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Green Fabrication of Iron oxide nanoparticles utilizing *Aspergillus Niger*

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KEY WORDS

Aspergillus niger, iron oxide nanoparticles, Scanning electron microscope, XRD

ABSTRACT

This study demonstrates the efficacy of *Aspergillus niger* (*A. niger*) in the green synthesis of iron oxide nanoparticles (IONPs). *A. niger* filtrate was employed as a biogenic source for IONP production. Characterization of the synthesized IONPs using UV-Vis spectroscopy revealed a characteristic absorption peak around 320 nm, confirming nanoparticle formation. FTIR analysis identified the presence of fungal biomolecules, such as proteins and carbohydrates, on the nanoparticle surface, suggesting their role in capping and stabilization. XRD analysis confirmed the crystalline nature of the IONPs and indicated the predominant presence of the hematite $(\alpha - Fe_2O_3)$ phase. Microscopic examination using SEM revealed a uniform, quasi-spherical morphology with particle sizes ranging from 36 to 44 nm. These findings highlight the potential of *A. niger* as a sustainable source to produce stable and biocompatible IONPs, suitable for diverse biotechnological and biomedical applications.

Introduction

Iron oxide nanoparticles (IONPs), especially hematite (α-Fe_2O_3), have attracted significant interest due to their unique properties including magnetic behavior, high stability, and biocompatibility **(Fernandez-Lafuente, 2023; Saied et al., 2022)**. These features, combined with their high surface area **(Maghraby et al., 2023)**, make them suitable for diverse applications spanning biotechnology, medicine, environmental remediation, and catalysis **(Fernandez-Lafuente, 2023)**. Also, IONPs particularly in their hematite (α-Fe_2O_3) form demonstrate significant advantages for biocatalyst immobilization **(Saied et al., 2022)**. Hematite offers high stability, crucial to ensure the long-term performance of immobilized lipases, and its excellent biocompatibility makes it a suitable candidate for various biotechnology and pharmaceutical applications **(Fernandez-Lafuente, 2023)**. The high surface area characteristic of nanomaterials provides ample sites for efficient enzyme immobilization and enhanced catalytic activity **(Maghraby et al., 2023)**. While conventional IONP synthesis methods like co-precipitation and thermal decomposition offer some control over particle properties, they often rely on high energy inputs, utilize hazardous chemicals, and generate undesirable byproducts **(Thakkar et al., 2010)**. These limitations necessitate extensive washing and purification steps, increasing production costs and posing challenges for scalability while also raising environmental concerns **(El-Khawaga et al., 2023)**. The need for sustainable and eco-friendly alternatives has driven the exploration of biological methods for nanoparticle synthesis.

Biosynthesis, particularly using fungal systems, provides a promising "green" approach to IONP production. Fungi are attractive bio-factories for several reasons: they are relatively easy to cultivate at large scales on simple, inexpensive substrates **(Castro-Longoria, 2016)**, and their metabolism generates a vast array of biomolecules **(Boroumand Moghaddam et al., 2015)**. Crucially, some of these molecules, such as certain enzymes and secondary metabolites act both as reducing and capping agents by reducing metal salts, thus influencing the nanoparticles morphology. Furthermore these metabolites have crucial part on stabilizing resulting nanoparticles, preventing aggregation and enabling precise control over particle size and shape, contributing to a better dispersion state and also prevents toxicity effects by reducing metal oxide related cytotoxicity **(Priya et al., 2021)**.

Additionally, the primarily extracellular nature of fungal nanoparticle production facilitates downstream processing by simplifying extraction compared to intracellular synthesis routes **(Šebesta et al., 2023)**. In numerous studies different strains from genus *Aspergillus*, were investigated, their extracts proved capability to create several nanoparticles with good size, dispersed nanomaterials **(Hasanin et al., 2023; Zúñiga-Miranda et al., 2023)** Which confirms its superiority for biogenic fabrication process. Within different species from the genus, *Aspergillus niger*, is specifically suitable for biosynthesis applications with rapid growth rate and non-pathogenic effect **(Rai et al., 2022)**.

Material and Methods

Peptone water and Yeast extract were supplied from HIMEDIA, India. Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), iron (II) was purchased from AL-Gomhoria for chemicals, Egypt.

The fungi strain *Aspergillus niger* ATCC 16878: *(A. niger)* was purchased from the Center of Fermentation Biotechnology & Applied Microbiology, Al-Azhar University, Egypt. The strain was deposited in a gene bank under accession number ATCC 16878.

Green Fabrication of Metal-based Nanomaterials Using Microbial Systems

Selection and Preparation of Biological Mediator

The microorganism was propagated in liquid potato dextrose medium under controlled environmental conditions (temperature: 28°C, orbital motion: 120 rpm) for a period of 144 hours **(Chatterjee et al., 2020)**. Following the growth phase, the mycelia were separated and subjected to multiple washing cycles using laboratory-grade deionized water to ensure purity. Subsequently, a precise quantity of purified fungal mass (15 grams) was introduced into 100 milliliters of sterilized deionized water. This suspension underwent agitation at 150 rpm while maintaining 28°C for 48 hours **(Saied et al., 2022)**. The resulting cell-free extract was harvested through filtration and reserved for subsequent nanoparticle biosynthesis procedures.

This study investigates the green synthesis of iron oxide nanoparticles using *Aspergillus niger* and evaluating the efficiency of this fungal bioproduction route to get stabilized IONPs with great features which facilitate it uses in industrial and pharmaceutical application.

Biological Fabrication of Iron Oxide Nanostructures

The production of iron-based nanomaterials utilized the metabolite-rich fungal extract (100 mL) obtained from *Aspergillus niger* ATCC culture. Following established optimization protocols for Aspergillus-mediated synthesis **(Fouda et al., n.d.)**, ferric chloride hexahydrate was introduced to achieve a final concentration of 6 mM in the reaction mixture. The solution underwent continuous agitation while its pH was modified and stabilized at 9.0. The biosynthesis process proceeded in darkness with precise temperature control (40°C) under shaking conditions (200 rpm) for 24 hours.

A reference sample containing only the fungal metabolites was maintained parallel to the synthesis reaction.

The successful formation of nanoparticles was visually confirmed by the development of a characteristic rusty-brown coloration. The synthesized particles underwent an initial drying step at 50°C for 24 hours, followed by a washing sequence involving 70% ethanol and multiple rounds of water washing. The final product dried through overnight dehydration at 70°C ("Harnessing the Biomedical Properties of Ferromagnetic α-Fe_2O_3 NPs with a Plausible Formation Mechanism," 2020).

Characterization of Biosynthesized Iron Nanoparticles

The physicochemical properties of the fabricated iron oxide nanostructures underwent comprehensive characterization through multiple analytical techniques.

UV-VIS analysis

Utilizing a Biowave 3-WPA system to examine the optical absorption characteristics across wavelengths ranging from 200 to 750 nanometers, with baseline correction performed using an appropriate control sample.

FTIR analysis

Fourier transform infrared spectroscopy (FTIR) was recorded from 400-4000cm^{-1} with samples prepared as KBr pellets using NICOLET-iS50 FT-IR spectrophotometer (Thermo Fisher Scientific).

XRD analysis

Crystallographic examination was conducted on finely pulverized samples using an EDX 8000 diffractometer (SHIMADZU, Russia), scanning through diffraction angles (2θ) from 10° to 90°. The resulting diffraction patterns were evaluated against JCPDS-ICCD reference data for phase identification.

Scanning electron microscope analysis

Morphological features and elemental distribution were examined using a TESCAN VEGA COMPACT scanning electron microscope equipped with

tungsten electron emission and EDX capabilities (JEOL JSM-6510 LV system) (Kohoutovice, Czech Republic). The imaging was performed under an acceleration potential of 30 kilovolts to achieve optimal resolution and compositional contrast.

Result and discussion

Biosynthesis and Characterization of Iron Oxide Nanoparticles

Previous investigations have successfully demonstrated the capability of various *Aspergillus niger* strains to facilitate the generation of iron oxide nanostructures through biological pathways **(Fouda et al., 2018; Sidkey, 2020; Saied et al., 2022)**. The introduction of ferric chloride hexahydrate and ferrous sulfate heptahydrate precursors into the fungal-derived solution, combined with alkaline pH adjustment to 9.0, triggered an immediate chromatic transformation in the reaction medium.

The development of distinctive rusty-brown coloration aligns with documented observations across multiple studies involving fungal-mediated synthesis of iron oxide nanoparticles **(Reshmy et al., 2021; Chen et al., 2017)**. This characteristic color modification serves as a preliminary indicator of successful nanoparticle formation. The validity of this observation was reinforced by maintaining a control sample containing only the metabolite-rich fungal extract, which retained its original transparency throughout the experimental duration, thereby confirming that the observed color transformation was specifically associated with the iron salt reduction process as shown in Fig.(1 A and B).

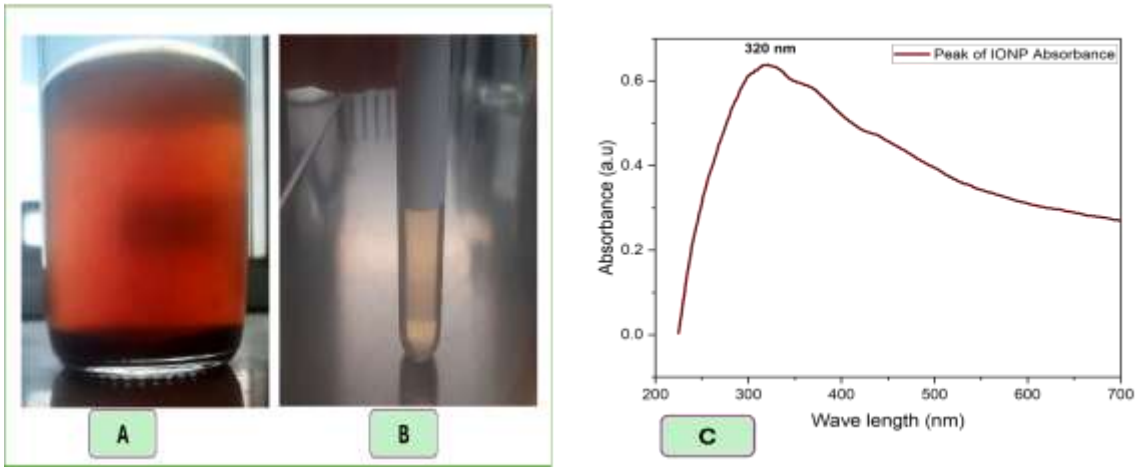

Fig. (1): IONP Biosynthesis using the biomass filtrate of *Aspergillus niger* ATCC 16878. Bottle (A) is a Test, and Tube (B) is a control contains only fungal filtrate without salt precursor. (C) UV-Visible spectra of IONP synthesized by fungal filtrate incubation with $FeCl_3.6H_2O$ as salt precursor.

Ultraviolet – Visible (UV-Vis) Characterization

The successful transformation of iron precursors into nanoscale structures was initially indicated by the transformation of the reaction mixture's appearance, shifting from a light yellow solution to a characteristic auburn coloration. Quantitative validation of this observation was obtained through absorption spectroscopy measurements.

Spectral analysis revealed a distinctive absorption maximum at 320 nanometers, as illustrated in Fig. (1C). This observation aligns with established literature, where biologically derived iron oxide nanostructures typically exhibit absorption bands within the 290-370 nanometer range **(Bouafia & Laouini, 2020; Karpagavinayagam & Vedhi, 2019)**. The specific peak position can vary based on multiple synthesis parameters, including nanoparticle dimensions, initial metal ion concentration, and the specific composition of the biological reducing agents employed **(Abdel Maksoud et al., 2022)**.

Comparative analysis with previous research reveals diverse absorption patterns for similar biosynthetic approaches. For instance, investigations by Ahmed et al. documented broad spectral features spanning 300-550 nanometers for hematite nanoparticles **(Ahmed et al., 2021)**. In related studies, Asoufi et al. reported characteristic absorption at 450 nanometers for iron oxide nanostructures produced using *Ailanthus excelsa* extracts **(Asoufi et al., 2018).**

Fourier Transform Infrared (FTIR) Spectroscopy

The biological synthesis of metallic nanostructures typically proceeds through multiple enzymatic pathways, involving various biological catalysts including oxidoreductases, NADH-dependent reductases, nitrogen-processing enzymes, and hydrolytic enzymes interacting with metallic precursors **(Senapati et al., 2005; Narayanan & Sakthivel, 2010)**. Infrared spectroscopic examination of the biosynthesized particles, as depicted in Fig.(2), revealed distinctive vibrational signatures indicating complex surface functionalization. The spectral profile demonstrated characteristic absorption bands at multiple frequencies, providing evidence of diverse molecular interactions at the particle interface.

The mid-infrared region exhibited several significant absorption features: vibrations at 1083.00, 1402.95, and 1653.06 cm^{-1}, attributable to C-O elongation, C=C stretching, and N-H

deformation modes respectively **(Mathur et al., 2015)**. These spectral signatures suggest the presence of biomolecular stabilizing agents, potentially including proteinaceous compounds, carbohydrate structures, and aromatic constituents. derived from the fungal metabolites. characteristic band at 2361.31 cm^{-1}, indicative of nitrile or isocyanate functionalities, while a broad absorption centered at 3396.17 cm^{-1} corresponds to hydroxyl and amine stretching vibrations **(Yusuf, 2023)**. The higher frequency region (3400-3650 cm^{-1}) indicates surface hydration and hydroxylation **(Yusuf, 2023)**. Notably, the low-frequency region revealed diagnostic bands at 534 and 476 cm^{-1}, characteristic of Fe-O bond vibrations **(Bhat et al., 2022)**. The presence of metal-oxygen vibrational modes in the 500-1000 cm^{-1} region provides definitive confirmation of successful iron oxide nanoparticle formation **(Periakaruppan et al., 2021)**. Similarly, peaks where previously reported in studies that synthesis different types of IONP from biological sources **(Chatterjee et al., 2020; Saied et al., 2022; Tarafdar & Raliya, 2013)**.

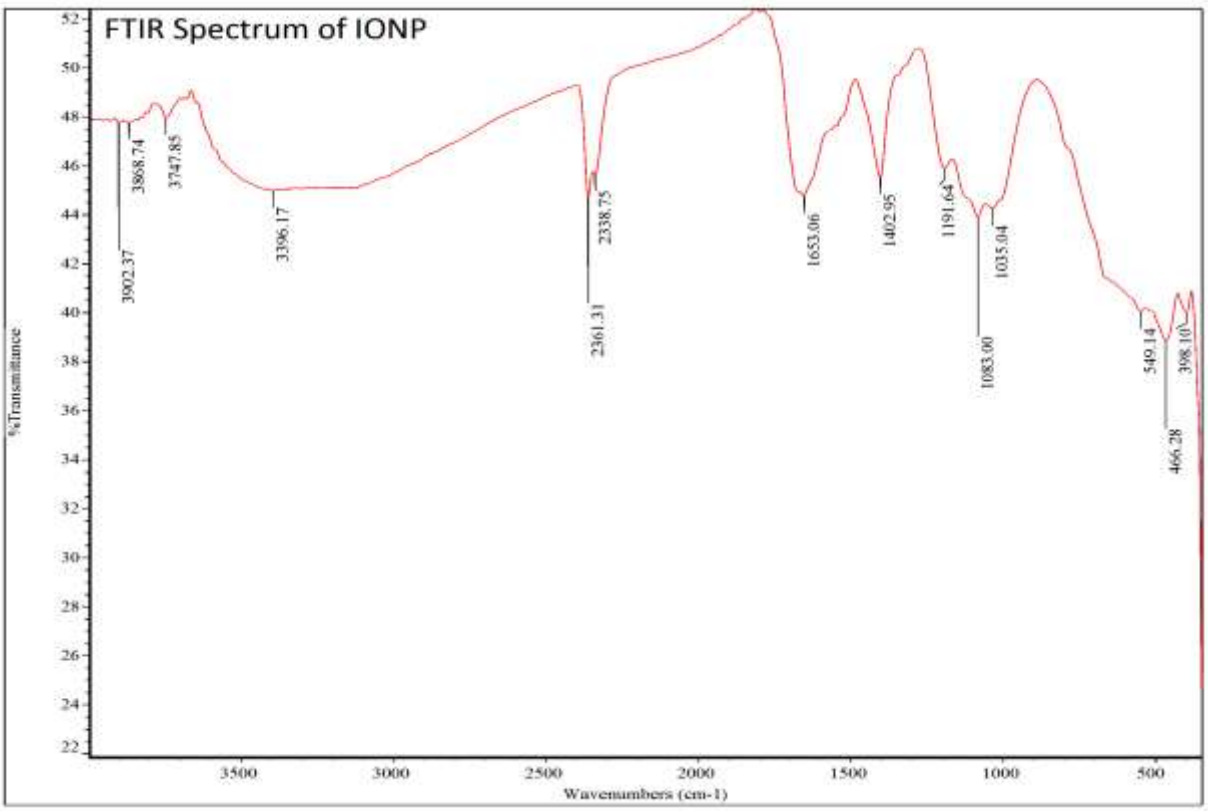

Fig. (2): FTIR Spectrum of Biosynthesyzied IONP by using *A. niger*

X-ray Diffraction (XRD) Characterization

X-ray diffraction (XRD) analysis was conducted to determine the crystalline phases present in the sample. The resulting XRD pattern is presented in Fig.(3). The observed diffraction peaks suggest the formation of the hematite (α-Fe_2O_3) phase.

Peaks were detected at 2θ values of 33.18°, 35.65°, 49.51°, 54.16°, and 57.38 °, corresponding to d-spacings of 2.698 Å, 2.516 Å, 1.840 Å, 1.692 Å, and 1.604 Å, respectively. While some of these peaks are consistent with hematite (α- Fe_2O_3), as indicated by PDF card number 01-089-0598, the presence of additional peaks suggests the possible contribution of other phases or impurities. Similarly, Said et al. reported hematite formation using *A. niger* extracts with reflections indexed as (012), (104), (110), (113), (024), (116), (122), (214), and (300) **(Saied et al., 2022)**. These findings suggest that proteins play a vital role in the synthesis of Iron Oxide nanoparticles, serving as both capping and stabilizing agents during their fabrication. These observations align with previous research that has been employed in the creation of hem-NPs **(Zhang et al., 2011; Abdeen et al., 2016)**.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) examination of the biologically derived iron oxide nanostructures revealed detailed surface features and particle characteristics. The SEM (Fig.4) demonstrated the presence of predominantly spheroidal particles with minor deviations from perfect sphericity, consistent with previous morphological observations **(Sasani et al., 2023)**. Dimensional analysis indicated particle measurements between 36.44 and 43.72 nanometers, with some evidence of particle clustering, a common phenomenon in bio-mediated synthesis protocols **(Siddiqi & Husen, 2016)**.

The observed dimensional parameters align with established literature values for fungal-derived iron oxide nanomaterials **(Fouda et al., 2018; Bashir et al., 2019)**. The consistent particle dimensions suggest effective growth regulation during the biosynthetic process, likely facilitated by surface-active metabolites produced by the fungal system **(Guilger-Casagrande & Lima, 2019)**.

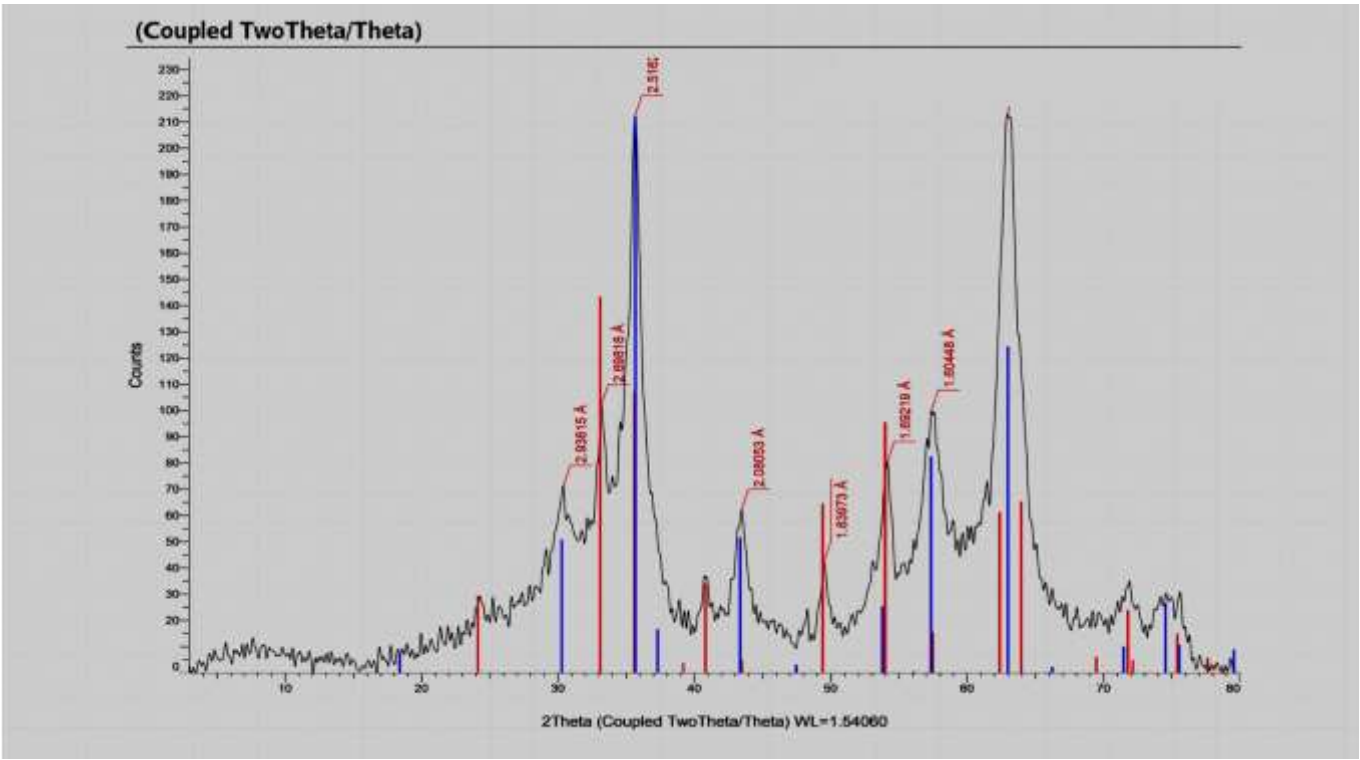

Fig. (3): XRD spectrum of Biosynthesized Hematite nanoparticle

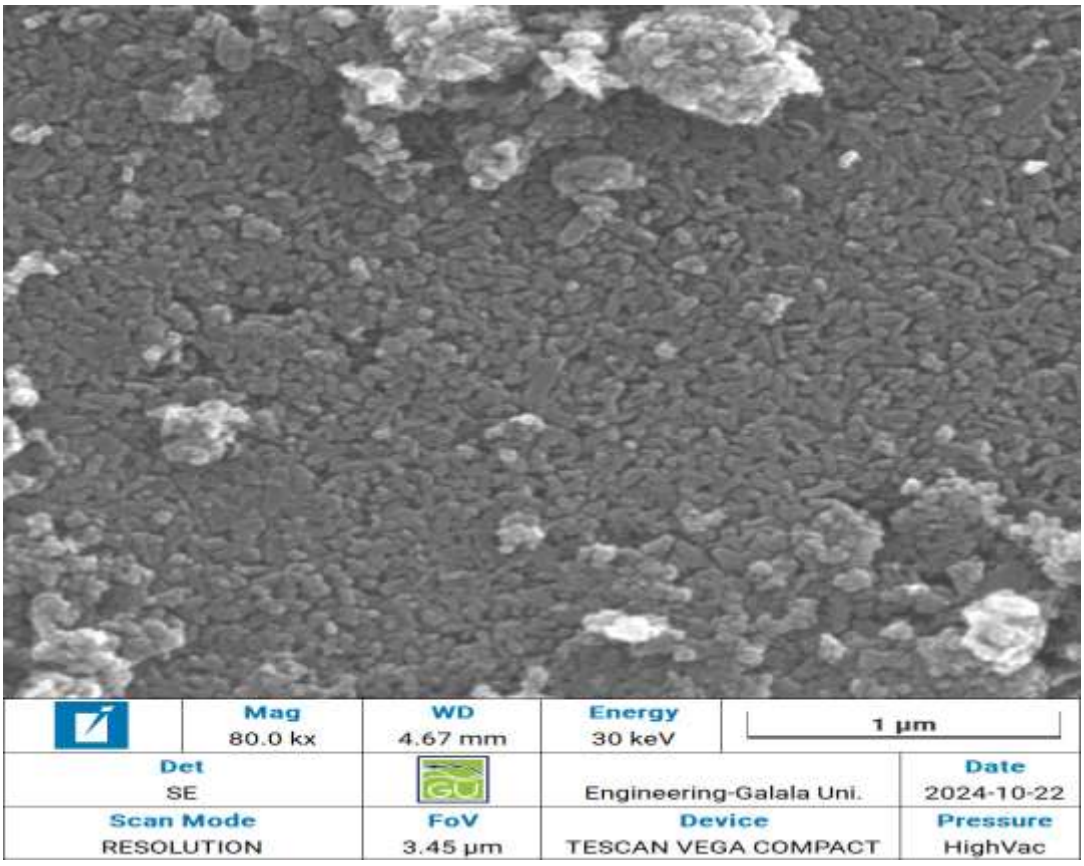

Fig. (4): SEM image of biosynthesized IONP by *Aspergillus niger* ATCC 16878.

Conclusion

This study successfully demonstrated the green synthesis of iron oxide nanoparticles (IONPs) using *Aspergillus niger* ATCC 16878. The biosynthesized IONPs exhibited a uniform quasi-spherical morphology with dimensions range of 36 to 44 nm and were confirmed as crystalline hematite (α-Fe_2O_3) by XRD. Characterization via UV-Vis, FTIR, and SEM further confirmed successful nanoparticle formation and revealed surface functionalization by fungal biomolecules, contributing to their stability. This green synthesis approach offers a sustainable alternative to conventional methods and demonstrates *A. niger's* potential as a bio-factory for producing functionalized IONPs. These green approaches will open a new door of synthesizing more bio compatible IONP from fungal source that will have multiple green applications.

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التصنيع الصديق للبيئة لجسيمات أكسيد الحديد النانوية باستخدام فطر (*Aspergillus niger*)

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[3]قسم الفيزياء، كلية العلوم، جامعة الجلالة، السويس ٤٣٥١١، مصر.
[4]قسم الفيزياء، كلية العلوم، جامعة المنصورة، المنصورة ٣٥٥١٦، مصر.

تتناول هذه الدراسة التخليق الحيوي لجزيئات أكسيد الحديد النانوية باستخدام فطر ***(Aspergillus niger)*** . أظهرت النتائج تخليق جزيئات نانوية متجانسة وشبه كروية بقطر يتراوح بين ٣٦ و٤٤ نانومتر، وتم تأكيد طبيعتها البلورية كهيماتيت (α- Fe_2O_3) باستخدام حيود الأشعة السينية. كشفت تقنيات التحليل الطيفي للأشعة فوق البنفسجية، والأشعة تحت الحمراء، والفحص المجهري الإلكتروني عن تكوين ناجح للجسيمات النانوية مع وجود مجموعات وظيفية من جزيئات حيوية فطرية على سطحها، مما يساهم في استقرارها. تقدم هذه الطريقة الحيوية بديلًا مستدامًا للطرق التقليدية، وتسلط الضوء على إمكانات *A. niger* كمصنع حيوي لإنتاج جزيئات أكسيد الحديد النانوية الوظيفية التي لها تطبيقات محتملة في مختلف المجالات العلمية والطبية.