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BIOSYNTHESIS AND CHARACTERIZATION OF NANO-IRON BY BEAUVERIA BASSIANA FUNGUS

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Abstract:

Iron nanoparticles were obtained in a biologically reaction method using the Beauveria bassiana fungus. X-ray diffraction (XRD), scanning analysis (SEM) and atomic energy microscope (AFM) showed that the characterization of the iron particles was verified, as the average grain size was 13.9 nanometers. The topography and features of the surface were also studied, and it was found that it was somewhat regular. The optical properties of iron oxide were studied using UV/Vis spectroscopy, and the energy gap was found to be (4.8 eV). The FTIR spectrum showed a transverse band at (1640 cm-1) which belongs to the groups of primary amines (N-H) belonging to the Bavaria extract. Other bands appeared at the (3500-3460) cm-1 . This work confirm the effectiveness of the Bavaria bassiana fungus as an aid in the process of preparing iron nanoparticles.

Keywords: *Beauveria bassiana fungus, iron oxide nanoparticles, topography ,optical properties, energy gap, the group of amines.*

Introduction

1. Introduction

Beauveria bassiana is a cosmopolitan genus of soil-borne entomopathogenic fungi, usually facultatively necrotrophic, but which can also be found as a saprophyte or as an endophyte. B. bassiana is one of the most widespread entomopathogenic fungi but an important feature is that B. bassiana is sensitive to the disturbing effects of cultivation. [1]. Fungi of the genus Beauveria are also known to produce biologically active secondary metabolites such as polyketides (e.g., osporin, bacyanin, and tinilin), non-peptide pigments and other metabolites (e.g., oxalic acid). Non-ribosomal peptide antibiotics (such as buviricin, bacyanolides, and buvirilides). These secondary metabolites are mainly insect pathogens and can be applied in agriculture. In fact, Beauveria bassiana is marketed under many different formulations as a biological control agent against different types of invertebrate pests. Some metabolites produced by fungi can also have medical applications and are used in many chemical and pharmaceutical industries [2].

Biosynthesis of nanoparticles using fungal, bacterial, or plant systems is environmentally safe and economical. Inorganic ions are converted into metallic nanomaterials using microorganisms such as yeasts, fungi, and bacteria. In our work, we have adopted the use of the Bavarian Basania fungus to create oxide nanoparticles [3].

The biosynthesis of nanoparticles using the fungus B. bassiania depends on complex chemical reactions. Biosynthesis begins with the secretion of enzymes from the fungus Bavaria bassiania [4]. These enzymes act as catalysts for chemical reactions. Where inorganic minerals are transformed into metal nanoparticles. This is done through chemical reactions that occur in a medium inside the fungus. Nanoparticles are formed based on the molecular structure of minerals. The size and shape of nanoparticles are regulated by enzymes [5].

The biosynthesis of nanoparticles using the fungus Bavaria bassiania considered a distinctive and promising step towards the development of nanotechnology in different applications [6]. In this work, Bavaria Bassania fungi were used to synthesize iron nanoparticles for the first time.

2. Materials and methods

- **Preparation of broth and culture of Beauveria bassiana"**

We obtained Bavarian Basania mushroom powder from the Department of Agriculture/Ministry of Higher Education and Scientific Research. In order to obtain the extract of the Bavaria Bassania mushroom solution, the following steps were followed:

1. Cucumber pieces were cut to a thickness not exceeding 3 mm, a diameter of 2 cm, and a weight of 20 g.
2. Prepare the sugar solution by dissolving 10 gm per 100 ml at a temperature of 60 degrees Celsius for 30 minutes.
3. Add 2 grams of Bavaria powder equally to 8 pieces of cucumber plants and supplement shown in the figure (1) .
4. All the cucumber pieces were collected and placed in the sugar solution
5. Place the fungal content in a container at a temperature of 20 degrees Celsius for 14 days
6. Filter the extract using medical cotton (four times) to obtain the extract

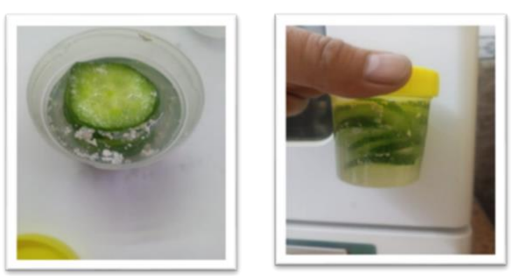

Figure 1 Beauveria Bassiana On PDA

- **Synthesis of nano-iron oxide particles**

Following 14 days of incubation, the fungal biomass was removed from the medium by filtering it using Whatman No. 1 paper. The following steps were taken: place 500 mL of deionized water in an incubator set up at 25 °C and 80% relative humidity for 120 hours; strain the solution through Whatman No. 1 filter paper to remove any unwanted substances; mix 2.4186 g of iron nitrate

($Fe(NO_3)_3$) (molecular weight = 241.86 g/mol) with 100 mL of deionized water using a 0.2 µm Millipore filter. Before adjusting the pH to 7, the solution is heated to 50 °C for 30 minutes. Drops of OH solution, the Bavarian extract that was previously obtained, are added, and the mixture is placed on a heated magnetic stirrer. A dark location was used to incubate the flask for 120 hours at 25°C with 50%±80% relative humidity, while the pH was continuously monitored. The creation of FeNPs was indicated by the solution turning a dark brown colour, as seen in Figure 2. The transformation of iron nitrate into iron nanoparticles is accomplished by means of the anti-oxidant properties of proteins, enzymes, and other chemicals found in the fungal extract

Figure 2 shows the color transformation of the iron nitrate solution

Characterization

X-ray diffraction of FeNPs

Samples of iron nanoparticles coated by spin deposition on glass slides were characterized by an X-ray diffraction system (Aeris research edition) using Cu Kα radiation. In a scattering range (2ϴ) from 20 - 80 degrees.

Ultra violet-visible (UV-VIS) spectroscopy of FeNPs

By ultraviolet and visible spectroscopy (UV-VIS). Iron concentrations were monitored by UV-VIS spectrometry of the reaction medium for time periods from 24 h to 120 h and was recorded at 420 nm using a UV-VIS spectrometer..

Scanning electron microscopy (SEM) analyses of FeNPs

SEM measurements were done with Panalytical company SEM. SEM provide further insight into the morphological details of the iron nanoparticles.

Fourier transform infrared spectroscopy (FTIR) of FeNPs

FTIR spectra of FeNPs were obtained with a Fourier transform infrared spectroscopy (FTIR). The FeNPs substance was characterized in stages at a spectrum range of (1550-1650) cm-1.

Atomic force microscopy (AFM) measurements of FeNPs

The surface topography of the nanoscale iron samples was studied using an atomic force microscope (Model TT-2) in order to obtain the characteristics of the iron particles and know the distribution of these particles on the surface, as well as other important information.

4. Results and discussions

4.1 X-ray diffraction result

The crystalline nature of the biosynthesized iron nanoparticles was analyzed, using X-ray diffraction method (Fig. 3). Two intense peaks were recorded over the spectrum of 2Θ.whereas the angles (24.1, 49.1554) represent miller coefficients (020,003) and are identical to the material Fe CI_2 according to specification 00-054- 0489, and the angles (33.08, 35.7024) are identical to the material Fe_2 O_3 according to specification 00-054-0489 and the angles (53.6764,63.4334) represents the Miller coefficients (300,223) are identical to the material Fe_2 O_3 according to specification 00-040-1139.

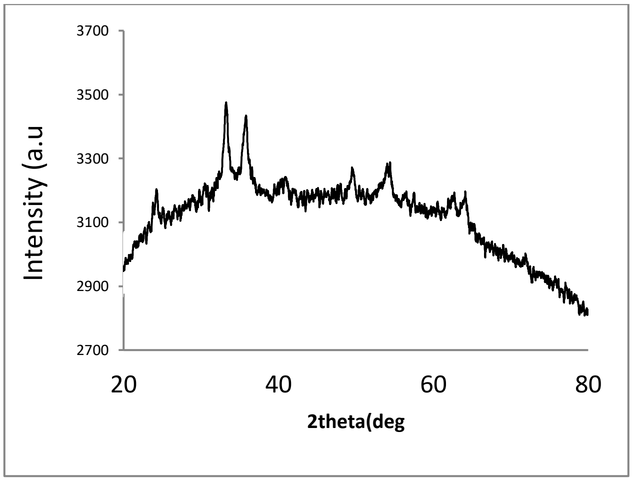

Figure 3 XRD patterns of iron nanoparticles biosynthesized by B. bassiana

The crystal size and inter distances were calculated as shown in Table (1). Due to the absence of any impurities of another substance, the method is suitable for preparing iron oxide, with a slight difference in the shape of the spectrum of reflected rays, which is due to the effect of the extract. The average of crystal size (D_{ave}) was calculated according to Schreier's equation

$$D_{ave} = \frac{0.94\lambda}{\beta cos\Theta}$$

also listed in Table 1 where: β - represent the full width at half maximum in the unit (radian) θ - represent the Braque diffraction angle

Table 1 shows the synthetic properties of a substance $Fe(NO_3)_2$

2Θ	hke	FWHM	D nm	σ line\m.^{2}	$\eta * 10^{-3}$
24.1	0.2	0.029	4.43	50.7	7.068
33.0		0.04	1.92	268.7	5.710
35.7		0.04	0.858	1358.1	2.944
49.1	0.0.3	0.059	1.620	380.8	10.237
53.6	3.0	0.064	0.490	4151.5	3.6533
63	2.2	0.074	1.739	330.3	17.560

4.2 Atomic force microscopy results

It is clear from the process of scanning with an atomic force microscope (AFM) of the surface structures of the films prepared using the biosynthesis method using the Beauveria bassiana fungus Figure (4), that there are uniformly and regularly spread ripples on the surface, which indicates that there are no voids and that the deposited film has a uniform topography. The zigzags represent the particles of the material that line up horizontally towards the peaks. The highest, where the highest peak represents an increase in the average roughness and reaches (61.24 nm). Increasing the roughness leads to an increase in the surface distances, which leads to an increase in the surface distances per volume unit. Atomic force microscope measurements also showed that the grain size of iron particles has an average grain size of 13.9, as shown in Table 2 and Figure 4. Table (2) shows the surface roughness rate and square root values of the mean roughness square and grain size rate according to the AFM measurements of films.

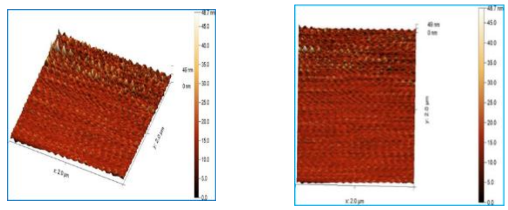

Figure 4 Two and three-dimension images, mediated by the atomic power microscope of the sample

Table 2 Values of surface roughness rate and square root values of the mean roughness square and grain size rate according to the AFM measurements of Fe $(NO_3)_2$

Sample	*Average Grain size (nm)*	*Roughness average (nm)*	*Root mean square (nm)*
$Fe(NO_3)_2$	13.9231	61.24	16.04

4.3 SEM Result of FeNPs

SEM images show the morphological and crystallographic details of the prepared FeNPs. Figure (5) shows the surface morphology of the material prepared using the biosynthesis method using the Beauveria bassiana fungus. It was found that the material has a nanostructure, as the sample contained clusters with a size of approximately 500 nm and a fairly regular distribution of iron nanoparticles, which have a particle size of 16.6 nm, also noting some crystalline structures and shapes that were formed due to agglomerations of the plant extract material.

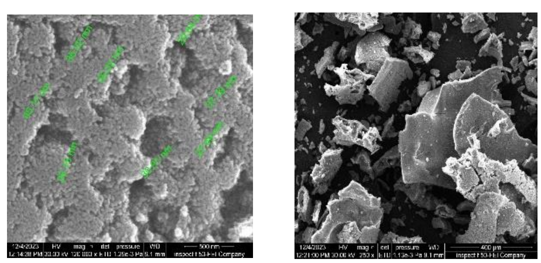

Figure 5 shows images of the sample Fe NPs prepared by Bavarian bassiana fungus .

4.4 FTIR Analyses of FeNPs

Figure (6) shows the IR spectroscopic analysis for the chemical functional groups in the FeNPs sample. The Iron nanoparticles (FeNPs) were analyzed for the presence of biomoleucles firmly attached with them through the FTIR , where the $Fe(NO_3)_2$ substance was characterized in three stages at a spectrum range of (1550-1650) cm-1. The results showed a transverse band at (1640 cm-1) which belongs to the groups of primary amines (N-H) belonging to the Bavaria extract, and other bands appeared at the (3500 – 3460) cm-1and another group belonging to the groups of primary amines (N-H) belonging to the Bavaria extract.

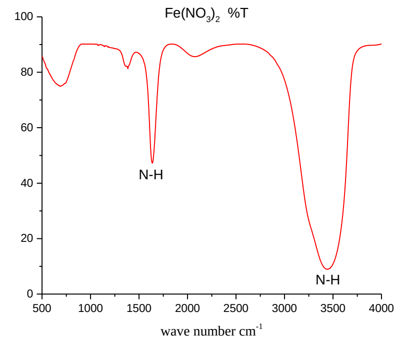

Figure 6 shows the spectrum (FTIR) used in determining the functional clusters in the plant extract.

4.5 Optical Measurements of FeNPs

Figure (7) represents the results of optical measurements, which included the ultraviolet absorption spectrum of (FeNPs) prepared by the biosynthesis method using the Beauveria bassiana fungus and deposited on the glass by the spin coating method, The maximum range of peak wavelength was (200-900 nm) was observed. It was found that the energy gap is equal to 4.8 eV according to Tauc plot. It was noted that the absorption spectrum increases towards short wavelengths after using the Bavarian extract, and this is attributed to the phenomenon of quantum confinement (figure 8)

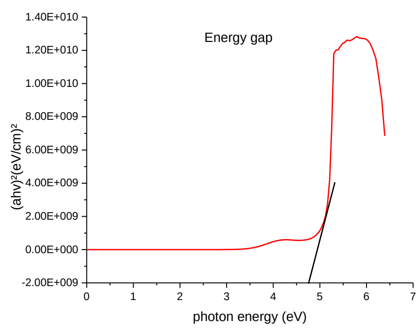

Figure 7 shows the amount of the energy gap for sample FeNPs.

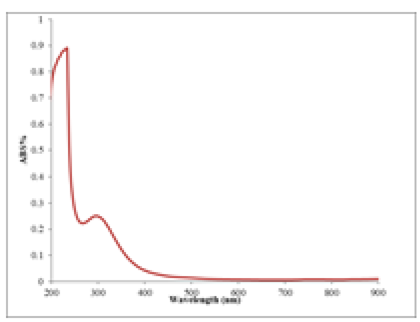

Figure 8 shows the amount of the absorbent coefficient for sample FeNPs

5 .Discussion:

In this work, iron nanoparticles were prepared by Beauveria bassiana fangus at room temperature. The FeNPs were completely stable without the use of any toxic chemicals, as biological agents were used. The reaction started after 24 h of incubation in the dark, FeNO3 with the color of the filtrate changing to dark brown, indicating the formation of iron nanoparticles. In addition, they gave a characteristic band at 240 nm. Hence, it indicates the complete reduction of iron ions to dark brown in aqueous solution due to the excitation of surface plasmon vibration in FeNP [7]. It was found that the absorption peak in the ultraviolet range corresponds to 220 nm and this may be due to absorption by the amide bond or it may be attributed to tryptophan and tyrosine residues in proteins, which indicates the secretion of some protein components into the medium by a fungus. AFM measurements, it was found that the shape of the composite iron NPS resembles the spherical shape in the clusters

This means that Beauveria bassiana fangus has the potential to synthesize FeNPs. The biosynthesized FeNPs showed suitable homogeneity and stability at substrate concentration of 50 mM at room temperature with biomass weight of 20 g at pH 7 with biomass culture for 14 days..

6. Conclusions:

We have prepared nano iron particles as safe, economically viable through their successful manufacture using bioaccumulative filters from the highly persistent , Beauveria bassiana fangus. The green method of producing nanoparticles that quickly replace traditional chemical formulations is a major concern due to environmental hazards. Currently, different types of biological units that serve a dual role as reduction agents and stabilizers in bioactive nanoparticle formation are considered to be an effective and safe fungal protocol with the ability to convert iron nitrate. To nanoparticles of the iron oxide component through a mechanism outside the cell. From this study, it can be concluded that the summation of the Beauveria bassiana recorded in acidity and temperature is the appropriate effective means by which it is considered to be effective in the green formation of the nanodioxide molecules. In addition, Beauveria bassiana fungus are an innovative way to produce nanobodies.

7. References

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