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BIOSYNTHESIS OF NANO GOLD PARTICLES FROM *FUSARIUM SOLANI* ISOLATED FROM KARBALA

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ABSTRACT: *This study was conducted to explore the possibility of biosynthesis of gold nanoparticles by using the fungus Fusarium solani isolated from soil sample in Karbala , Iraq. The cell filtrate of F.solani reacted with HAuCl4 ions, resulting f Appearance of a burgundy red color in solution containing the biomass was a clear indication of the formation of gold nanoparticles after 6 hr . The gold nanoparticles were characterized by Visual analysis, The results showed After HAuCl4 addition with fungus filtration, a well-defined absorption peak at 530 nm appears. The spectra obviously show the upsurge in intensity of gold solution with period. The data obtained from micrograph images by Transmission electron microscopy (TEM) found distinct shape and size of poly disperse nanoparticles Mostly particles were spherical and ellipsoidal and in shape in the range of 18-24 nm and average size 21.82 nm in size without significant agglomeration . The synthesized gold nanoparticles has been evaluated for antibacterial activities by well method against selected human pathogens. The results of antibacterial efficacy are depicted in table 1. Biosynthesized gold nano patrticles had antibacterial activity against selected gram negative bacterial pathogens. gold nanoparticle (23µl/disc) impregnated disc exhibited highest antibacterial activity against Klebsiella pneumoniae (20.8mm) followed by Eschertia coli (18.4 mm) by well method. gold nanoparticles were subjected at different concentration to study the minimal inhibitory concentration (MICagainst the selected pathogens were portrayed in table 3. MIC revealed that 16µl/ml of Ag-NPs exhibited highest inhibition activity against Klebsiella pneumoniae followed by Eschertia coli .*

KEYWORDS: Biosynthesis, Nano Gold, Fusarium Solani, Karbala and Antibacterial Activity

INTRODUCTION

Nanotechnology has actively developed as an imperative ground of modern research with potential effects in electronic and medicine (Boisselier et al.,2009). Nanoparticles(NP) are typically collections of atoms in the size range of 1-100 nm . It is unstated that the possessions of a metal NP are determined by its size, figure, arrangement, crystallinity, and construction (Sun and Xia, 2002). Nanoparticles appear a talented choice when associated to the conservative materials used, with the variety of submissions that nanoparticles discovery in varied fields of engineering and science. Their individuality arises specifically from higher surface to capacity ratio and augmented percentage of atoms at the grain boundaries. They characterize an imperative class of materials in the expansion of different devices that can be used in numerous physical, biological biomedical and pharmaceutical requests (Sigel,1993; Lee et al.,2003). . Nanoparticles can be made from vast range of materials such as gold, silver, metal oxides, inorganic material polymeric materials and lipids (Fadeel and Bennett ,2010). Both unicellular and multicellular organisms are known to produce inorganic materials either intra or extra cellular (Shankar et al.,2004) .Various microbes are recognized to reduce metal ions to the metals. Although it is known that bacteria, yeast, cyanobacteria and actinomycetes can reduce toxic metals through reduction of metal ions. Among these fungi are extremely

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good candidate group of organisms in the synthesis of metal nanoparticles ,it is play an important role in remediation of toxic metals through reduction of the metal ions this was considered interesting as nano factories very recently (Fortin and Beveridge, 2000). The biosynthesis of inorganic nano materials by eukaryotic organisms such as fungi may be used to develop nanoparticles of gold (Mukherjee et al.,2001). The current examination for Gopinath and Arumugam,(2014) designates the extracellular synthesis of gold nanoparticles from F. solani culture filtrate synthesized gold nanoparticles were painstaking. Compared with other fungi, Fusarium are comparatively more repeatedly used for the biosynthesis of nanoparticles. The spherical or triangular gold nanoparticles of size 20-40 nm were well-dispersed and no important reunification was originate straight after a month (Mukherjee et al.,2002). Nanoparticles have involved extreme interest in their expansion as probable antibacterial drugs (Yacoby and Benhar,2008; Rao,2008) .

It has been described that biophysical communications happen between NPs and bacteria counting biosorption, NPs breakdown or accumulation, and cellular uptake, with effects with membrane injury and toxicity (Amstad et al.,2011). The instruments of NPs constraining bacterial development remain fewer well understood. It has been described that the size and superficial alterations of NPs could affect their antibacterial heights (Bandyopadhyay et al.,2011; Li et al.,2008). Inclusive empathetic of antibacterial mechanisms is desirable to nimprove the efficiency of NPs in disease behavior ,the essential for actual antibacterial managers is acknowledged.

MATERIAL AND METHODS

Isolation and Identification of *F. solani*

The fungus *F.solani* was isolated from soil samples collected randomly from Karbala , Iraq and maintained on potato dextrose agar (PDA) medium at 28Cº. Identification depended on Macroscopic and microscopic comment finished increasing by lacto phenol cotton blue was used recognized isolated fungus. Pure culture of *F. solani* was maintained on potato dextrose agar slants at 28Cº.

Bacteria

Tow gram negative bacterial pathogens were Eschertia coli and Klebsiella pneumoniae were collected from department of Biology, College of Science, University of AL- Mustansarya, Baghdad, Iraq , for the present antibacterial susceptibility study.

Inoculation Preparation

Biomass of *F.solani* was ready in potato dextrose broth (PDB). The flask was inoculated by spores of *F. solani* and incubated at 28ºC on a rotatory shaker (120 rpm) for 7 days, biomass was collected by filtration over filter paper and then washed with distilled water, biomass of *F. solani* was transported in sterilized flask in contact with 100 mL of double distilled water for 3 days at 28°C and agitated once more at120 rpm. The crude cell filtrate was gotten by filtering through filter paper.

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Biosynthesis of Gold Nanoparticles

The crude cell filtrate was treated with 1 mM HAuCl4 solution in Erlenmeyer flask as 1:1 proportion and incubated at 28°C, Control containing cell-free filtrate without Chloroauric acid solution was track concurrently as standard with the investigational flask.

Spectroscopy

The color of crude cell filtrate develops different after the incubation . Chloroauric acid solution was observed above every 6 h through 2 days. Gold ion bio-reduction was monitored by sampling of aliquots (1mL) at different time intervals. Absorption measurements were carried out on UV-visible spectrophotometer ,absorbance was measured between 350-750nm.

Transmission Electron Microscope

TEM micrographs of the sample were taken using the *F. solani* synthesized AuNPs drop was placed on the carbon coated copper grids and kept for 5 min to dry. Then sample dried copper grid loaded on to a specimen holder for TEM images .

Antibacterial Determination by Well Diffusion Method

Gold nanoparticles were assayed for antibacterial activity by well diffusion method against selected bacteria . The overnight broth cultures of each test bacteria were prepared in Muller Hinton Broth at 37oC. Each bacteria (106 cells) were mixed with sterile Muller Hinton Agar (MHA) and poured on to the sterile Petri disc separately. The plates were left for few minutes for solidification. After solidification in each plate 5 wells of 5 mm diameter size with equal center to center distance were made using gel puncture aseptically. Using variable micropipette 5µl, 8µl, 15µl , 18µl & 23µl of gold nanoparticle solutions were poured onto each well in triplicates for each test bacteria. The inoculated plates were incubated at 37oC for 24hours.After incubation the different levels of zone of inhibition was measured and recorded.

Determination of Minimal Inhibitory Concentration (MIC)

Gold nanoparticles were determined for minimal inhibitory concentration (MIC) by standard micro dilution method against selected bacteria. The test bacteria (103cells/ml) were inoculated in MHB. Different concentration of gold nanoparticles viz., 2µl, 4µl, 6µl, 8µl, 10µl, , 12µl, 14µl & 16 µl were tested for MIC leading to the inhibition of bacterial growth. The MIC was examined in spectrophotometer at 600nm after 24 hours of incubation at 37oC .

RESULTS AND DISCUSSION

Visual Observation

The color of HAuCl4solution was golden and *F. solani* crude cell filtrate was colorless. The reaction combination of 1 mM aqueous solution of gold chloride with *F.solani* crude cell filtrate show exaggerated color indicates reduction of gold ions to gold nanoparticles (Figure 1) the color entirely turns into purple color It is observe that the color of gold nanoparticles change from colorless to purple which clearly indicates the synthesis of gold nanoparticles. Appearance of a burgundy red color in solution containing the biomass was a clear indication of the formation of gold nanoparticles in the reaction mixture and was due to the excitation of

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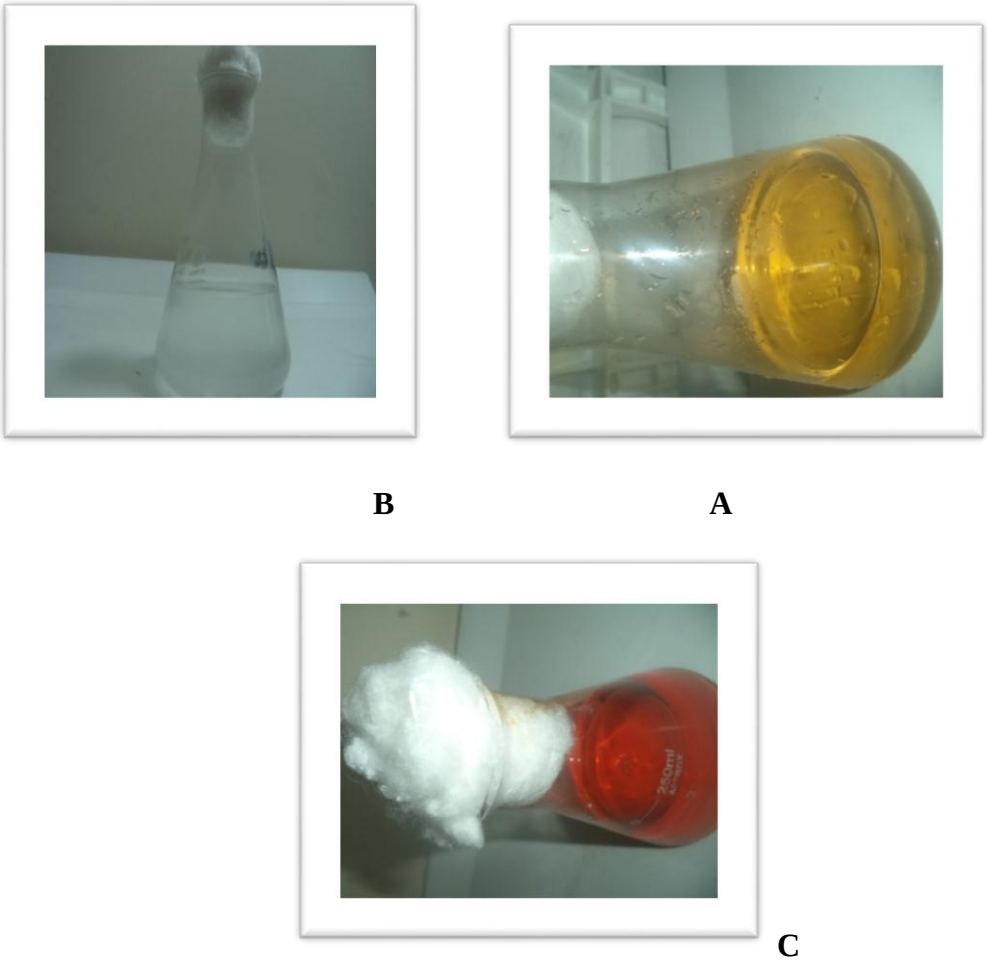
surface plasmon vibrations in the nanoparticles (Ahmad et al.,2003).This modification in color was owing to the shared coherent oscillation of conduction electrons at the surface of the gold nanoparticles when these particles interrelate with the oscillating electric field of the incident light, a phenomenon called surface plasmon resonance (SPR). This change in color indicates the reduction of HAuCl4 to nano gold which is the first step in the formation of AuNPs (Verma et al.,2011; Chauhan et al.,2011). This significant observation indicates the reduction of the Au+ ions and the biosynthesis of gold nanoparticles. Beforehand, Singaravelu et al. (2007) described that the gold nanoparticle synthesis development was started at 1h and the process was accomplished at 15 hr. Nevertheless, in the current study, the gold nanoparticle synthesis procedure is speedily happening at 30 min of incubation time.



B **A**

C

Figur1: (A) Chloroauric acid solution (B). crude cell filtrate of F. solani (C) crude cell filtrate after immersion of 1mM aqueous solution of HAuCl4

UV–spectroscopy

The spectra recorded from *F.solani* reaction container at diverse reaction times are described, UV–vis absorption spectroscopy was used to measure the absorbance of gold nanoparticles every 6 hours for 2 days . The results showed that after HAuCl4 addition with fungus filtration, a well-defined absorption peak at 530 nm appears. The spectra obviously show the upsurge in intensity of gold solution with period, representative the formation of augmented number of

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gold nanoparticles in the solution. Inbakandan et al. (2010) found that the sharp absorption peak at 527 nm indicates the presence of gold nanoparticles in the solution .

The most commonly used method for checking the formation of nanoparticles is UV-Vis spectroscopy. UV Visible absorption dimensions in the range 350-600 nm can deliver an insight into size, distribution, surface possessions and optical possessions of the nanosized Au particles. The surface plasmon bands for the gold nanoparticles typically ranges between 510 and 560 nm in aqueous solution contingent upon the function of their morphology, since plasmon bands are very sensitive to the length and sharpness of the tips of nano materials.(Nachiyar et al.,2015). As substantiated by Huang and Yang (2003), the spherical nanoparticles have strong absorption at about 520 nm with almost no absorption after 600 nm; however, the triangular shape has absorption at 540 nm which extends well in near infrared region (NIR). The wavelength of peak absorption depends upon several factors such as particle size, dielectric constant of surrounding media and the inter particle distance .

TEM Analysis

TEM micrograph showed details morphology of gold nanoparticles. The data obtained from micrograph images found distinct shape and size of polydisperse nanoparticles Mostly particles were spherical and ellipsoidal and in shape in the range of 18-24 nm and average size 21.82 nm in size without significant agglomeration (Fig2). TEM results revealed that the gold nanoparticles from F.solani are highly stable in the diameter range between 20 and 50 nm (Gopinath and Arumugam,2014). Further, studies conducted by Verma et al (2011) also substantiate these results herein an endophytic fungus Aspergillus clavatus was used for the synthesis of gold nanoparticles.

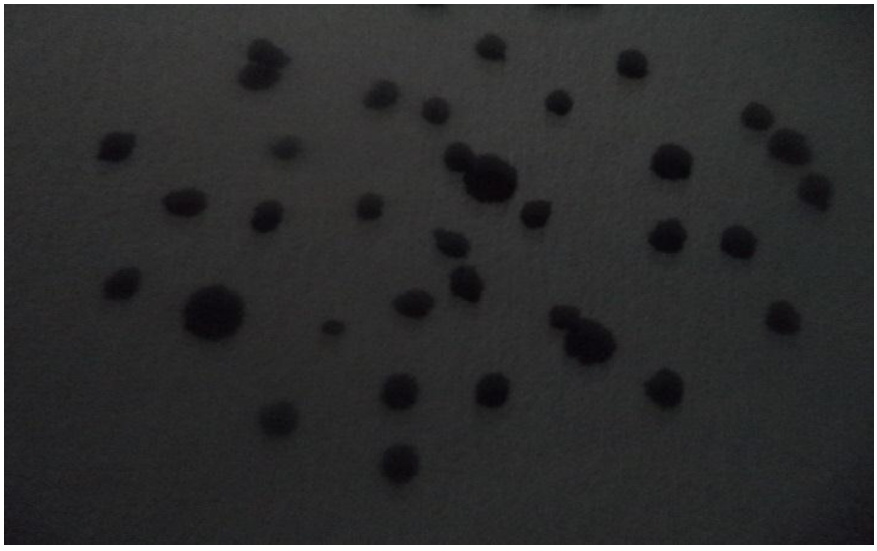

Figure 2:TEM micrograph of gold nanoparticles synthesized by F. solani

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Antibacterial activity of nano gold synthesized from *F.solani* against selected bacteria by well method

The results in table 1 show antibacterial activity of biosynthesized GNP against the bacterial cultures, biosynthesized GNP showed a distinct inhibition zone of gram negative bacteria .The synthesized gold nanoparticles has been evaluated for antibacterial activities by well method against selected human pathogens. The results of antibacterial efficacy are depicted in table 1. gold nanoparticle (23µl/disc) impregnated disc exhibited highest antibacterial activity against Klebsiella pneumoniae (20.8mm) followed by Eschertia coli (18.4 mm) by well method .The result thus indicates that the biosynthesized GNP was toxic to tested gram negative bacteria. Works studies have exposed that GNPs constrain bacterial growth by making holes in the cell wall, subsequent in release of cell contents, or they bind with the DNA inhibiting its unraveling thereby transcription (Rai et al.,2010) .It is balanced to state that the necessary of gold nanoparticles to the bacteria be depending on the surface area available for the communication. Nanoparticles have large surface area nearby for connections which enhances bactericidal consequence than the great sized particles; henceforth they inform cytotoxicity to the micro organism (Orendorff et al.,2005).

Table 1: Antibacterial activity of gold nanoparticles synthesized against bacteria by well method

Bacteria	**Zone of inhibition in [mm] in diameter**				
	Concentration of gold nanoparticles				
	5µl	**8µl**	**15µl**	**18µl**	**23µl**
Eschertia coli	**2.3**	**6.5**	**12.7**	**14.6**	**18.4**
Klebsiella pneumoniae	**3.1**	**7.4**	**14.1**	**17.3**	**20.8**

Similar study by Azam et al.,(2009) found that the antibacterial action of gold nanoparticles as a purpose of particles attentiveness against gram negative bacterium Escherichia coli was carried out in solid growth media. Antibacterial possessions of gold nanoparticles are accredited to their entire surface area, as a greater surface to capacity ratio of nanoparticles and it delivers a more effectual means for improved antibacterial movement. Gold nanoparticles demonstration high antimicrobial and antibacterial action with zone of inhibition of about 22 mm.

Minimal inhibitory concentration (MIC) of of nano gold synthesized from *F.solani* against selected bacteria

Gold nanoparticles were subjected at different concentration to study the minimal inhibitory concentration (MIC) against the selected pathogens were portrayed in table 2. MIC revealed that 23µl/ml of exhibited highest inhibition activity against Klebsiella pneumonia it was20.8 followed by Eschertia coli18.4 .These results clearly indicate that biosynthetic gold nanoparticles could provide an eco-friendly and safer alternative for the conventional chemotherapeutic agents to treat infectious disease caused by gram negative bacteria .

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Table 2: Minimal inhibitory concentration (MIC) of gold nanoparticles synthesized aginest bacteria

Bacteria	Zone of inhibition in [mm] in diameter Concentration of gold nanoparticles in µl/ml							
	2µl	4µl	6µl	8µl	10µl	12µl	14µl	16µl
Eschertia coli	1.6	1.4	0.95	0.81	0.77	0.70	0.63	0.51
Klebsiella pneumoniae	1.4	1.1	0.89	0.76	0.6	0.54	0.5	0.41

Lie t al.,(2014) presented the use of functionalized gold nanoparticles (AuNPs) to combat multi-drug-resistant pathogenic bacteria. Tuning of the functional groups on the nanoparticle surface provided gold nanoparticles that were effective against both Gram-negative and Gram-positive pathogens including multi-drug resistant pathogens. These AuNPs exhibited low toxicity to mammalian cells, and bacterial resistance was not observed after 20 generation. A strong structure-activity relationship was observed as a function of AuNP functionality, providing guidance to activity prediction and rational design of effective antimicrobial nanoparticles.

Grace and Pandian ,(2007) investigated that the gold nanoparticles have a great bactericidal effect and it possess well-developed surface chemistry, chemical stability and appropriate smaller size which make them easier to interact with the microorganisms. In specifically, the nanoparticles bind to the building elements of the outer membrane causing structural changes, degradation and finally cell death. Percentage fold increase of gold nanoparticles in the presence of various stabilizers is given in. Both methods have been reported for enhanced activity against bacteria, showing decreased minimum inhibitory concentration (MIC) in comparison with use of free antibiotics. The improved performance is proposed to result from polyvalent effect of concentrated antibiotics on the NP surface as well as enhanced internalization of antibiotics by NPs. (Zhao and Jiang,2013).

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