni De Filpo, 2013, pp. 217-222). But using fungicides has many effects on the environment. Therefore, the author used safe alternative materials to improve leather durability in line with the ageing cycle (Rodica Roxana Constantinescu, 2015, pp. 2-14). Nanotechnology is a great term that covers the functional structures of materials at dimensions measured in nanometers which were highly improved in different properties due to the limited size of their new molecules as properties of nanoparticles differ according to size, distribution and morphology (Raghad DH. Abdul Jalill, 2016, pp. 204-222). In various recent studies the use of nanomaterial as a resistance factor for organic materials against different microbiological degradation has been explained. Nanoparticles (NPs) including dimensions between 1 and 1000nm in size are applied widely in the conservation field nowadays. Metal NPs have been intensively studied in other fields and also in conservation but not studied or applied enough in archaeological leathers, so the author chose to use them here in the study to compare both zinc and titanium nanoparticles in order to evaluate the antifungal activity on the tanned leather (Clausen C. , 2007, p. p. 10) (Freeman & Mcintyre, 2008, pp. pp.6-27) (Kartal, Green III, & Clausen, Do the unique properties of nanometals affect leachability or efficacy against fungi and termites?, 2009, pp. 490–495) (Yu, Jiang, Wang, & Song, 2010, pp. pp.385-390) (Clausen, Green III, & Kartal, Weatherability and Leach Resistance of Wood Impregnated with Nano-Zinc Oxide, 2010, pp. pp.1464–1467) (Clausen, Kartal, Arango, & Green III, 2011, pp. 465-470) (Placeholder1) (Saha, Kocaefe, Sarkar, Boluk, & Pichette, 2011, pp. 183-190) (Placeholder3) (Sahin & Mantanis, 2011, pp. 41-48) (Afrouzi, Omidvar, & Marzbani, 2013, pp. 1200-1203).

The author chose to compare them according to the following reasons. ZnONPs is a white powder which is not soluble in water, but is soluble in mineral acids (either weak or strong), ammonia, acetic acid or formic acid. It has unique antibacterial and antifungal activities because it has an essential trace metal that has antioxidant properties which are very helpful against microorganisms. It is should be noted that ZnONPs has been chosen for many reasons: a biocompatible agent, cheap, has low toxicity and has a good conductive coating efficacy (Kartal, Green III, & Clausen, 2009, pp. pp.490–495) (Placeholder4) (Yu Gao, 2019, pp. 17-26).

Titanium dioxide (TiO_2) is a semiconductor. Its nanoparticles (NPs) are manufactured worldwide and applied in conservation. As TiO2 NPs have a lot of physicochemical characteristics in comparison with their fine particle (FP) (Hongbo Shi, 2013, pp. 18-20). TiO2 is naturally obtained from anatase, rutile, and brookite. TiO_2 nanoparticles became a new generation of advanced materials and are widely used in conservation because of their excellent optical and photocatalytic properties, which exhibit unusual structural, optical, electronic, magnetic and chemical properties (Dalvandi, Ghasemi, & Hossain, 2013, pp. 68-72) (Chaudhary, Srivastava, & Kumar, 2011, pp. 855-874) (Martin Šebesta M. U., 2020, pp. 26-30).

Hence, using antimicrobial agent is necessary for the conservation of tanned leather to ensure the preservation process of the leather. Thus, this study aims to provide evidence of the importance of using antimicrobial agents to avoid the growth of fungi which cause loss of protein substance, the appearance of pigmented stains, as well as microstructural and physical-mechanical damage on tanned leather. It also aims to evaluate the efficacy of nanoparticles against the fungi causing decay. The comparison between ZnONPs and TiO_2 NPs according to their antifungal activity on tanned leather has not been applied and studied enough, so they were chosen to evaluate the extent of their anti-fungal activity against fungi such as (*Aspergillus)* and the extent to their effect on the properties of leather.

1. MATERIALS AND METHODS

1.1 Nanoparticles used:

Zinc oxide nanoparticles (ZnONPs) (White Powder; Brand: NT-ZONP; Batch number: 4016-S2; Solubility: Dispersed in water and suspension in ethanol /Methanol; Avg. Size (TEM): 30 ± 5nm ; Shape (TEM): Spherical like shape) and Titanium dioxide nanoparticles (TiO_2 NPs) (White Powder, Brand: NT-TiO_2-NP, Product Code: 4080-1, Solubility: Dispersed in Water / Ethanol; Avg. Size (TEM): 15 ± 2 nm; Shape (TEM): Quasi-Spherical like shape) were obtained from Nanotech Egypt for Photo-Electronics Company in powder form, but the suspension used in the experiment was prepared in a concentration of 5000 ppm showed in figs.1 (0.25 gm/ 50 ml).

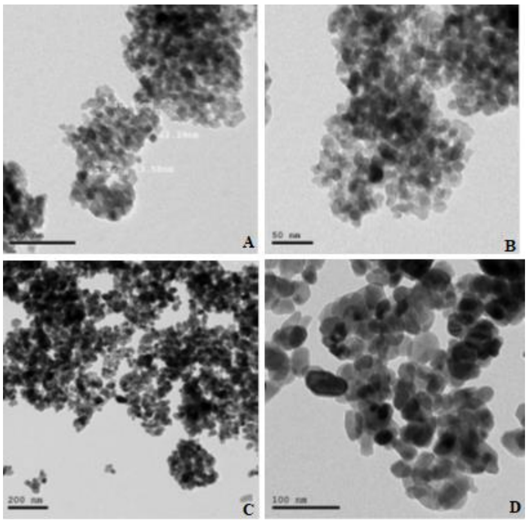

Fig.1. showing TEM micrographs of ZnONPs and TiO_2 NPS nanoparticles: A, B- showed TEM micrographs of Titanium dioxide (TiO_2 NPS); C, D- showed TEM micrographs of Zinc oxide (ZnONPs)

2.2. *Aspergillus sp.* isolation:

A pure isolate of *Aspergillus Niger* fungi was prepared in The College of Biotechnology, MISR University for Science and Technology, Egypt (local isolate). Some filaments of *Aspergillus Niger* strain (local isolate) were grown using a growth chamber (dark, 28˚C) in Potato Dextrose Agar (PDA) in 42 gm/l flasks for 72 hours.

2.3. Preparation of Nanoparticle Suspensions of (ZnONPs) and (TiO_2 NPs) and

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application technique:

Nanoparticles of ZnONPs and TiO_2 NPs used in the experiment were in the form of suspensions, and their application was by spraying onto the samples. The suspension used in the experiment was prepared at a concentration of 5000 ppm. The solution was prepared by adding 0.25 gm of powder to 50 ml of the nanoparticle solvent Thewhite powder of ZnONPs is dispersed in distilled water and a suspension of ethanol /Methanol. Still the white powder of TiO_2 NPs is dispersed in distilled water/Ethanol. So, the solutions were prepared in a volumetric flask then using a stirring device to mix the powder with the solvent. The samples of tanned leather were then soaked in the prepared nanoparticle suspension of ZnONPs and TiO_2 NPs for 72 hours.

2.4.*Aspergillus Niger* cultivation on control/treated tanned leather samples with the selected nanoparticles (Accelerated microbiological ageing):

New tanned leather samples were obtained from tanneries in Egypt (vegetable tanned leather from sheep animal samples) which were prepared following the ancient prescription found in the references of this field (Reed, 1972, pp. pp. 314-319).

The PDA medium was used in the experiment. Also, there was negative control in all experiments. The spreading spores technique was applied to samples using metal wire as spore spreaders sterilized ina flame then left to cool. The spores were spread rapidly but gently back and forth through the surface of the agar while rotating the petri dish slowly with the other hand which would be in random arrangement (Sanders, 2012).

The spraying technique was used to apply the nanoparticles to the leather samples.

Petri dishes (plates containing the control/ treated with selected nanoparticles leather, culture media, and fungal spores) were inoculated and incubated at 28°C and regularly checked every day for 8-10 days to determine fungi growth.

Stains and Damage Caused by fungal attack on the samples of vegetable-tanned leather were evaluated. The antifungal activity of selected nanoparticles was checked as the antifungal activity of ZnONPs and TiO_2 NPs were evaluated against *Aspergillus sp.* The results of fungal growth in the colonies were measured including the percentage of inhibition growth was calculated Then the percentage of the spore's inhibition rate was calculated. Observations were also made using a microscopic optical lens camera. .

Then the antifungal activity of the ZnONPs and TiO_2 NPs against *Aspergillus Niger* was evaluated. The diameter of mould growth (in cm), in each Petri plate, was then measured.

The inhibition of growth (%) of *Aspergillus Niger* was calculated from the formula:

Inhibition of growth of test fungi = [(the average increase in mycelial growth in the control sample - the average increase in mycelial growth in the test samples)/ the average increase in mycelial growth in the control] x 100

2.4. Color changes by spectrophotometer:

Color changes were measured using CIE L*a*b* system for the treated leather samples using a UV spectrophotometer at the Textile Testing Lab., Division of Chemical Metrology, National Institute for Standards, Egypt.

2.5. Mechanical properties (tensile strength – elongation):

Mechanical properties of leather samples were tested using a tensile testing machine model H5KT, Tinius Olsen Co. SDL-UK of capacity 5kN (1,000 lbf) in the Textile Testing Lab., Division of Chemical Metrology, National Institute for Standards, Egypt.

2.6. pH value:

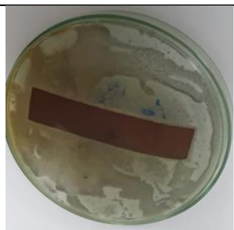
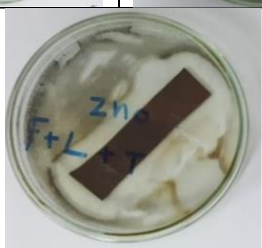
Using pH meter in the Research and Development Center - MISR University for Science and Technology (MUST), Egypt.

3. RESULTS

3.1. Accelerated microbiological ageing with the treated leather samples:

The author evaluated the efficiency of different nanoparticles in resisting and inhibiting fungal activity on tanned leather samples by applying the following equation,
Inhibition of growth of test fungi = [(the average increase in mycelial growth in the control sample - the average increase in mycelial growth in the test samples)/ the average increase in mycelial growth in the control] x 100. The results obtained and attached to the table according to these lab measurements: the diameter of the inhibition halo in the zinc oxide dish is approximately 5.5 cm, and 2.5 cm is subtracted for the leather area, so the diameter of the aura will be 3 cm, and then the resulting number is divided by 13.5 cm and then multiplied by 100 to get the percentage of the diameter of the inhibition halo.
The results showed that Nano-ZnO and Nano-TiO_2 had good effects on preventing samples of leather from fungal decay but at different rates. The result explained that both ZnONPs and TiO_2 NPs possess an inhibitory effect on the growth of *Aspergillus Niger*. However, ZnONPs antifungal activity was more effective than TiO_2 NPs against *Aspergillus Niger.* There are a lower number of conidia on the surface of the leather samples in the petri dish treated with ZnONPs because the growth was further inhibited by ZnONPs antifungal activity.

Table 1 showing inhibition percentage of *Aspergillus Niger* growth in tanned leather samples treated with Nano materials (Zinc oxide and Titanium dioxide) compared to control samples

Inhibition of growth	Negative Control		The fungi with the leather sample without any treatment of nanoparticles
	Control	 	0%
	Zinc oxide (ZnONPs) nanoparticles against *Aspergillus* in tanned leather (soaking technique)		78%

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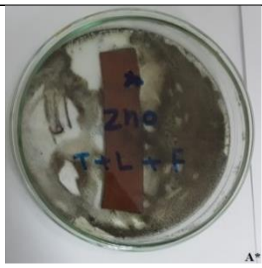
	Zinc oxide (ZnONPs) nanoparticles against *Aspergillus* in tanned leather (spraying technique)		32.4%
	Titanium dioxide (TiO_2 NPs) nanoparticles against *Aspergillus* in tanned leather (soaking technique)		22.2%
	Titanium dioxide (TiO_2 NPs) nanoparticles against *Aspergillus* in tanned leather (spraying technique)		18.6%

In comparison with the control sample, the inhibition growth percentage was 0%, but the inhibition of growth ZnONPs against *Aspergillus* in tanned leather treated by the soaking technique was 78%. Also, the inhibition of growth TiO_2 NPs against *Aspergillus* in tanned leather treated by same soaking technique was 22.2%. However, in the case of spraying, the results were not as satisfying, as the inhibition of growth ZnONPs against *Aspergillus* in the tanned leather samples was 32.4%. In comparison, the inhibition of growth TiO_2 NPs against *Aspergillus* in the tanned leather samples was 18.6%. Therefore, the author recommendedZnONPs in controlling the *Aspergillus Niger* also preferring the soaking technique rather than applying it to the materials via spraying.

3.2. Color changes by spectrophotometer:

Aspergillus Niger causes extensive color changes in the leather substrate according to the stains produced by mould growth. The results in table 2 showed that there is a decrease in the effects of both Zinc and Titanium nanoparticles compared to the control samples (from 5.57 to 1.51 in the case of ZnONPs using the soaking technique and from 5.57 to 1.42 using the spraying technique but from 5.43 to 3.32 and 3.02 of TiO_2 NPs in lightness for both soaking and spraying techniques). However, the results of ZnONPs are better than TiO_2 NPs in lightness (L value), red-green color (a value) and yellow-blue color (b value). The results of a total color difference are decreased in both Zinc and Titanium nanoparticles compared to the control samples (from 4.85 to 2.18 of ZnONPs and from 4.78 to 3.23 of TiO_2 NPs). Also, the results of the soaking technique are better than the spraying technique (1.51 compared to 1.42 in ZnONPs and 3.32 compared to 3.02 in TiO_2 NPs

Table 2 showing the Color changes results of the treated leather samples with nanoparticles against *Aspergillus*

Nanoparticles against *Aspergillus* in tanned leather samples	Color values			Total color difference
	L	a	b	ΔE
Control	5.57	1.27	3.15	4.85
Zinc oxide (ZnONPs) nanoparticles **(soaking technique)**	1.51	1.43	2.08	2.23
Zinc oxide (ZnONPs) nanoparticles **(spraying technique)**	1.42	1.33	2.00	2.18
Control	5.43	1.25	2.14	4.78
Titanium dioxide (TiO_2 NPs) nanoparticles **(soaking technique)**	3.32	0.82	4.11	3.74
Titanium dioxide (TiO_2 NPs) nanoparticles **(spraying technique)**	3.02	0.18	4.08	3.23

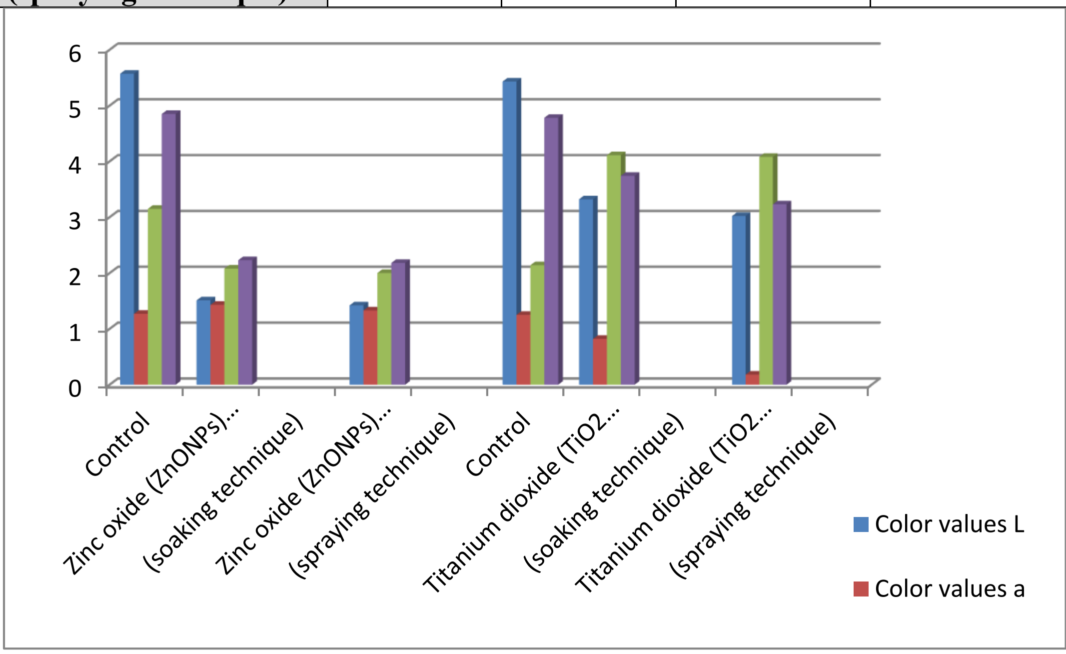


Fig.2. showing Color changes results of the treated leather samples with nanoparticles against *Aspergillus*

3.3. Mechanical properties (tensile strength – elongation):

The mechanical properties (the elongation and the tensile strength) have been measured in order to estimate the resistance of the treated leather samples with nanoparticles against *Aspergillus Niger* compared to the control samples. Results showed a a difference between the ZnONPs and TiO_2 NPs in elongation and the tensile strength (from 11.14 of ZnONPs to 10.08 of TiO_2 NPs in Elongation and from 21.76 of ZnONPs to 20.23 of TiO_2 NPs in Tensile Strength). Also, the results of the soaking technique are better than the spraying technique in both ZnONPs and TiO_2 NPs.

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Elongation		Tensile Strength	
Control	12.18	**Control**	22.05
Zinc oxide (ZnONPs) nanoparticles (soaking technique)	11.14	**Zinc oxide (ZnONPs) nanoparticles (soaking technique)**	21.76
Zinc oxide (ZnONPs) nanoparticles (spraying technique)	11.08	**Zinc oxide (ZnONPs) nanoparticles (spraying technique)**	21.33
Titanium dioxide (TiO_2 NPs) nanoparticles (soaking technique)	10.08	**Titanium dioxide (TiO_2 NPs) nanoparticles (soaking technique)**	20.23
Titanium dioxide (TiO_2 NPs) nanoparticles (spraying technique)	10.00	**Titanium dioxide (TiO_2 NPs) nanoparticles (spraying technique)**	20.04

3.4. pH value:

It was essential to measure the pH value to estimate the ability of *Aspergillus Niger* todamage the leather samples due to the enzymes produced by the fungi. The results showed an increase in the results compared to the control samples in case of ZnONPs from 4.15 to 5, but there is a decrease in the results compared to control samples in the case of TiO_2 NPs from 5.13 to 4.23. Also, the soaking technique results are better than the spraying technique in both ZnONPs and TiO_2 NPs.

Nanoparticles against *Aspergillus* in tanned leather samples	pH value
Control	4.15
Zinc oxide (ZnONPs) nanoparticles (soaking technique)	5
Zinc oxide (ZnONPs) nanoparticles (spraying technique)	4.50
Control	5.13
Titanium dioxide (TiO_2 NPs) nanoparticles (soaking technique)	4.23
Titanium dioxide (TiO_2 NPs) nanoparticles (spraying technique)	4.10

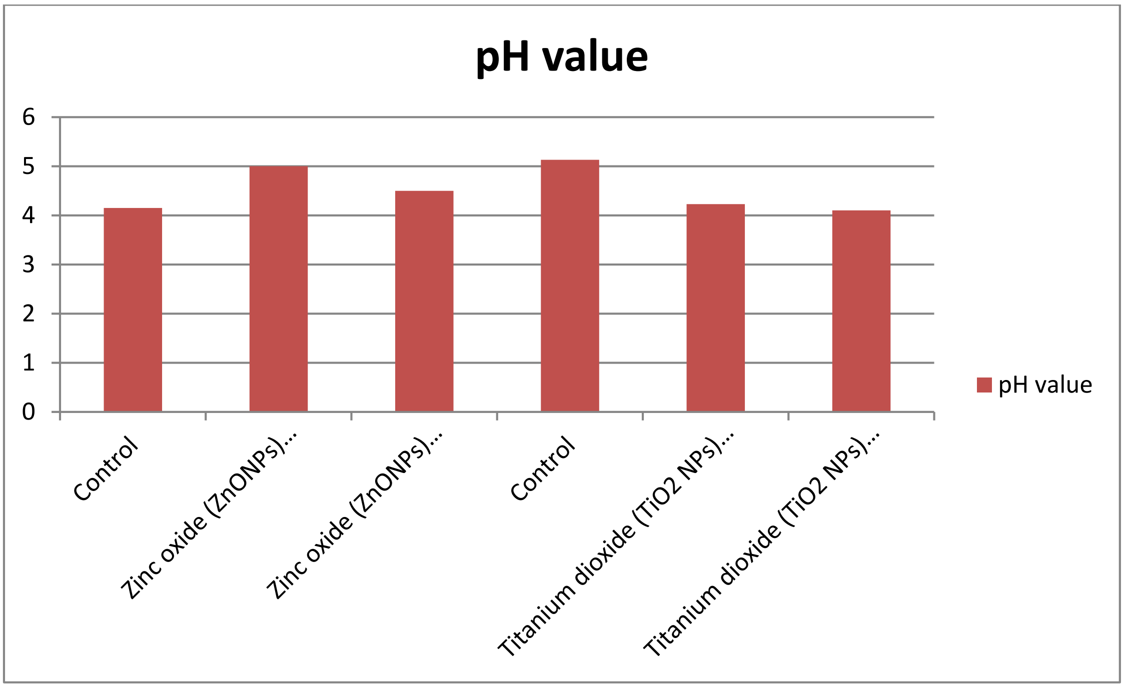

Fig.3. showing pH values measurements of ZnONPs and TiO_2 NPs Nanoparticles against *Aspergillus* in tanned leather samples.

4. DISCUSSION

Aspergillus Niger is the most aggressive and invasive species, which develops rapidly. A colony of *Aspergillus* appeared and covered the surface of the sample. All leather samples inoculated with *Aspergillus Niger* were entirely covered by mould. The growth of fungi on leather can lead to a variety of undesirable defects such as pigmented spots, which are difficult to remove, non-uniform dyeing and finishing, reduction of physical and mechanical properties and harmful changes in the leather surface. The results revealed that ZnONPs can prevent the hyphal growth of *Aspergillus* which solely depends on the dose in comparison to control sample ZnONPs. The antifungal characteristics of nano-ZnONPs are related to entering the cell wall structure; also, some metabolism processes resulted in cell death. The filament of *A. Niger* during the 7-day cultivation period resulted in a decrease of pH from 5.6 to 2.4 according to presence of Zn. The resistance against the *A. Niger* increased with the increment of nano-ZnO concentrations and was almost 15 times lower than the control sample. In comparison, the rates in the nano-Ti_2O concentrations were lower than nano-ZnO-NPs in resistance against the *A. Niger* (Lia, et al., 2009, pp. 504-209) (Meghmala S. Waghmode, 2019) (Martin Šebesta M. U., 2020).

Color change; and Mechanical properties (tensile strength – elongation); pH values are important values to be measured to evaluate the microbiological degradation in the leather samples. *Aspergillus Niger* affects the appearances of leather leading to the color fading, discoloration and other deteriorations aspects according to the stains produced by mould growth. The pH values referred to high concentration of the hydrogen $(H)^+$ Ions in the samples. The high concentration of the hydrogen Ion indicated to breaking of the inner connected bonds of the collagen resulted from the fungi enzymes. Mechanical properties have been measured to evaluate the leather resistance against fungi.

The results proved a decrease in the color change in both Zinc and Titanium nanoparticles in comparison with the control samples. However, the results of ZnONPs are better than TiO_2 NPs nanoparticles in the lightness (L value), red-green color (a value) and yellow-blue color (b value). Besides the results of a total color difference is the reduction in both Zinc and

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Titanium nanoparticles compared to the control samples. The results of the soaking technique are better than the spraying technique, but there is an increase in the pH value results of ZnONPs and a decrease of TiO_2 NPs compared to the control samples. Also, the results of the soaking technique are better than the spraying technique in both of ZnONPs and TiO_2 NPs. Besides there are differences between the ZnONPs and TiO_2 NPs in the elongation and tensile strength results.

5. CONCLUSION

It has been concluded by this study that no leather type is completely resistant to fungal attack which led us to show the importance of antifungal nanomaterials. Additionally, the application of antifungal nanomaterials using the soaking technique is better than the spraying technique.

After measuring the color change, mechanical properties (tensile strength – elongation); and pH values the results proved that the antifungal properties of zinc oxide nanoparticles are better than Titanium dioxide nanoparticles against *Aspergillus* in tanned leather. In addition, the author recommended the soaking technique for applying antifungal nanomaterials.

Also, the author recommended putting a protective film layer of zinc oxide nanoparticles as it is super oxidative, hydrophilic and becomes an unseen film which can be applied to leather as an antifungal agent. Therefore, it can be used as a suitable preservative layer in organic materials. Thus, new research ideas have been opened for the study of protecting leather from fungal damage in the future by placing a protective layer of nanomaterials, and these studies should be taken into consideration.

6. RECOMMENDATIONS

The most common fungi contaminating museums was *Aspergillus*. From the study results, the author recommended that ZnONPs can be used as a disinfectant for the surface of archaeological collectables which is in accordance with the requirements of world heritage preservation and storage.

Hence, we advise researchers to broaden research horizons and open fields to study nanomaterials generally in controlling fungi, even though the author has faced some laboratory obstacles and financial difficulties in obtaining experimental samples of leather, fungi and nanomaterials and applying them in the field of microbiological resistance.

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دراسة مقارنة في النشاط المضاد للفطريات بين جزيئات أكسيد الزنك وثاني أكسيد التيتانيوم النانوية ضد الرشاشيات النيجرية في الجلود المدبوغة

السيدة نفيسة الشامي
كلية الاثار والارشاد السياحي،
جامعة مصر للعلوم والتكنولوجيا
alsaydanafesa@hotmail.com

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الكلمات الدالة

تثبيط؛
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جلد مدبوغ؛
فطريات؛ النقع؛
اكسيد الزنك؛
ثاني أكسيد التيتانيوم

الملخص

تعد المشغولات الجلدية المدبوغة مواد حساسة لنمو التلف الميكروبيولوجي عند توافر الظروف المناسبة من درجة الحرارة والرطوبة بسبب احتواء الجلد على وسط غذائي من البروتين والدهون. تهدف هذه الدراسة إلى مقارنة الفعالية المضادة للفطريات بين جزيئات أكسيد الزنك (ZnONPs) وثاني أكسيد التيتانيوم (TiO2 NPs) ضد فطر الاسبرجلس على عينات الجلد المصابة بالتلف الفطرى. كما طبق المؤلف طريقتين مختلفتين لتطبيق المواد النانوية عند طريق النقع والرش. تم قياس التغيير اللونى والخواص الميكانيكية (قوة الشد - الاستطالة) وقيمة الأس الهيدروجيني من اجل تقدير مدى ثبات الجلد ومقاومته ضد فطر الاسبرجلس. أوضحت النتائج أن الجزيئات النانوية لاكسيد الزنك يمكن أن تمنع النمو الفطرى مقارنة بالعينة غير المعالجة بسبب كفاءة اكسيد الزنك فى مقاومة الفطريات وذلك مقارنة بعينة الكنترول (العينة الضابطة) حيث كانت نسبة نمو التثبيط فيها ٠٪ بينما كانت نسبة التثبيط للنمو لعينات الجلد المدبوغ المصاب فى حالة تطبيق تكنيك النقع بالمواد النانوية لجزيئات اكسيد الزنك ٧٨٪. بينما كانت النسبة فى حالة المواد النانوية لثاني أكسيد التيتانيوم مع نفس التكنيك ٢٢,٢%. الا انه فى حالة تطبيق تكنيك الرش النتائج كانت غير مرضية فكانت نسبة تثبيط النمو فى حالة الجزيئات النانوية لاكسيد الزنك ٣٢,٤% بينما فى حالة الجزيئات النانوية لثاني أكسيد التيتانيوم ١٨,٦%. اثبتت النتائج انخفاضًا في التغير اللونى في كل من جسيمات الزنك والتيتانيوم النانوية مقارنة بعينات الكنترول. وجد المؤلف أن الجزيئات النانوية لاكسيد الزنك أفضل من الجزيئات النانوية لثاني أكسيد التيتانيوم في النتائج كما أن نتائج تقنية النقع أفضل من تقنية الرش في كليهما.

اثبتت قيمة الأس الهيدروجيني فعالية الجزيئات النانوية لاكسيد الزنك أكثر من الجزيئات النانوية لثاني أكسيد التيتانيوم في مقاومة فطر الاسبرجلس والذى بدوره يتلف الجلود المصابة نتيجة الانزيمات التي تنتجها الفطريات. لذلك أوصى المؤلف بأفضلية استخدام الجزيئات النانوية لاكسيد الزنك في مقاومة فطر الاسبرجلس كما يفضل أيضًا التطبيق من خلال تقنية النقع بدلاً من الرش لتطبيق المواد النانوية.