AL-Qadisiyha Journal For Science Vol.20 No. 1 Year 2015
Saba A. \ Taif M. \ Asmaa M. ISSN 1997-2490

Antifungal Activity of Silver Nanoparticles Synthesized by *Ttichoderma harizanum*

Receved :18\12\2014 **Accepted :8\2\2015**

Saba A. Al- Ziadi **Taif M. Al- Kalidy** **Asmaa Maen**

Sabaameer_8692@yahoo.com

College of Science / University of Qadisiyah

Abstract:-

In this study, Antifungal potential of silver nanoparticles synthesized from by filtrate *Ttichoderma harizanum* on different pathogenic fungi was investigated. 10 mL of filtrate *Ttichoderma harizanum* on was mixed with 90 mL of (1 mM) and (2 mM) aqueous $AgNO_3$ and heated at 70 °C for 10 min. A change color solution to dark brown color was observed .

Characterization using UV-VIS spectrophotometery. The UV-Vis spectral analysis showed silver surface plasmon resonance band at (299.5 – 435.5) nm in 2 mM aqueous $AgNO_3$. Antifungal activity against three fungi was tested using well Poisoned food method. The synthesized silver nanoparticles efficiently inhibited various pathogenic fungi in a dose dependent

The approach of dark brown synthesis seems to be cost effective, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis. The powerful bioactivity demonstrated by the synthesized silver nanoparticles leads towards the clinical use as antifungal.

Keywords: Silver Nanoparticles , Antifungal activity , *Ttichoderma harizanum*

Microbiology classification : QR:502

INTRODUCTION

Nanotechnology is the first major worldwide research initiative of the 21st century.

Nanotechnologies are general purpose technologies that act as both the basis for technology solutions across a range of industrial problems or as a nexus for the convergence of other enabling technologies like biotechnologies, computational sciences, physical sciences, communication technologies, cognitive sciences, social psychology and other social sciences **[1;2; 3; 4]**

Accordingly, these environmentally-friendly biological systems may be considered as benign nanofactories. It must be pointed out that many such microorganisms are biologically poisonous to humans, animals and plants, and care must be taken in their choice for production of nanoparticles.

It is demonstrated that using the dissimilatory properties of an eukaryotic organism such as fungi may be used to biosynthesize and grow nanoparticles. It is shown that certain fungi have the ability of producing extracellular metabolites that serve as agent for their own survival when exposed to such environmental stresses like toxic materials (such as metallic ions), predators and temperature variations**[5].**

In the biosynthesis of metal nanoparticles by a fungus, the fungus mycelium is exposed to the metal salt solution. That prompts the fungus to produce enzymes and metabolites for its own survival. In this process the toxic metal ions are reduced to the none-toxic metallic solid nanoparticles through the catalytic effect of the extracellular enzyme and metabolites of the fungus. The presence of hydrogenase in fungi, such as *Fusarium oxysporum* **[6],** *Trichoderma reesei* **[7]** and *Trichoderma viride***,** was demonstrated with washed cell suspensions that had been grown aerobically or anaerobically in a medium with glucose and salts amended with nitrate **[8].** The nitrate reductase was apparently essential for ferric iron reduction **[9].**

Many fungi that exhibit these characteristic properties, in general, are capable of reducing *Au* (III) or *Ag* (I) **[10**]. Besides these extracellular enzymes, several naphthoquinones **[11;12]** and anthraquinones **[13]** with excellent redox properties, were reported in *Fusarium oxysporum* that could act as electron shuttle in metal reductions **[14;15].** Specifically the following results towards production of nanoparticles have been achieved using fungi:

i. Biosynthesis of magnetite using the fungus *Fusarium oxysporum* and *Verticillium species* **[16].**

ii. Production of gold nanotriangles by actinomycete, which is a bacteria resembling fungi **[17];**

iii. Intracellular synthesis of gold and silver nanoparticles in *Verticillium* fungal cells **[18; 19 ; 20].**

iv. Extracellular production of gold, silver and bimetallic *Au-Ag* alloy nanoparticles by the fungus *Fusarium oxysporum* **[21;22].** It has been observed that the exposure of aqueous solutions of metal salts or a mixture of metal salts to *Fusarium oxysporum* resulted in extracellular formation of nanoparticles of dimensions 5–50 *nm* and alloy nanoparticles of dimensions 8–14 *nm* **[23;24].**

v. Extracellular production of silver nanoparticles using the fungus *Aspergillus fumigatus* **[25].**

vi. Production of silver nanoparticle as a result of the reduced state of pretreated fungus Phoma Species **[26].**

vii. Extracellular enzymatic reduction of MnO_2, nitrate, selenite and ferric ions using fungus *Trichoderma reesei* **[27].**

In the present paper we report extracellular production of silver nanoparticles using *Trichoderma harizanum*. In what follows, the main advantages of *Trichoderma harizanum* over other fungi are reported.

MATERIALS AND METHODS

Materials

The chemical silver nitrate ($AgNO_3$) , fungus *Trichoderma harizanum* , Distilled water, Potato's Dextrose Agar (PDA) .

Preparation of the filtrate of the fungus *Trichoderma harizanum*

Filtrate of the fungus *Trichoderma harizanum* was prepared by it was growth in Distilled water About 100 ml of about 5 days at 27 C° , That filtrate of the fungus *Trichoderma harizanum* were filtered with the help of filter paper , Then filtrate of the fungus *Trichoderma harizanum* was kept in refrigerator at 4 C° for future experiments.

Synthesis of silver nanoparticles

Aqueous solution of silver nitrate was prepared by adding 2mM of $AgNO_3$ to 90 ml of distilled water at room temperature. The aqueous solution was mixed with 10 ml of filtrate of the fungus *Trichoderma harizanum* at a temperature of 70 ° C while stirring magnetically at 1000 rpm for 10 min. The bio-reduced aqueous component was used for the UV-Vis spectroscopy characterization.

Characterization of silver nanoparticles

UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (CE7200) UV/Vis spectrophotometer, England) from 200-900 nm at a resolution of 2nm.

Evaluation of antifungal activity

The silver nanoparticles synthesized using filtrate *Trichoderma harizanum* was tested for antifungal activity by poisoned food method**(28)** against different pathogenic fungi *Aspergillus niger*, *Aspergillus ochraceus*, *Fusarium solani*. The pure cultures of fungi were sub cultured on PDA. Each fungi was transfer disk from colony of fungi using Piercing cork diameter 7.5 on to each well on all plates . After incubation at 27◦C for 7 days, the diameter of colony (radial growth) was measured in millimeter.

Results and Discussion

Figure(1) shows a tube of filtrate *Trichoderma harizanum* immersion in 1 mM AgNO3 solution and 2 mM AgNO3 solution. The pale brown color of the fungal cells can clearly be observed in Figure 1. A picture of the tube containing the fungal cells after immersion in 1*M AgNO3* solution for 10 min is shown in Figure1(A). The tube containing the fungal cells after immersion in 2*M AgNO3* solution for 10 min is shown in Figure1(B). It can be observed that the previous yellow color of the reaction mixture is changed to the brownish color after 10 min of reaction. The appearance of a yellowish-brown color in solution containing the biomass is a clear indication of the formation of silver nanoparticles in the reaction mixture. The color of the solution is due to the excitation of surface plasmon vibrations (essentially the vibration of the group conduction electrons) in the silver nanoparticles

Optical spectroscopy is widely used for the characterization of nanomaterials. In the present study we use three different spectroscopy techniques to fully characterize the silver nanoparticles we have produced. They include absorption UV-Visible light spectroscopy, fluorescence emission spectroscopy and Fourier transform infrared spectroscopy.

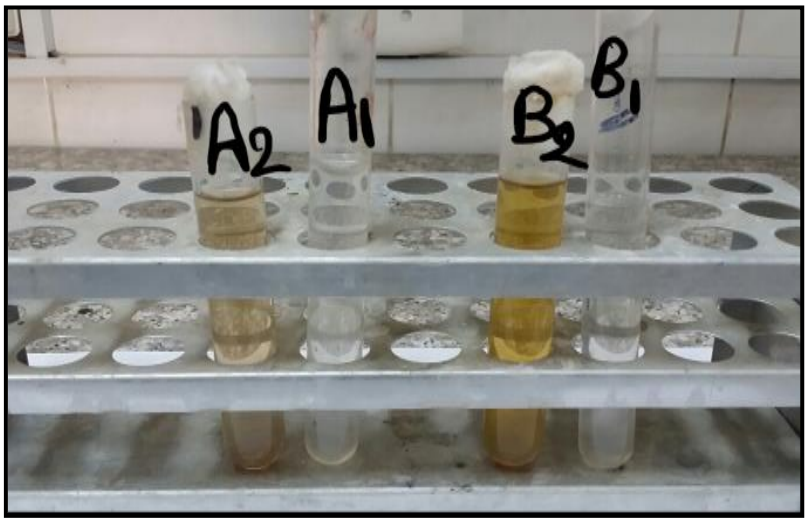


Figure 1: Picture of tube containing filtrate of the fungus *Trichoderma harizanum* with aqueous $AgNO_3$ (1m)(A1) before and (A2) after heated at 70 °C for 10 min and tube containing filtrate *Trichoderma harizanum* with aqueous $AgNO_3$ (2m)(B1) before and (B2) after heated at 70 °C for 10 min.

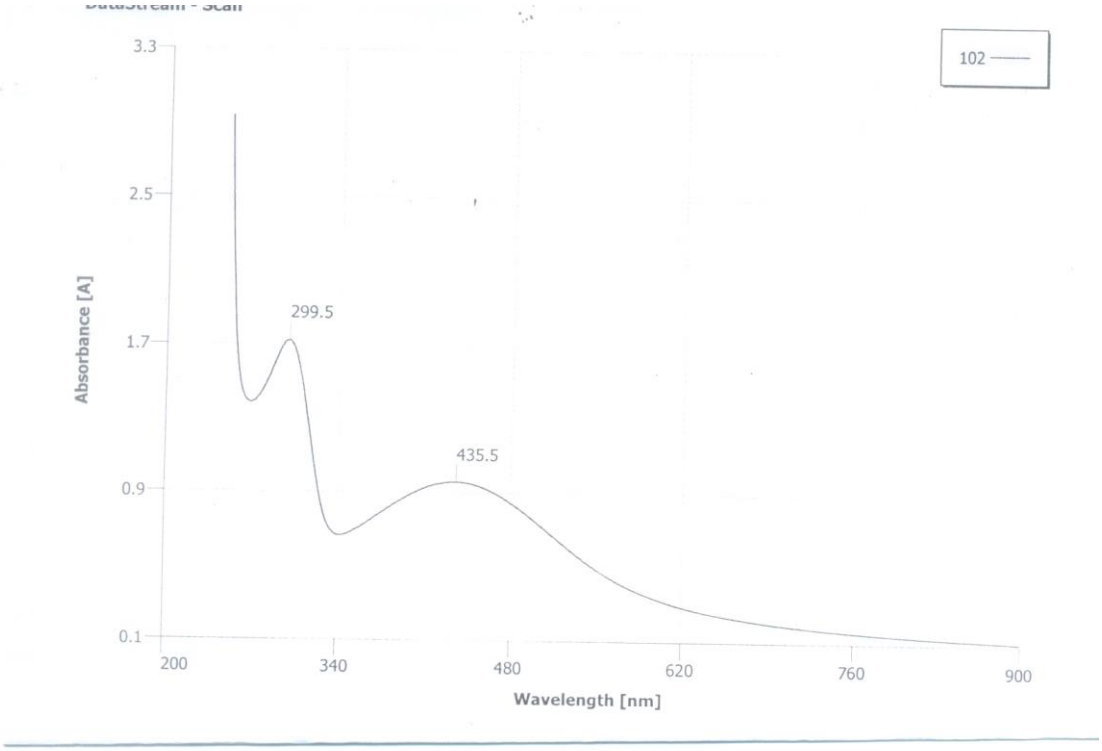


Figure (2) UV/Vis absorption spectra of reduction of silver ions to silver nanoparticles after heating at 70 °C for 10 min

In this study, the application of silver nanoparticles as an antifungal agent was investigated and demonstrated that the zone of inhibition increased according to concentration of silver nanoparticles in all pathogenic fungi Figure (4) , we have shown for the first time the use of *Trichoderma* in the extracellular synthesis of silver nanoparticles. In the biosynthesis of metal nanoparticle by a fungus, enzymes are produced which reduce a salt to its metallic solid nanoparticles through the catalytic effect. Compared to other filamentous fungus, the *Trichoderma* is considered to be the most efficient extracellular enzyme producer, and has a long history in the production of industrial enzymes**(29)**.

Extracellular secretion of enzymes offers the advantage of obtaining large quantities in a relatively pure state, free from other cellular proteins associated with the organism, and can be easily processed by filtering of the cells and isolating the enzyme for nanoparticles synthesis from cell-free filtrate. Our measurements indicate that extracellular biosynthesis of silver nanoparticle by *Trichoderma harizanum* have inhibitory ability of pathogenic fungi reached at Concentration 1M $AgNo_3$ (*1.85 , 2.7 ,6.9)* for *F.solani ,A.ochraceous ,A.niger* respectively Figure(3,4) , While reached at Concentration 2M $AgNo_3$ (1.25 ,1.4 , 1.55) for all fungi respectively Figure (3,4) ,While treatment comparison (without addition) (3.85 ,9.0 ,8.8) for all fungi respectively Figure (3 ,4). we compare the size ranges methods of AgNP produced through various fungi together with the environmental biological and economical implications of the use of each fungus. According to biosynthesis of silver nanoparticles by fungus *Trichoderma harizanum* is preferred from the points of view of safety, economy and the large-scale production potential. As discussed above, we can biosynthesize silver nanoparticles on a large scale through *Trichoderma harizanum*, which is a major advantage over other fungus methods. It should be mentioned that *Trichoderma harizanum* is not known to be harmful to humans. According to previous studies on *Trichoderma harizanum*, the production of extracellular enzyme and nanoparticles in this fungus is more efficient than other fungi. It is also shown that *Trichoderma harizanum* has easier and cheaper cultivation requirements and higher growth rates on both industrial and laboratory scales, thereby having a lower cost in large-scale production. It should be pointed out that large-scale production of silver nanoparticles by other techniques, such as chemical vapor deposition, irradiation, and liquid solution reduction, usually produces particles larger than a few micrometers in size. These other techniques also involve lower production yields and higher expenses **[30 ; 31; 32]** compared to large-scale biosynthesis through *Trichoderma harizanum* Because of the significant commercial value of the findings

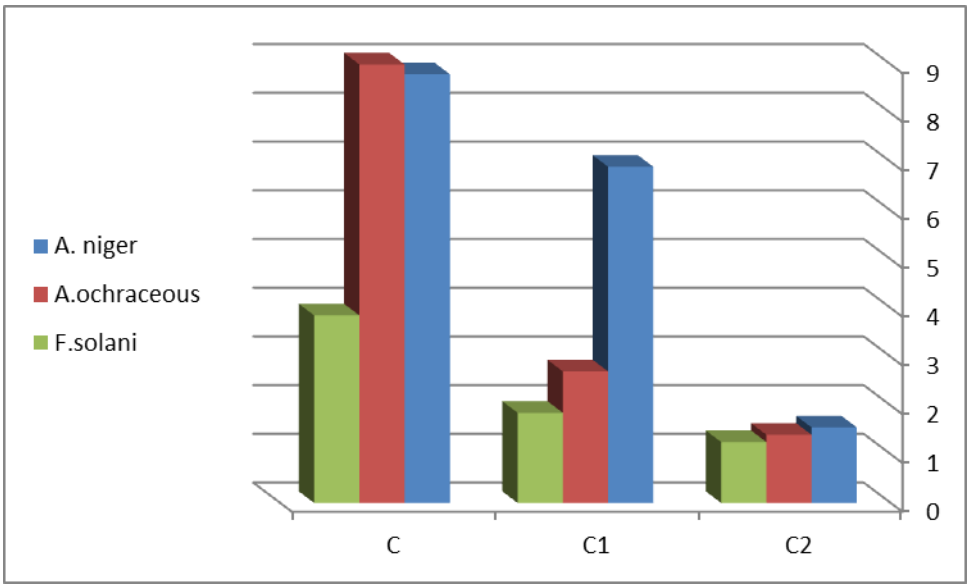
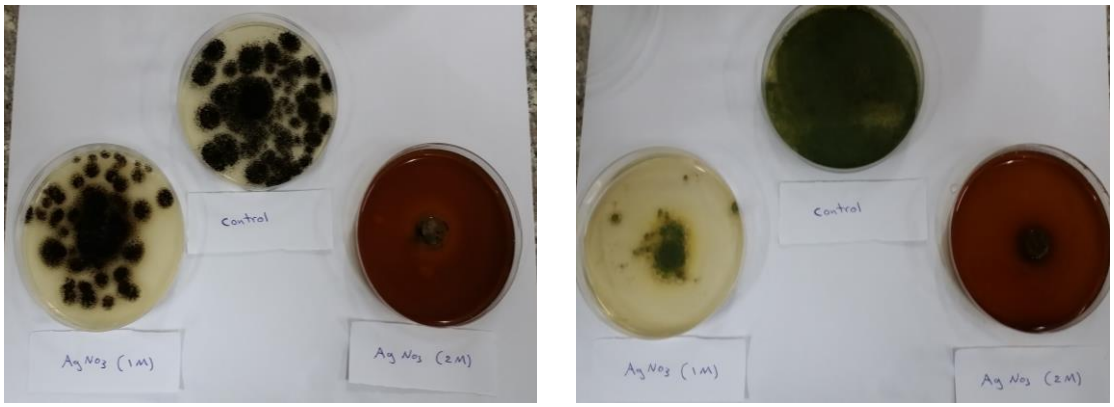


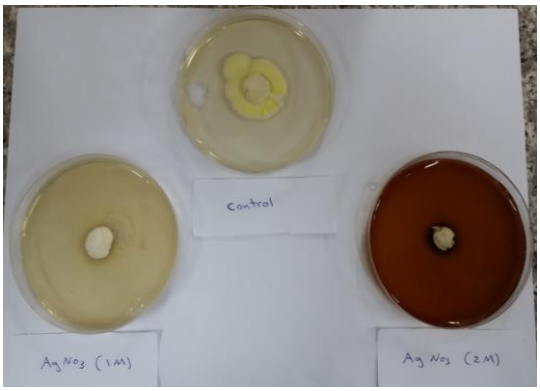
Figure 3 : aqueous $AgNO_3$ with filtrate of the fungus *Trichoderma harizanum* treatment had significant inhibited effect for growth of tested fungi
C : Control (without addition)

C1: (1 mM) aqueous $AgNO_3$ with filtrate of the fungus *Trichoderma harizanum*

C2: (2 mM) aqueous $AgNO_3$ with filtrate of the fungus *Trichoderma harizanum*



(A) *Aspergillus niger* (B) *Aspergillus ochraceus*



(C) *Fusarium solani*

Figure 4 : aqueous $AgNO_3$ with filtrate of the fungus *Trichoderma harizanum* treatment had significant inhibited effect for growth of tested fungi(A ,B ,C ,)

Reference:

1- Linton JD, Walsh S. 2004. Integrating innovation and learning curve theory: An enabler for moving nanotechnologies and other emerging process technologies into production. R&D Management 34: 513-522.
2- Kautt M, Walsh S, Bittner K. 2007. Global distribution of micro-nano technology and fabrication centers: A portfolio analysis approach. Technology Forecasting and Social Change 74:1697-1717.
3- Freitas Jr RA. 2010. The Future of Nanomedicine. Futurist 44(1): 21-22
4- Hyungsub C, Mody CCM. 2009. The Long History of Molecular Electronics: Microelectronics Origins of Nanotechnology. Social Studies of Science (Sage) 39(1): 11-50.
5- Mehra RK, Winge DR 1991 Metal Ion Resistance in Fungi: Molecular Mechanisms and their Regulated Expression. *J. Cell. Biochem*, 45 30-40
6- Gilbert B, Zhang H, Huang F, Finnegan MP, Waychunas GA, Banfield JF 2003Special Phase Transformation and Crystal Growth Pathways Observed in Nanoparticles. *Geochem. Trans*, 4 20-25.
7- Rautio J, Smit BA, Wiebe M, Penttila M, Saloheimo M 2006 Transcriptional Monitoring of Steady State and Effects of Anaerobic Phases in Chemostat Cultures of the Filamentous Fungus *Trichoderma Reesei*. *BMC Genomics*, 7 247-249.
8- Chovanec P, Kalinak M, Liptaj T, Pronayova N, Jakubik T, Hudecova D, Varecka L 2005 Study of *Trichoderma Viride* Metabolism under Conditions of the Restriction of Oxidative Processes. *Can. J. Microbiol*, 51(10) 853-862.
9- Ottow JCG, Von Klopotek A 1969 Enzymatic Reduction of Iron Oxide by Fungi. *Appl. Microbiol*, 18 41-43.
10- Lloyd JR 2003 Microbial Reduction of Metals and Radionuclides. *FEMS Microbial. Rev*, 27 411-425.
11- Medentsev AG, Alimenko VK 1998 Naphthoquinone Metabolites of the Fungi. *Photochemistry*, 47 935-959.
12- Bell AA, Wheeler MH, Liu J, Stipanovic RD, Puckhaber LS, Orta H 2003 United States Department of Agriculture-Agricultural Research Service Studies on Polyketide Toxins of *Fusarium Oxysporum f sp Vasinfectum:* Potential Targets for Disease Control. *Pest Manag Sci*, 59 736-747.
13- Baker RA, Tatum JH 1998 Novel Anthraquinones from Stationary Cultures of *Fusarium Oxysporum*. *J Ferment Bioeng*, 85 359-361.
14- Misko TP, Schilling RJ, Salvemini D, Moore WM, Currie MG 1993 A Fluorometric Assay for the Measurement of Nitrite in Biological Samples. *Anal Biochem*, 214 11-16.
15- Kumar CV, McLendon GL 1997 Nanoencapsulation of Cytochrome c and Horseradish Peroxidase at the Galleries of Alpha-Zirconium Phosphate. *Chem Mater*, 9 863-870.
16- Bharde A, Rautaray D, Bansal V, Ahmad A, Sarkar I, Mohammad Yusuf S, Sanyal M, Sastry M 2006 Extracellula Biosynthesis of Magnetite using Fungi. *Small*, 2(1) 135-41.
17- Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M 2003 Extracellular Biosynthesis ofMonodisperse Gold Nanoparticles by a Novel Extremophilic Actinomycete, *Thermomonospora* sp. *Langmuir*, 19(8) 3550.
18- Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajaykumar PV, Alam M, Sastry M, Kumar R 2001

Bioreduction of AuCl4-ions by the Fungus, *Verticillium sp.* And Surface Trapping of the Gold Nanoparticles formed. *Angew Chem Int Edu*, 40 3585-3588

19- Kowshik M, Vogel W, Urban J, Kulkarni SK, Paknikar KM 2002 Extracellular Synthesis of Silver Nanoparticles by a Silver-Tolerant Yeast Strain MKY3. *Adv. Mater.*, 14 812-815.

20- Naik RR, Stringer SJ, Agarwal G, Jones SE, Stone MO 2002 Biomimetic Synthesis and Patterning of Silver Nanoparticles. *Nat Mater*, 1 169-172

21- Duran N, Marcato, PD, Alves OL, Souzaand G, Esposito E 2005 Mechanistic Aspects of Biosynthesis of Silver Nanoparticles by Several Fusarium Oxysporum Strains. Journal of Nanobiotechnology, 3:8 doi:10.1186/1477-3155-3-8.

22- Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M 2003 Extracellular Biosynthesis of Silver Nanoparticles using the Fungus *Fusarium Oxysporum*. *Colloid Sorf B*, 28313-318

23- Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, Sastry M 2002 Extracellular Synthesis of Gold Nanoparticles by the Fungus *Fusarium Oxysporum*. *Chem Biochem*, 3 461-463.

24- Sastry M, Ahmad A, Islam NI, Kumar R 2003 Biosynthesis of Metal Nanoparticles using Fungi and Actinomycete. *Urent Sci*, 85 162-170

25- Bhainsa KC, D'Souza SF 2006 Extracellular Biosynthesis of Silver Nanoparticles using the Fungus *Aspergillus* Fumigatus. *Colloids Surf B Biointerfaces*. 47(2) 160-164.

26- Chen JC, Lin ZH and Ma XX 2003 Evidence of the Production of Silver Nanoparticles via Pretreatment of Phoma sp.3.2883 with silver nitrate. *Lett Appl Microbiol*, 37 105–108

27- Klittich CJR, Leslie JF 1988 Nitrate Reduction Mutants of *Fusarium Moniliforme* (*gibberellafujikuroi*). *Genetics*, 118 417-423.

28- Dixit , S. N. and Tripathy , S. C. and upadyyey, R. R. (1976).The antifungal substance of rose flower (*Rose indica*) Economic Botany . 30 : 371 – 373.

29- Oksanen T, Pere J, Paavilainen L, Buchert J, Viikari L 2000 Treatment of Recycled Kraft Pulps with *Trichoderma Reesei* Hemicellulases and Cellulases. *J Biotechnol*, 78(1) 39–44.

30- Balaprasad A, Chinmay D, Ahmad A, Sastry M 2005 Biosynthesis of Gold and Silver Nanoparticles Using *Emblica Officinalis* Fruit Extract, Their Phase Transfer and Transmetallation in an Organic Solution. *J Nanosci Nanotechnol*, 5(7) 1665-1671.

31- Senapati S 2005 Biosynthesis and Immobilization of Nanoparticles and their Applications. Ph.D. thesis, University of Pune.

32- Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R,Ajaykumar PV, Alam M, Sastry M, Kumar R 2001 Bioreduction of AuCl4-ions by the Fungus,*Verticillium sp.* And Surface Trapping of the Gold Nanoparticles formed. *Angew Chem Int Edu*, 40 3585-3588.

الفعالية المضادة الفطرية لدقائق الفضة النانوية المتشكلة بواسطة راشح الفطر *Ttichoderma harizanum*

تاريخ الاستلام :- 18\12\2014 تاريخ القبول : 8\2\2015

صبا عبد الأمير كاظم الزيادي طيف مظهر مسلم الخالدي أسماء معن نعمة
كليـة العلـوم / جامعـة القادسيــة
Sabaameer_8692@yahoo.com

الخلاصة :
تم في هذه الدراسة اختبار الفعالية المضادة الفطرية لدقائق الفضة النانوية المتشكلة بواسطة راشح الفطر *Ttichoderma harizanum* على بعض الفطريات الممرضة اذ تم مزج 10 مل من راشح الفطر مع 90 مل من محلول نترات الفضة المحضرة بتراكيز (1 مولاري و2 مولاري) وتم تسخين المزيج عند درجة حرارة 70 م ° ولمدة 10 دقائق فلوحظ تغير اللون من الاصفر الى البني الداكن ، ولتشخيص الدقائق المتشكلة تم استخدام المقياس الطيفي UV-VIS اذ ظهرت حزمة التصوير الطيفي عند قراءة (299.5 – 435.5) نانوميتر لتركيز 2 مولاري ، كما درست الفعالية المضادة الفطرية على ثلاث انواع فطرية ممرضة باستخدام طريقة الغذاء المسموم واظهرت دقائق الفضة النانوية المتشكلة تثبيط لمختلف الانواع الممرضة المختبرة واعتمادا على تركيزها وبذلك يعد تخليق دقائق الفضة النانوية بهذه الطريقة البيولوجية سهلة وغير مكلفة ويمكن استخدامها سريريا كمضادات فطرية .

الكلمات المفتاحية : دقائق الفضة النانوية ، الفعالية المضادة للفطريات ، راشح الفطر *Trichoderma harizanum*

Microbiology classification : QR:502