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Original article

BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES FROM FUNGI ISOLATED FROM DETERIORATED HISTORICAL GILDED CARTONNAGE AND ITS APPLICATION IN CLEANING

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Abstract:
Fungal biosynthesis of nanoparticles is considered an eco-friendly and safe tool for the synthesis of nanoparticles applied in various fields. In this work, iron oxide nanoparticles were biosynthesized by fungi isolated from fungal attacked archaeological cartonnage and used to clean deteriorated cartonnage. Six fungal strains were isolated and molecularly identified. These identified fungi were used for the biosynthesis of nanoparticles of iron oxide from both ferrous sulfate and ferric chloride. The most appropriate nano-particles for cleaning artworks were produced from Penicillium commune MH5. Iron oxide nanoparticles were characterized using colorimeter, Ultraviolet-visible spectroscopy, X-ray diffraction, and Transmission electron microscope studies. Iron nanoparticles were added to magnetic nano gel, microemulsions micelles solutions (Oil in water microemulsion made with sodium dodecyl sulfate (SDS), 1-pentanol (PeOH) as co-surfactant, propylene carbonate (PC), pure ethyl acetate are replaced by less toxic solvents), and compared with nano gel which uses in cleaning cartonnage which fungi isolated from it. The Fourier transform infrared spectroscopy, scanning electron microscopy, and coloring measurement was used to evaluate the successful effect of the new gel with the magnetic nanoparticles in cleaning the artwork surfaces.

1. Introduction

Nanotechnology is a growing and universally developing material science. Nano structure-based materials and the study of physicochemical phenomena at the nanoscale are introducing new approaches to conservation science [1]. Nanoscience is a unique resource to the restoration process as it recovers the mechanical properties of archeological materials due to their small particle size that penetrates well into the material to be treated [2]. As well as their large surface area, leading to new characterization that can slow down the degradation processes of artworks or even recover them from the damages introduced by detrimental rest-oration campaigns [3].

The cleaning process of painted artifacts is an important process to remove superficial layers that may encourage their further degradation or hide pictorial scenes. Traditional materials might cause damage to the artifact, or lead to conservator health hazards on the long-term application [4]. Recently, conservators tend to apply nanomaterials to avoid these problems and one of the most important scientific developments was nanoscience [5]. In this work, not only the advantages of using nanomaterials but also the bio-synthesis of nanoparticles in the laboratory in simple easy ways to treat the archeological artifacts were discussed. Biosynthesis of nanoparticles is considered as the most popular bottom-up green synthesis and biogenic [6]. Biological methods of synthesis of nanoparticles attracting many researchers due to their feasibility, less toxicity, environmentally friendly, simple, rapid, stable, less cost-effective, biocompatibility, and scalability [7]. A physicochemical way for the synthesis of nanoparticles used harsh chemicals and needs high energy demand [8]. Microorganisms are known as important factories for the biosynthesis of nanoparticles due to their ability to accumulate and detoxify heavy metals through the production of various reductase enzymes which can reduce metal salts to metal nanoparticles with narrow size distribution leading to less polydispersity [9]. Different nanoparticles were biosynthesized extra- or intracellularly by different microorganisms including fungi, bacteria, actinomycetes, and yeasts [10]. The extracellular synthesis has received much attention because it involves trapping the metal ions on the surface of the cells and reducing ions in the presence of enzymes [11]. Magnetic Fe and Fe_3O_4 (magnetite) nanoparticles are successfully synthesized using fungi. The magnetic properties are measured to exhibit super paramagnetic and ferromagnetic-like behaviors for Fe and Fe_3O_4 nanoparticles, respectively. The obtained results open a new route for using the biophysical method for large-scale production of highly magnetic nanoparticles to be used for biomedical applications [12]. Iron oxide nanoparticles have been widely favored because of their low cytotoxicity, biodegradable and reactive surface that can be modified with biocompatible coatings. The reaction process was simple, eco-friendly, inexpensive, and easy to handle. Green chemicals were employed to synthesize iron nanoparticles [13]. Iron is the magnetic metal that represents a feasible approach in different fields including the cleaning of the archaeological cartonnage [14]. Iron nano-particles represent a new magnetically responsive compartmentalized nano system. Functionalized magnetic nanoparticles have been chemically incorporated into gel structure that can be loaded with microemulsions or micelles solutions called a nanomagnetic gel [15]. Magnetic nano gel was imposed by the dominating scat-tering of the magnetic nanoparticles embedded into the polymeric matrix of the gel. The microemulsion was recovered by applying a magnetic field to the nanomagnetic gel, which causes shrinkage of the gel and the release of the microemulsion [16]. Magnetic nano gel has a lot of advantages due to the addition of metallic nanoparticles. It can be shaped as desired and applied to a specific area with fine spatial control of the area, easily moved with tweezers or cut with a knife and flexibility during the cleaning process, the residues of nano gel are limited by manipulation of magnetic nano gel by an external magnet, increase the activity of removing undesired layers out of the surfaces [17-19]. In the present investigation, an attempt was made for rapid, low-cost, and eco-friendly iron nanoparticle biosynthesis using the six fungi isolated from archeological cartonnage and using magnetic iron nanoparticles in the cleaning process to make nanomagnetic gel which used to clean

deteriorated cartonnage. The present study includes morphological and elemental characterization of the biosynthesized iron nanoparticles and nonmagnetic gel.

2. Materials and Methods

2.1. *Sampling, fungal isolation and identification*

Fungi were isolated from cartonnage dating back to a late-era period using the following method: Sterile cotton swabs were wiped across fungal colonies then transferred to the laboratory in sterile tubes and used for fungal isolation [20]. Furthermore, some other deteriorated pieces were scattered above the agar plate media and some other tiny dust was suspended in sterile distilled water and used to inoculate the agar medium. All samples were transferred to the laboratory on the same day of collection and immediately processed. Each swab of fungal growth was immersed in a sterile glass vial containing 5 ml of sterile distilled water and shake for 2 h on a reciprocal shaker. Aliquots (100 µl) of spore suspension were spread on each 10 cm Petri dishes (3 plates per sample) containing Potato-dextrose agar medium (g/l): peeled potato (200), glucose (20), and agar (20). This medium has been supplemented with the antibacterial agent (Penicillin G, 0.1%) and Rose Bengal [21] to limit the fungal growth. Plates were incubated for 7 to 14 days at 30 ^{o}C in the dark and the appearing single colonies were picked up cultivated on slants of the same medium (PDA) and incubated at 30 ^{o}C for 7 days and maintained at 4 ^{o}C as pure cultures.

2.2. *Fungal identification*

Fungal cultures (MH1, MH2, MH3, MH4, MH5, and MH6) were identified molecularly by DNA isolation, amplification (PCR), and sequencing of the ITS region [22]. The primers ITS2 (GCTGCGTTCTTCAT CGATGC) and ITS3 (GCATCGATGAA-GAACG CAGC) were used for polymerase chain reaction (PCR), while ITS1 (TCCG-TAGGTGAACC TGCGG) and ITS4 (TC-CTCCGCTTATTGATATGC) were used for base sequencing. The purification of the PCR products was done to eliminate separate PCR primers and dNTPs from PCR products using a Montage PCR Clean-up kit (Millipore). Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied Bios stems, USA) and *Candida* sp. was used as a control. Sequencing results were individually inputted online into the nucleotide BLAST program through the NCBI database to identify the isolates [23,24]. The gene sequences of the fungal isolates were deposited in the GenBank database.

2.3. *Biosynthesis and characterization of iron nanoparticles*

The isolated and identified fungi were screened for their ability to the biosynthesis of iron oxide nanoparticles and the produced nanoparticles were characterized.

2.3.1. Fungal cultivation

The isolated and molecularly identified 6 fungal strains were cultivated on Sabouraud Dextrose broth (g/l): dextrose (20), Peptone (10), and the final pH (5.6) with pH (5.1). One of the 10-days old slant was used to inoculate two Erlenmeyer flasks of a total volume of 250 ml each containing 50 ml of the broth medium. The inoculated flasks were incubated on a rotary shaker (150 rpm) for 7 days. The mycelia were separated from the culture by centrifugation at 4000 rpm on a cooling centrifuge model (Centerion). The culture supernatants were screened for their ability to biosynthesized iron oxide nanoparticles.

2.3.2. Biosynthesis of iron oxide nanoparticles

The culture was kept under shaking conditions (200 rpm) for 96 h at 28 ^{o}C. After 96 hours, the mycelia were separated from the cultural broth by centrifugations (500 rpm) at 10 °C for 20 min and the mycelial free culture supernatant was used as a source of reducing agent for nanoparticles

biosynthesis. Ferrous sulfate and ferric chloride solutions of 2×10^{-4}M concentration were prepared in 1000 ml distilled water and the pH was adjusted to pH 5.5. From this 100 ml solutions were taken in a 250 ml conical flask to which 10 ml mycelial free culture supernatant was added and shake for 24-72 h [25].

2.3.3. UV/Visible spectroscopy

The bio-reduction of soluble salts to NPs was monitored periodically by UV-vis spectroscopy (Shimazu 2401PC) after the dilution of the samples with deionized water [26]. A UV-vis spectrograph of the iron nanoparticles was recorded by using a quartz cuvette with water as a reference. The UV-vis spectrometric readings were recorded at a scanning speed of 200-800 nm [27].

2.3.4. X-Ray Diffraction

Powdered sample of iron nanoparticles was used for X-ray diffraction; the coherently diffracting Crystallography domain size of the iron nanoparticle was calculated from the width of the XRD peaks using the Scherrer formula. X-ray diffraction (XRD) measurements of *Penicillium commune* SH19 reduced iron nanoparticles were carried out on drop-coated films of the respective solutions onto glass substrates by a Phillips PW 1830 instrument operating at a voltage of 40 kV with Cu Ka radiation [28].

2.3.5. Transmission Electron Microscope

TEM analysis of iron NPs has been evaluated using the JEOL model 1200 EX electron microscope. The samples were prepared by placing a drop of the suspension of Fe-NPs solutions on carbon-coated copper grids and allowing water to evaporate. The samples on the grids were allowed to dry for 4 min. The shape and size of iron nanoparticles were determined from TEM micrographs [29].

2.4. *Experimental study*

2.4.1. Sample preparation

This study was conducted on experimental samples similar to the components of the archaeological cartonnage studied in the analytical study. The support is made of linen fabric; sample sizes are 5×10 cm. Then cut into rectangles and it was thoroughly washed and ironed not to shrink during preparation of gesso as a ground layer. The linen was soaked in animal glue solution. The ground was prepared in two layers composed of calcium carbonate and animal glue. Samples are painted according to the archaeological cartonnage; it was a form of mineral oxides (Egyptian blue-goethite-carbon black), then gilding leaf paste to the surface piece by cotton. The archeological cartonnage is simulated through an examination of the cartonnage deteri-oration phenomena by putting different stains on the surface, tab. (1).

Table (1) Methods of applying deterioration stains on the surface of experimental samples

Stain	Method
Dust and dirt	Cartonnage is exposed to the blurring of colors and distortion of the general appearance by the dirt and dust (Inside cracks located on the surface). Samples were left in the open air for three months (natural aging).
Salts Effloresces	The salt solution was made and sprayed the samples three times between each one-half hour, then put the samples in the open air for three months of natural aging.
Linen charred on the cartonnage	The bands were made by cut linen cloth into pieces lengthwise, then put the pieces into the oven at 200°C for one month. Charred linen was put in the sample then moisten to make a stain.
Stain arising from fungal damage	We cultivated swaps which wiped from archeological cartonnage and molecularly identified for 5 fungal strains on each sample on Sabouraud Dextrose broth (g/l): dextrose (20), Peptone (10), and the final pH (5.6) and potato dextrose broth (g/l): potato infusion (200), dextrose (20), and the pH (5.1). Then, we put the sample in a plastic box, closed this box with tape then leave it for one week to fungi grow on the sample. 1-*Aspergillus terreu,* 2-*Aspergillus Oryza,* 3- *Aspergillus tamari,* 4-*Aspergillus niger,* 5-*Penicillium commune,* and *Aspergillus ovoparasiticus.*
Previous restoration	**Presence of adhesive paper sticks with gum Arabic.** Some cartonnage exposed to previous restoration (study subject) where the restoration was made by support of paper and glued by Arabic gum, which we made in experimental samples. **Spots of using paraloid P 72.** Put a layer of paraloid B72 on the surface of samples and make thermal aging. **Using beeswax in restoration. Johnson, C.,** mentioned that some cartonnage were restored by a layer of wax, the problem of wax that on this material could sweat and develop brown film or a white bloom in the surface, some waxes can be brittle**)** put a layer of was in the samples [30].

2.4.2. Thermal aging cycles

Samples were twice exposed to artificial aging cycles. The first one before applying deterioration stains and the second was before applying cleaning materials to become substantially similar to the case of the archaeological cartonnage. Samples were exposed to the frequency at temperature and humidity stability according to the standard specifications (Din 321-2002, ASTMD 1037-1999). These specifications showed that the frequency of the (T) and (RH) stability or vice versa helping to speed the collapse of the properties of materials**.** The accelerated aging plan that was used in this study consisted of 12 cycles of wet and dry periods with relative humidity (RH) changed between 90% to 30% and a constant temperature of 70 °C. This treatment was optimized for indoor conditions based on ASTM 1037-1999 and the guidance of DIN 321-2002. The aging treatment started with a wet step (90% RH) for 3 h, followed by a dry step (30% RH) for 3 h, a wet step again for 3 h, and finally a dry step for18 h. This process was repeated for 4 cycles for a week as presented in the tab. (2)

Table (2) Aging cycles conditions (T) vs. normal conditions (N) [31].

Treatment stages	Time	N (control of samples)
Wet 70 °C - 90% RH	180 min (3 h)	--
Dry 70 °C - 30% RH	180 min (3 h)	Standard climate Chamber
Wet 70 °C - 90% RH	180 min (3 h)	20 ± 1 °C
Dry 70 °C - 30% RH	180 min (18 h)	65 ± 3% RH
No. of cycles	4	Depends on samples, from 3 days to 3 weeks
Total time	5 days and 20 h	--

2.5. *Preparation of synthesis nano gel and nanomagnetic gel*

Nano gel: Oil in water micro-emulsion has been created using sodium dodecyl sulfate (SDS), 1-pentanol (PeOH) as a co-surfactant. Propylene carbonate (PC) and pure ethyl acetate (EtOAc) are replaced by less toxic solvents. They are partially water-soluble (EtOAc 8% w/w and PC 20% w/w). The addition of SDS and 1-PeOH either to the EtOAc or PC aqueous solutions provides a drastic improvement in the efficiency of the removal process, due to the presence of micelles that provide the large interfacial area necessary for spot uptake [32]. The microemulsions were mixed in the ratio 1: 0.75 (v/v) with cellulose powders forming gelatin film [33]. The system used is composed (wt %) of water (73.3), SDS (3.7), 1-PeOH (7), PC (8), and EtOAc (8). *Nano-magnetic gel*: The nonmagnetic gel is a result of adding nanomagnetic particles, previously prepared by fungi, to nano gel to control the gel and make sure not to leave residue on the surface and the gel can be removed completely by the magnet. The nanomagnetic gel was prepared briefly as follows: nano gel (2.5 g) was added stepwise to pre-dissolved Fe_3O_4 (0.5 g in 100 ml distilled water. After 30 min the temperature was raised to 35 °C for 24 h to complete the polymerization process. The microemulsion was mixed in the ratio of 1: 0.75 (v/v) with cellulose powders forming gelatin film to be used in restoration.

2.6. *Application and evaluation of nano gel and magnetic nano gel*

2.6.1. FTIR Spectroscopy

It was important to verify if nano gel and nanomagnetic gel were effective. This was done by FT-IR measurements using a model analyzed as KBr pellets by Jasco FTIR 460 plus spectrometer. FTIR is a 4100 Jasco-Jap. The sample was prepared using KBr. The spectra in absorbance mode were recorded in the range from 400 to 4000 cm^{-1}, after 32 scans, with a resolution of 4 cm^{-1}. Calibration of the wave number was automatically done by the instrument using an internal polystyrene film. Spectra reported here were compensated from a background spectrum obtained with a blank KBr pellet.

2.6.2. Light microscopy

The samples were examined after mounting gel on a glass slide by the optical microscope with power magnification 500-X in National Research Center.

2.6.3. Scanning electron microscopy

A study on morphology was performed with scanning electron microscopy to obtain data on dispersion and particle agglomeration. A layer of Japanese tissue paper and gel materials were cut into square pieces and placed over the surface that needs cleaning. Then put a layer of polyethylene, to keep the moisture ratio of the gel. The poultice remained on the surface for 4-6 hours. After ending the cleaning process the layer was removed from the surface, but a magnetic nano gel magnet was used to remove the remains of compresses. In some spots, we must remove it mechanically before applying the cleaning materials. This spot like charred linen and wax. Surface stains were examined and analyzed before the application of conventional and nanoparticle cleaners to identify the shape of the spots and the extent of their adhesion and their impact on the cartonnage, then tests and analysis were carried out after the application of the cleaning materials and the removal of the surface stains, to judge the effectiveness of the cleaning materials used and the resistance between samples before and after cleaning. SEM was used to examine the surface of experimental samples of cartonnage after application stain and procedure artificial aging, and that to compare between this sample and the samples after applying the cleaning materials and remove surface stains.

2.6.4. FTIR

The experimental samples were analyzed by FTIR before and after application of cleaning materials to monitor changes in the chemical bonds of the surface and the impact before and after cleaning of the aging process of the samples.

2.6.5. Colorimetric determination

Colorimetric measurements were performed by the Commission International de I'Eclaraige "CIE" lab color system "1976" using Switzerland Spectro densitometer "Exact X-Rite".

3. Results

Table (3) clarifies the different samples collected from different parts of deteriorated cartonnage and the way how the samples were collected either by swab or using the rotten pieces. In this respect, nine samples were collected and used for fungal isolation. The microbiological examination of the foot case cartonnage showed that it was invaded with the following fungal micro flora *Aspergillus flavipes*, *Aspergillus ochraceus*, *Aspergillus terreus* *Penicillium chrysogenum*, *Penicillium steckii*, and *Scopulariopsis candida* [34].

Table (3) Samples, parts used, and manner of isolation of fungi from historical cart-onnage.

Serial	Part used	Manner of sampling
1	Support	linen pieces
2	Gilding layer	swab
3	Ground layer	swab
4	Ground layer	ground pieces
5	Cartonnage debris	powder
6	Coloring layer	swab
7	Support and ground layer	swab
8	Coloring layer	swab
9	Ground layer	support stick

3.1. *Molecular identification of the fungal isolates*

Fungal isolates MH1, MH2, MH3, MH4, MH5, and MH6 with nucleotide sequences of 604, 594, 597, 596, 564, and 585bp (respectively) were identified using the whole 18S rRNA gene of the fungal spp in both strands. Blast search revealed 100% similarity of MH1, MH2, MH3, MH4, MH5, and MH6 to *Aspergillus terreus* strain PAS3 (Acc. no. KY806124.1), *Aspergillus oryzae* isolate CF (Acc. no. KY006837.1), *Aspergillus tamarii* isolate AV11 (Acc. no. MH 517369.1), *Aspergillus niger* strain CMX 23807 (Acc. no. MG991648.1), *Penicillium commune* strain 2.5.4.5 (Acc. no. KX67462 6.1) and *Aspergillus novo-parasiticus* isolate DTO 223-C5 (Acc. no. MH279415.1), respectively. These fungi were identified as *Aspergillus terreus* MH1, *Aspergillus oryzae* MH2, *Aspergillus tamarii* MH3, *Aspergillus*

niger MH4, *Penicillium commune* MH5 and *Aspergillus novo parasiticus* MH6, respectively with the Gene Bank accession numbers MH562044, MH562045, MH562 046, MH562047, MH 562048, and MH56 2049, respectively. The phylogenetic tree of the identified fungi to each other is illustrated in fig. (1). The identification of fungi up to the genus level could be attributed by macroscopic and microscopic examination by studying colony color, shape, hyphae, conidia conidiophores as well as the spores arrangement, whereas molecular identification of fungi reach the species level in which the extracted fungal DNA was amplified by PCR using specific internal transcribed spacer primer (ITS1/ ITS4). The PCR products were sequenced and compared with the other related sequences in GenBank (NCBI) Alsohaili and Bani-Hasan, 2018). Molecular identification protocols, 18 SrRNA, by DNA extraction provide a unique fingerprint for the identification of different fungal isolates up to a species level [35]. Molecular identification is an essential tool for mycologists who studying fungal taxonomy, molecular development, population genetics, or fungus-plant relations [36]. Fungal identification using molecular techniques is achieved by the sequencing of PCR amplified part of 18S rRNA genes using universal primers to fungal species [37,38].

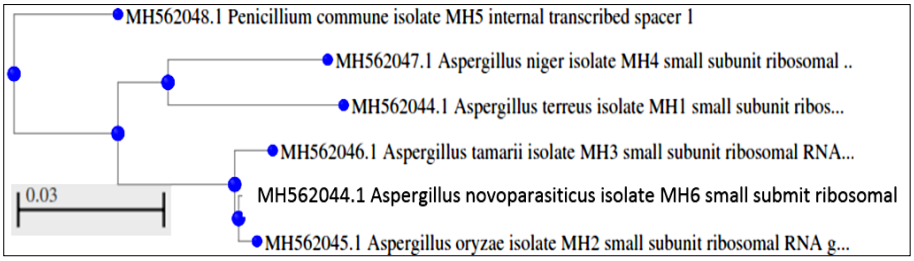

Figure (1) Shows the phylogenetic tree of different isolated and molecularly identified (18SrRNA) fungi from the deteriorated cartonnage.

3.2. *Biosynthesized iron nanoparticles color and UV/Vis*

Ferric chloride, ferrous sulfate were added to the culture filtrates of the isolated fungi that were grown on Saboraud for 7 days. The different fungal filtrates of mycelial free culture filtrate were screened for their ability to biosynthesize iron oxide nanoparticles by mixing these fungal filtrates with both ferric chloride ($FeCl_3$, 1mM) and ferrous sulfate ($FeSO_4$, 1mM). Results in fig. (2) exhibited a clear color change in the case of *Penicillium commune* MH5 for both $FeCl_3$ and $FeSO_4$. UV/Vis spectroscopy analysis showed that the maximum UV/Vis absorbance for the biosynthesized iron nanoparticles was found to be around 200-300nm for that biosynthesized by *P. commune* MH5 for $FeCl_3$ and $FeSO_4$ solution. More characterization including XRD and TEM have been used for iron nanoparticles biosynthesized by *Penicillium novoparasiticus* MH5. Iron nanoparticles biosynthesized by *Aspergillus niger* exhibited a color change for $FeSO_4$ solution with deposition of dark particles but for $FeCl_3$ the solution became dark and no deposition took place [39]. Iron nanoparticles biosynthesized by *Pleurotus* sp. culture filtrate added to $FeSO_4$ solution exhibited maximum absorbance at 216 and 268 nm [40]. *Bacillus negaterium* was used for the biosynthesis of iron nanoparticles with maximum absorbance at 200-300 nm [41,43]. Revealed that the maximum absorbance of iron nanoparticles biosynthesized by *Alternaria alternate* was around 238 and 265 nm. Biosynthesis of iron oxide nanoparticles by fungi, bacteria, yeast, or plant extracts has been previously studied [44-48].

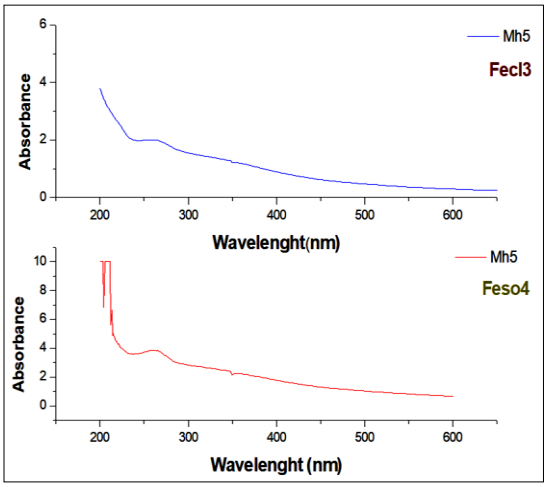

Figure (2) Shows color change and UV/vis absorption of iron oxide nanoparticles biosynthesized by culture filtrate of the fungus *Penicillium novoparasiticus* MH5

3.2.1. X-ray diffraction

XRD analysis was performed to prove the formation of iron nanoparticles, fig. (3). Based on the obtained results of x-ray editing at the angle θ2 and in degrees with a range of 10-60, 5 couriers were shown at degrees 30, 35/36, 44/45, 53/54, and 59/54, which were related to the levels of 220, 311, 400, 422, and 511, respectively. The phase and the crystallographic parameters of iron nanoparticles biosynthesize by *Streptomyces* sp. were characterized by XRD patterns. XRD peaks at 23, 31, 37, 43, 63,76 with lattice plan (111), (220), (311), (400), (422), and (440) were well matched with the JCPDS No. 653107 and confirms that the particles were in face centered cubic system [49]. The phase of the green synthesized nano-particles by flax (*Linum usitatissimum* L) was analyzed by using the XRD technique. The obtained 2θ values were at ~27.7°, ~36.1°, ~39.4°, ~45.9°, ~54.3°, ~66.7°, ~69°, ~77°, ~84.6°, ~91.1°, ~101.8°, ~120.6°, ~128.4°, and ~131.2°. These values matched well with the ICDD standard data files (JCPDS: 96-900-5817) for iron oxide (Fe_2O_3), thus confirming that the synthesized Fe Nanoparticles [50].

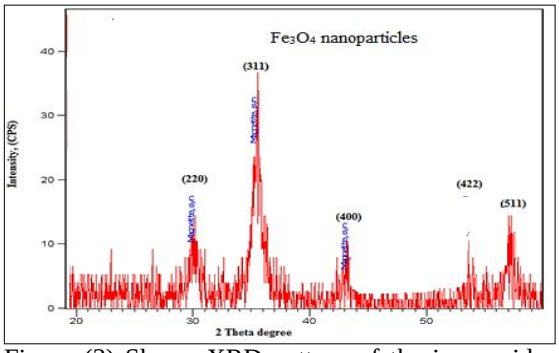

Figure (3) Shows XRD pattern of the iron oxide nanoparticles biosynthesis from $FeCl_3$ treated with culture filtrate of the fungus *Penicillium novoparasiticus* MH5

3.2.2. Transmission Electron Microscope

TEM image for the biosynthesized iron nanoparticles formed by the culture filtrate of *Penicillium commune* MH5. Results in fig. (4) revealed that the biosynthesized nanoparticles exhibited an average size of about 30-50nm. TEM nanoscale images of iron nanoparticles biosynthesized by *Bacillus megaterium* have a cubic shape and the size of nanoparticles, at a magnification of 200 nm, were between 40 and 60 nanometers [51]. The plant pathogenic fungus *Fusarium oxysporum* and the endophytic fungus *Verticillium sp*. culture filtrates were mixed with the aqueous solution of ferricyanide/ferrocyanide for 24 h. TEM images were recorded at different magnifications of particles resulting from the *F. oxysporum*–iron precursor reaction medium after 24 h of reaction. The particles formed are irregular in shape presenting an overall quasi-spherical morphology with a particle size range of 20–50 nm [52].

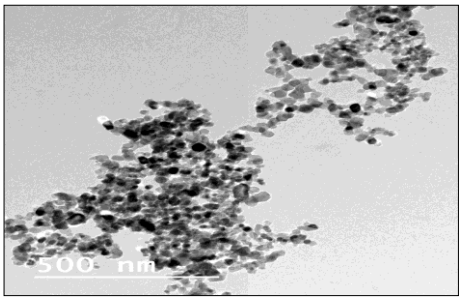

Figure (4) Shows a TEM image of iron nanoparticles biosynthesized by *Penicillium commune* MH5 filtrate

3.3. *Nano gel and nanomagnetic gel*

3.3.1. FTIR spectroscopy

The result of the infrared nano gel shows absorption at the following areas (N-H) 3341cm^{-1} and (C=C) (1650, 1456, 1424, 1328, 1193, 1124 cm^{-1}). Furthermore, absorption of nanomagnetic gel was similar to nano gel with different effects of traces of the characteristic curvature of the vehicles like 1123, 1182, 1329, 1424 cm^{-1}, fig. (5).

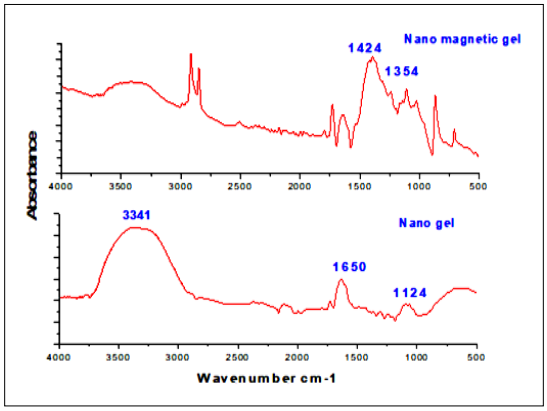

Figure (5) Shows results FTIR patterns of nano gel and nonmagnetic gel

3.3.2. Light microscope

The samples were examined after mounting gel on a glass slide; they were examined by the optical microscope with power magnification 500 X in National Research Center. Under the microscope, the nano gel is shown as transparent material but the nanomagnetic gel is shown as a transparent material with iron metal particles which appear like black grains, fig. (6).

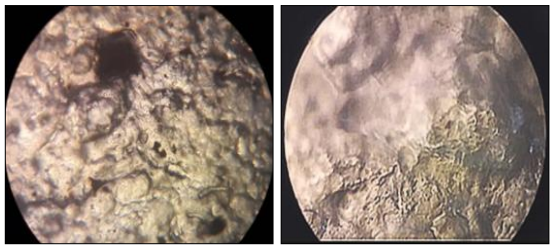

Figure (6) Shows result of the optical microscope of nano gel and nanomagnetic gel.

3.4. *Evaluation of cleaning materials on stain removal*

3.4.1. Scanning electron microscope

Samples of dirt, Paraloid B72, Arabic gum, and charred linen stains were applied on the surface and some of it was penetrated into the pores. They were examined by SEM of before cleaning, as for the halite, their granules crystallized within the pores of the samples and also fungal cells were branched within the sample granules. Samples cleaned by nanogel 5%, the investigation by SEM shows that the salts, charred linen, dirt, Paraloid B72 stains and paper which adhesive by Arabic gum was removed. Nano gel 5% proved to remove this stain, but it didn't eliminate the wax stain and parts of fungi stains. Through the examination of samples observed that the residues were found on the surface in large quantities and penetrate the pores. Nanomagnetic gel 5% proved it success in removing different stains except for wax spots with no residues left on the surface or in the pores, which aided in its removal from the treated surfaces by use of an external magnet which has didn't fumble the treated surface directly, fig. (7).

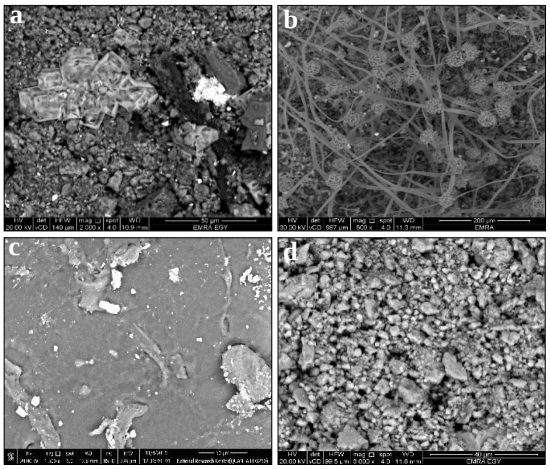

Figure (7) Shows SEM investigated samples before applying stains, **a.** salt crystallization on samples before applying stains, **b.** fungi stain, **c.** Sample after cleaning by nano gel, **d.** sample after cleaning by nano manetic gel.

3.4.2. FTIR spectroscopy

The experimental samples were analyzed before and after the cleaning process to identify the efficiency of the cleaning material in removing different spots by

recognizing the change in the chemical bonds and the impact before and after cleaning. Nano gel 5% has proven its ability to remove almost all stains but it remains residue on the surface. This follow-through disappearance of absorption bar of (linen1636 cm^{-1} , salts 1500 cm^{-1} 1650 cm^{-1}, dust 2450 cm^{-1} ,stain arizing 1450 cm^{-1} 1650 cm^{-1} 950 cm^{-1} Arabic gum 3600 cm^{-1} of 2800 cm^{-1} ,1650 cm^{-1} and paraloid B72 1740 cm^{-1} 1620 cm^{-1} but the absorption of wax at 2800 cm^{-1}, 1400 cm^{-1}, 900 cm^{-1} in the infrared pattern, fig. (8).

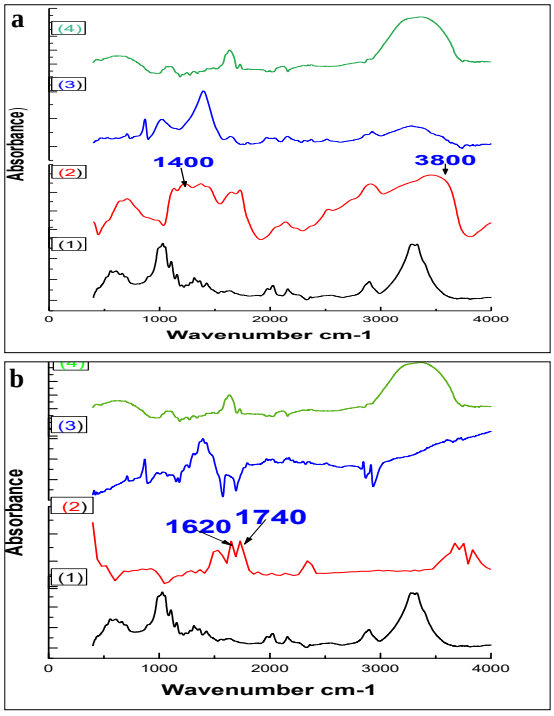

Figure (8) Shows FTIR of the experimental sample which cleaning **a.** Arabic gum by nano gel, **1**) samples before applying stains. **2**) surface of the sample after applying wax stain, **3**) sample after cleaning by nano gel 5%, **4**) standard sample of nano gel, **b.** paraloid B72 by nano gel, **1**) samples before applying stains. **2**) surface of the sample after applying paraloid stain, **3**) sample after cleaning by nano gel 5%, **4**) standard sample of nano gel.

Nanomagnetic gel is the most efficient materials that removed all spots expet wax spots especially didn't remain any residues in the samples and that shows through the disappearance of the absorption of spots after cleaning processlinen 1636 cm^{-1}, salts 1500 cm^{-1} 1650 cm^{-1}, dust 2450 cm^{-1}, stain arizing 1450 cm^{-1} 1650 cm^{-1} 950 cm^{-1} Arabic gum 3600 cm^{-1} of 2800 cm^{-1} ,1650 cm^{-1} and paraloid B72 1740 cm^{-1} 1620 cm^{-1} but the absorption bar of wax 2800 cm^{-1}, 1400 cm^{-1}, 900 cm^{-1} in the infrared and compared between the peaks there is no trace of nanomagnetic gel after cleaning fig.(9).

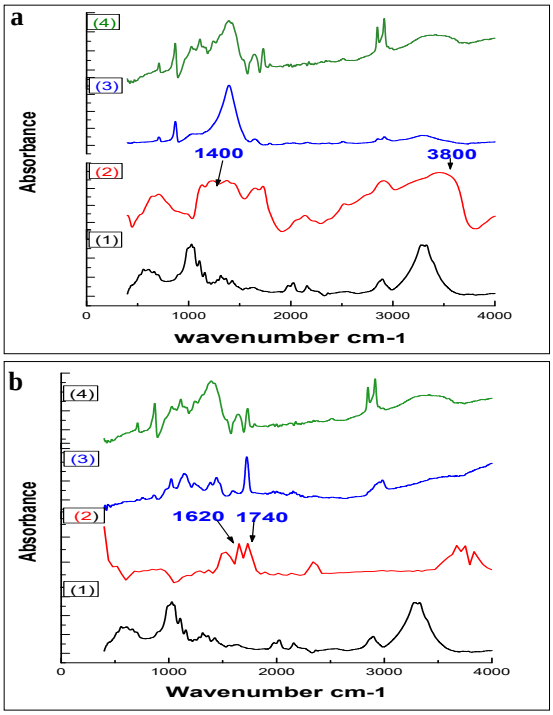

Figure (9) Shows FTIR of the experimental sample which cleaning **a.** Arabic gum by nano-magnetic gel, **1**) samples before applying stains. **2**) surface of the sample after applying wax stain, **3**) sample after cleaning by nanomagnetic gel 5%, **4**) standard sample of magnetic nano gel, **b.** paraloid B 72 by nano gel, **1**) samples before applying stains, **2**) surface of the sample after applying paraloid stain, **3**) sample after cleaning by magnetic nano gel 5%, **4**) standard sample of nanomagnetic gel.

3.4.3. Colorimeter changes

3.4.3.1. *Linen charred on the cartonnage*

The chromatic change was measured for three colors of experimental samples and

compared with standard samples, untreated and treated samples ΔE of untreated samples are 83.3, 52, 50. This is the high change of ΔE. Nanomagnetic gel with 5% is the best materials to remove these spots and didn't affect the samples ΔE are 2.1, 8.9, 2.3 nano gel with 5% that removed spots with a few change of colors.

3.4.3.2. *Dust and sunspots on the surface*

ΔE of untreated samples are 42.3, 46.2, 52.0 this is a huge change caused by these spots. ΔE of nanomagnetic gel with 5% are 5.2, 5.8, 4.6 the nano el with5%, ΔE 6.8, 1.8, 4.7 that removed spots with a few changes of color.

3.4.3.3. *Salts*

ΔE of standard 34.5, 48.4, 27.3, this indicates the great change caused by these spots. ΔE of nanomagnetic gel with 5% is 6.1 5.9, 3.9. ΔE nano gel with 5%, 7.9, 3.4, 10.0.

3.4.3.4. *Stain arising from fungal damage*

ΔE of untreated samples are 28.5, 57.8, 22.7 nanomagnetic gel with 5% that ΔE are 6.05, 3.0, 3.7 nano gel with 5%, ΔE 5.92, 6.0, 6.8.

3.4.3.5. *Previous restoration*

- (Arabic gum)

ΔE of untreated samples are 31.7, 48.0, 29.2. ΔE of nanomagnetic gel with 5% are 3.6, 5.5, 8.2, nano gel with 5%, ΔE 5.92, 6.0, 8.8 that removed.

- (Wax)

ΔE of untreated samples are 29.4, 65.9, 31.0. ΔE of nanomagnetic gel with5% are 3.3, 8.9, 5.1, nano gel with5%, ΔE 4.8, 8.7

- (Paraloid)

ΔE of untreated samples are 31.2, 50.5, 30. ΔE nano gel with 5% that is 5.6, 5.8, 4.2 nanomagnetic gel with 5%, ΔE 7.1, 5.6, 2.64. N.M.G removed the spots without color change and nano gel removed spots with a few changes in colors but nano gel 5% is the best after removing paraloid stains that is make a little change in color in the samples

3.5. *Application of nano-gel and nano-magnetic gel.*

The surface before the nano cleaning gel application has a different stain. The removal of the stains was performed by indirect application of the microemulsion gel and nanomagnetic gel onto the area to be cleaned by adding a layer of Japanese paper to preserve the colors layer then cover the cleaning materials with polyethylene. After the desired application time (variable from 4 to 6 h), the nanomagnetic gel was removed aid of a permanent magnet. The nano gel and nanomagnetic gel proved of removing different stains except for wax stain, but nano gel left residues on the treated surface which can attract the dust and turned into hard black spots. The treated area was encouraging to the growth of fungi and bacteria, which the surface turned to darken in color [15]. The nanomagnetic gel has different advantages. It can be shaped as desired and applied to a specific area with fine spatial control of the area. It is easily moved with Tweezers or cut with a knife and flexibility during the cleaning process. Spots on the surface of the mask have been identified. The nanomagnetic gel loaded with a micelle or microemulsion system to give a responsive chemical gel represents one of the most advanced, versatile systems for cleaning works of art, avoiding any side effects. The nanomagnetic gel can be easily removed by an external magnet. [17,52]. The nanomagnetic gel loaded with the microemulsion was used to clean the surface of a cartonnage sample by preparing nanomagnetic gel and cutting it the size of the stain. Covered the gel with a layer of polyethylene on the compresses. The poultice remained on the surface for 4-6 hours. Remains of compresses magnetic were removed by an extra magnet fig. (10).

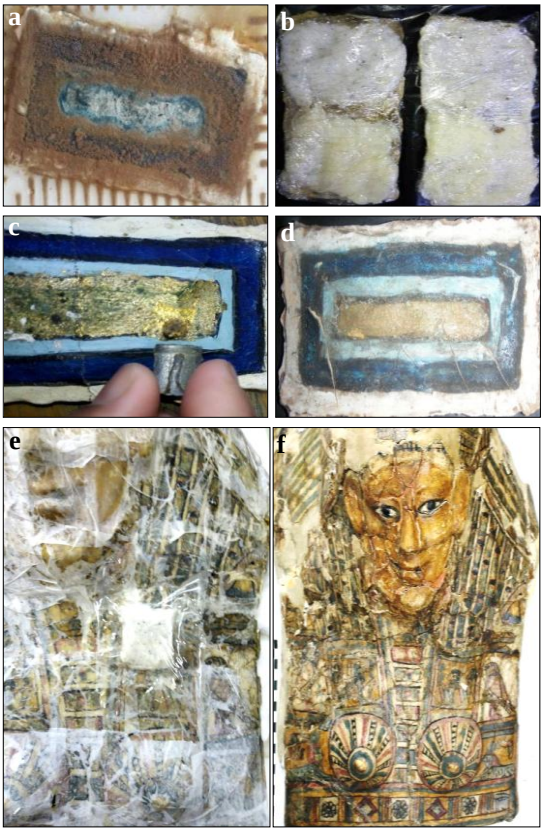

Figure (10) Shows application of nano gel and nanomagnetic gel on the samples **a.** sample with fungal stain, **b.** sample cleaning by nano gel, nanomagnetic gel, **c.** removing nano magnetic gel by extra magnet, **d.** sample after cleaning by nano gel. B72, **e.** mask during application the nanomagnetic gel to cartonnage mask for removing paraloid gel treatment, **e.** mask after cleaning of Paraloid B72.

4. Discussion

Fungi were isolated from cartonnage dating back to a late-era period using sterile cotton swabs and some other deteriorated pieces were scattered above the agar plate media, then molecular identification of isolated fungi fungal. The six isolated fungi and accession number are: **1**- *Aspergillus terreus* (Acc. no. MH562044). **2**- *Aspergillus oryzae* (Acc. no. MH562045) **3**- *Aspergillus tamarii* (Acc. no. MH562046). **4**- *Aspergillus niger* (Acc. no. MH562047). **5**- *Penicillium commune* (Acc. no. MH562048). **6**- *Aspergillus novoparasiticus* (Acc. no. MH562049). The identified six fungi were used for the biosynthesis of iron nanoparticles [26]. Several investigations have been focused on the biosynthesis of nanoparticles from fungi by color, UV-Visible spectroscopy, X-Ray diffraction studies, TEM analysis. Both $FeCl_3$ and $FeSO_4$ solutions have been found that a color change has been performed with some fungal filtrates from pale yellow color into dark color (reddish-black to brown). A visible color change has been noticed in the case of *Penicillium commune* MH5 and *Aspergillus novoparasiticus* MH6 for both $FeCl_3$ and $FeSO_4$ solutions. UV/Vis absorbance for the biosynthesized iron nanoparticles were found to be around 200-300 nm especially for that biosynthesized by *P. commune* MH5 and *A. novoparasiticus* MH6 for both $FeCl_3$ and $FeSO_4$ solutions. XRD peaks of iron nanoparticles at 23, 31, 37, 43, 63 and 76 with lattice plan (111), (220), (311), (400), (422) and (440). TEM image for the biosynthesized iron nanoparticles exhibited an average size of about 30-50 nm. TEM nanoscale images of iron nanoparticles biosynthesized by *A. niger* have a cubic shape and the size of nanoparticles, at a magnification of 200 nm, were between 40 and 60 nanometers. Iron nanoparticles used in the cleaning process in nanomagnetic gel and compared with other cleaning materials like nano gel, nano silver, saliva, alcohol. The spectral analysis of the infrared nano gel (N-H) 3341cm^{-1} and (C=C) (1650, 1456, 1424, 1328, 1193, 1124 cm^{-1}). Nanomagnetic gel was similar to nano gel with different effects of traces of the characteristic curvature of the vehicles like 1123, 1182, 1329, 1424 cm^{-1} [53]. The nano gel is shown as transparent material but the nanomagnetic gel is shown as a transparent material with the iron metal particles which appear like black grains by light microscope. Examination by scanning electron microscope of treated samples by cleaning materials shows that all cleaning materials effective to remove

dirt and dust but alcohol with water affected of inner layers that have lightened part of the ground layer, saliva, and nano gel residues were found in the surface. Nano silver, nano gel 3%, 5% Nanomagnetic gel 3%, removed salts, but nano silver has lightened some of the ground layers and, nano gel 3%, 5% had a few residues on the surface layer. Alcohol, nano silver, nano gel, and nanomagnetic gel were effective for removing charred linen stain. All cleaning materials were good for removing the adhesive paper stick with Arabic gum, but alcohol removed the paper which was used like support but did not remove the Arabic gum. Nano gel 3%, 5% removed Paraloid B72 with residues that were found in the surface and the pores, but Nanomagnetic gel 3%, 5% were very good at removing Paraloid B72 especially after being completely removed by magnets. All cleaning materials weren't effective of removed previous restoration by wax residues. Alcohol: water 1:1 led to removing the fungi and mycelium, but remained some mycelium on the surface and the pores. Saliva nano silver removed a high percentage of fungi, but saliva remained some of it. Some saliva materials remain on the surface. Nano gel 3%, 5%. Nanomagnetic gel 3%, 5% removed the all fungi and stains. The experimental samples were analyzed by infra-red before application of cleaning materials, and analysis after cleaning materials showed the disappearance of absorption bar of linen 1636 cm-1 ATR-FT-IR spectra of various materials refer to nanomagnetic gel efficiency of removing ancient restoration spots and disappearance ratio of spots in the surface. It had not remained of cleaning material on the surface. Alcohol, Nano gel, and nanomagnetic gel showed the disappearance of absorption bar of salts at 1500 cm^{-1} 1650 cm^{-1} this refers to nano silver efficiency removing of ancient restoration spots and disappearrance ratio of spots in the surface. Through analysis of experimental sample surface which was damaged by dust and sunspots in the pattern of infrared after cleaning by nanomagnetic gel showed the disappearance of absorption bar of dust 2450 cm^{-1} this refer to nanomagnetic gel efficiency of removing dust. Nano silver, nano gel, nanomagnetic gel the most efficient of removed fungal damage by the disappearance of absorption bar and others appearance of stain arising at 1450 cm^{-1}, 1650 cm^{-1} 950 cm^{-1}. All cleaning materials removed the paper adhesives by Arabic gum showed by the disappearance of absorption bar of Arabic gum at 3600 cm^{-1}, 2800 cm^{-1}, 1650 cm^{-1}. Nano-magnetic gel efficiency of removing ancient restoration spots and disappearance ratio of spots in the surface. It had not any remain of cleaning material on the surface, magnetic showed the disappearance of absorption bar of paraloid B72 at 1740 cm^{-1} of 1620 cm^{-1} especially nanomagnetic gel 5% and this refer to nanomagnetic gel removed all spots of paraloid and didn't leave residues, also it had no residue of nanomagnetic gel in the surface. All cleaning materials didn't remove the wax stain that showed the presence of absorption bar of wax at 2800 cm^{-1}, 1400 cm^{-1}, 900 cm^{-1}, this refers to cleaning materials inefficiency in the disposal of ancient restoration spots and presence ratio of spots in the surface. Chromatic change of samples of three colors was measured after applied cleaning materials. Nano-magnetic gel with 3% that ΔE are 2.1,8.9, 2.3 which means N.M.G removed the linen charred spots without what happens color change, nano gel with 5% that removed spots with a few change of colors, nano gel 5%, nanomagnetic gel 5% and finally saliva gave good results. Nano-magnetic gel with 3% that ΔE are 5.2, 5.8, 4.6 which means N.M.G removed the dust and dirt spots without color change, nano gel with 3%, ΔE 6.811.8, 4.7 that removed spots with a few change

of colored, nanomagnetic gel 5%, and finally nano gel 5% gave good results. Nano-magnetic gel with 3% that ΔE are 6.1, 5.9, 3.9 which means N.M.G removed the salts spots without color change, nano gel with 3%, ΔE 7.9, 3.4, 10.0 that removed spots with a few changes in colors, nano-magnetic gel 5%, and finally nano gel 5% gave good results. Nanomagnetic gel with 3% that ΔE are 6.05, 3.0, 3.7 which means N.M.G removed the stain arising spots without color change, nano gel with 3%, ΔE 5.92, 6.0, 8.8 that removed spots with a few change in colors, nanomagnetic gel 5%, and finally nano gel 5% gave good results. Nano-magnetic gel with 3% that ΔE are 3.6, 5.5, 8.2 removed the Arabic gum spots without color change, nano gel with 3%, ΔE 5.92, 6.0, 8.8 removed spots with a few changes in colors of nano-magnetic gel 5% are7.3, 3.1, 4.7, and nano gel 5% gave good results. Nanomagnetic gel with 3% that ΔE are 3.3,8.9, 5.1 which means N.M.G didn't remove the wax spots that color didn't change because this the color of the same spot, nano gel with 3%, ΔE 4.8,8.7, 4.5, nanomagnetic gel 5% are 10.7, 11.3,6.9. Nano gel with 3% that ΔE are 5.6,5.8, 4.2 which means N.G removed the paraloid B72 spots without making a color change, nano-magnetic gel with 3%, ΔE 7.1, 5.6, 2.64 that removed spots with a few change in colors of nano-magnetic gel 5% are7.2, 6.2, 6.60 and finally nano gel 5% gave good results. Nano-magnetic gel performed a good result in removing the spots, did not show the effect on pigments, and didn't find any residue in the surface because using after application nanomagnetic gel a magnet.

5. Conclusion.

In this study, iron nanoparticles biosynthesized from fungi which isolated from cartonnage mask. The best-produced nanoparticles, isolate Penicillium commune from both ferrous sulfate and ferric chloride. This method was friendly, simple, rapid, stable, and cost-effective technique biocompatibility, scalability less toxic nature of processes environmentally. Then magnetic nanoparticles were added to nano gel for use in the cleaning process i.e. green conservation. The mixture of nano gel and iron nano-particles can improve their role in removing undesired layers from the surface by earning a magnetic property as the magnetic nano gel can be removed from the treated surfaces using an external magnet. Nano gel and nano-magnetic can remove most of the dirt (salts, fungal, paraloid, animal glue stains) but can't remove the wax stain. (FTIR), SEM and coloring measurements were used to observe the success of the effect of the new gel with the magnetic nanoparticles in cleaning the cartonnage surfaces.

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