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ANTAGONISTIC ACTIVITY OF SILVER NANOPARTICLES SYNTHESIS BY *FUSARIUM OXYSPORUM* AGAINST *CANDIDA* SPP.

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ABSTRACT

Background and Objective: *Candida* infections are one of the most commonly occurring fungal infections in human. Silver nanoparticles (Ag-NPs) are a new kind of material with several applications, such as antimicrobial against bacteria, fungi and viruses. The current education remained achieved to appraise the extracellular synthesis of silver nanoparticles by *Fusarium oxysporum* against *Candida* spp.

Methods: Ag-NPs synthesis from *F.oxysporum* extracellularly and characterized by UV-Visible spectral analysis, X-ray Diffraction Analysis (X-RD), Atomic force microscopy(AFM) and Fourier Transform Infrare Spectroscopy (FTIR),effect of Ag-NPs at concentrations (10, 25, 50,100µg/ml) against 7 isolates of *Candida* spp. by 2 way: agar plate well diffusion assay and Cell Proliferation Assay (MTT).

Results: Ag-NPs shown extreme absorbance peak on 418nm in UV–Visible Spectral. Since the X-RD design showed the presence three peaks, Strong diffraction peaks were: 38.19 º (111), 48° (200) and 67° (220). The Ag-NPs surface morphology revealed from AFM images show, size diameter 73.73 nm, Roughness average (Ra) and Root mean square (Sq) of was 14.7 nm, 18.2 nm, respectively. The FTIR spectrum of biosynthesized Ag-NPs showed six distinct peaks, 3711.87, 1699.18, 1108.81, 834.71, 446.70 and 419.33 cm^{-1}. And altogether *Candida* spp. isolates remained vulnerable towards Ag-NPs and inhibition rate increases with the increase of concentration.

***Conclusion*:** Biological synthesis of Ag-NPs using *F.oxysporum* have potent antimicrobial action in contradiction of *Candida lambica, C.famata, C.glabrata, C.albicans, C.tropicalis, C.sphaerica* and *C.krusei.*

KEYWORDS: *Candida* species, Silver Nanoparticles Ag-NPs, *Fusarium oxysporum***.**

INTRODUCTION

The kind *Candida* has varied species that are shared inhabitants of soil and of the mucosal surfaces of human gastrointestinal tract, genital-urinary tract and the mouth, and then remain accomplished of producing oral thrush or vaginal thrush. The greatest shared vaginal isolate comprises *C. albicans* through an occurrence of 70-90%, then fewer often non-*albicans Candida* species such as *C. tropicalis, C. glabrata (Torulopsis glabrata), C. kefyr (C. Pseudotropicalis), C. krusei, C. famata, C. Parapsilosis*, and *C. lusitaniae* (Namkinga, 2013, Imran and Al-Asadi, 2014).

The greatest shared nanoparticles made through fungi are silver and gold, but fungi must have remained used in the synthesis additional kinds of nanoparticles counting, platinum, zinc oxide, zirconia, magnetite, titanium, silica and cadmium selenite quantum dots and Cadmium sulfide. Furthermore, the extracellular biosynthesis with fungi can similarly type downstream dispensation ample calmer than bacteria, stimulating instance of NPs biosynthesis by fungi remained that the cell related Biosynthesis of silver by *F. oxysporum* (Ahmad *et al.*, 2003). Present study needs exposed that microorganisms, plant extracts, and fungi container harvest nanoparticles done biological pathways (El-Nour *et al*., 2010; Ghorbani *et al.*, 2011). The panache of act of Ag-NPs happening fungi is through directing the yeast cell membranes and troublesome membrane possible. The show electron microscopy examination needs exposed that the interaction between Ag-NPs and the membrane construction of *C. albicans* cells through Ag-NPs contact consequences in variations in the membranes of *C. albicans*, which container remains empirical as the "pits" on the membrane exteriors. The creation of holes formerly principals to cell demise (Gajbhiye *et al*., 2009). The purpose of the study Biosynthesis of Ag-NPs from *F. oxysporum* and *In vitro* study of the inhibitory result of Ag-NPs against *Candida* spp by agar plate well diffusion assay and MTT assay.

MATERIALS AND METHODS

Extracellular Synthesis of Ag-NPs: *Fusarium oxysporum* isolate and grown-up on Potato dextrose Agar (PDA), formerly secondhand trendy current education toward examination their capability toward biosynthesize of silver nitrate $Ag(NO_3)_2$ nanoparticles. The mycelia of *F. oxysporum* remained immunized in 250mL Erlenmeyer flagons, respectively covering 100mL of potato dextrose broth (PDB) medium, then hatched at 25 ± 2°C for 7days. Future, mycelia remained gaining through filtration over whatman strainer newspaper no. 1 then eroded thrice by pasteurized purified water to eliminate the traces of the medium on fungal biomass. The eroded mycelia remained suspended interested in 100mL pasteurized puri-

fied water then incubated at 25°C aimed at 24hrs. Once more, mycelia remained reaped through filtration over Whatman strainer newspaper no. 1. Formerly, cell filtrate was preserved through 1.0 mM silver nitrate solution and hatched at room temperature in light. Positive controls covering cell free filtrate deprived of silver nitrate and lone 1mM silver nitrate by way of negative control remained too conserved (Birla *et al*., 2013).

Description of Ag-NPs: The discovery of Ag-NPs was mainly approved available through pictorial remark of color modification of the fungal filtrate afterward action by silver nitrate. Arrival of dark brown color of fungal cell filtrate designates the creation of Ag-NPs. Additional; Ag-NPs remained considered using following methods:

1-Characterization of Ag-NPs by UV-Visible Spectrophotometer: Biotransformation of silver ions remained watched through UV–visible spectroscopy measurement of response medium. Three milliliters of supernatant were occupied and 48hrs and absorbance was scanned by Labomed, UV–vis double beam (Labomed, Inc, USA) within the wave length ranging from 200 to 600 nm. The absorption of the visible is contingent directly on color of the chemicals in solution (Husseiny *et al*., 2015).

2- Characterization of Ag-NPs by X-RD Examination: The stage diversity, then ounce size of manufactured Ag-NPs remained strong-minded by X-ray diffraction spectroscopy (Philips PAN analytical). The manufactured Ag-NPs remained teachings by CUKα energy by a power of 30 kV and present of 20 MA through scan rate of 0.030/s. dissimilar stages current in the manufactured examples remained resolute through X′ pert high notch software by exploration then competition ability. The atom size of the prepared examples remained strong-minded through by Scherrer′s reckoning by means of shadows

$$t = k\lambda / \beta \cos\theta \quad \text{.....(1)}$$

Where: t: is the crystallite size (in nm), K: (= 0.9) was the Scherer's constant, λ:is the wavelength of X-ray, B is the full width at half maximum of the peak in radians and Θ: is the Braggs angle in radians (Panigrahi , 2013)

3- Characterization of Ag-NPs by Atomic force microscopy: Size, surface topography and granularity volume delivery of biosynthesized nanoparticles characterized using Atomic Absorption Spectroscopy (AA-680, Shimadzu-Japan), (characterized by Dr. Abdul Kareem Al-Samaraii Lab. Baghdad/Iraq (Naveen *et al*., 2010).

4- Characterization of Ag-NPs by Fourier Transform Infrare Spectroscopy (FTIR): FTIR analysis of the dried powder of Ag-NPs was carried out by scanning the spectrum in the range 400– 4,000 cm^{-1} at a resolution of 4 cm^{-1} (8400S / Shimadzu/Japan). FTIR measurements were made to locate the possible biomolecules, which are responsible for the reduction of silver ions to Ag-NPs and stabilization of Ag-NPs. To prepare dried powder of Ag-NPs, the fungal treated broth was centrifuged at 12000 g for 15 minutes. Supernatants were discarded, and pellets of Ag-NPs were washed three times with autoclaved distilled water. The dried powder of Ag-NPs was subjected to FTIR analysis (Birla *et al*., 2013).

Antagonistic action of Ag-NPs against *Candida* spp *in vitro:* The antifungal act of the Ag-NPs on Seven most virulent isolates of *Candida* spp (*C. famata, C.albicans, C.glabrata, C.sphaerica, C. lambica,* and *C.tropicalis)* were assayed by agar plate well diffusion assay and Cell Proliferation (MTT).

Preparation of yeast inoculum: *Candida* spp. isolates inoculated in SDA at 37°C for 24 hrs., then *Candida* spp colonies were suspended in 5ml of 0.85% normal saline, the suspension was mixed for 15 second with a vortex, and then its concentration was adjusted to $5x10^{6}$ CFU/ml by using haemocytometer slide**.**

Preparation of nanoparticles concentration: The prepare dried powder of silver nanoparticles synthesis from *F.oxysporum* mentioned in (Extracellular Synthesis of Ag-NPs according to Birla *et al*. (2013) with some modification, the fungal treated solution was centrifuged at 12000g for 15 minutes. Supernatants were discarded, and pellets of Ag-NPs were washed thrice with deionized water. The pellets of Ag-NPs poured into sterile petri dishes and put in oven at 50°C. Then prepare different concentration (10, 25, 50, 100 µg/ml) from dried powder of Ag-NPs.

Inhibitory effect of Ag-NPs against *Candida* spp through agar plate well diffusion assay: The method described by (Abood, 2013) was followed to notice of Ag-NPs inhibition activity by spreading 0.1 ml of the yeast suspension on the surface of SDA and left to dry at room temperature. 100 µL of an Ag-NPs solution at 10, 25, 50, and 100 µg/mL concentrations was added into 5 mm diameter wells, later that incubated for 24 hrs at 37C°, then the zone of inhibition has been measured using a ruler.

Inhibitory effect of Ag-NPs against *Candida* spp by Cell Proliferation Assay Kit

1- Principle of MTT assay: The feasibility of *Candida* spp. was unhurried through using MTT [3-(4, 5-dimethylthiazole 2-yl)-2, 5-diphenyltetrazolium bromide] cell explosion examine, is a colorimetric examine scheme, Viable cells thro-

ugh lively metabolism change MTT into formazan, deceased cells mislay the aptitude (bioVision,USA).

2- Kit Contents:

- 50ml from MTT Reagent
- 150ml from MTT Solvent

3- Procedure of MTT Kit: Added 1 ml, from each isolates of *Candida* spp. in 105 test tubs which divided into 35 groups each group had 3 repeaters for treatment and for control, added 1ml from each concentration of Ag-NPs (10, 25, 50, and 100 µg/mL) on treatment group tube and incubation at 37 °C in 24 hrs and zero time, the control group for each *Candida* spp. isolates, without addition Ag-NPs. The number of viability of *Candida* spp. was calculated by MTT cell proliferation assay as the following steps:

1. After treatment, put 50 µl per well of microplates 96 wells flat bottom from each treatment group and control group.
2. Added 50 µl of the MTT Reagent to each well. Incubate the microplate for 3 hrs in a CO2 incubator (e.g. 26 °C, 5% CO2).
3. Carefully remove media. Do not disturb cells and do not rinse with PBS.
4. Added 150µl of the MTT Solvent into each well.
5. Checked aimed at whole solubilization of the purple formazan crystals formerly slow the absorbance of the examples through using a microplate (ELISA) bookworm, on wavelength was 590 nm.

To measure number yeast for drawing stander curve to use in measured the viability of yeast by inserting in a standard curve, Different number of yeast were prepared (1.18×10^6, 5.92×10^6, 11.85×10^6, 17.77×10^6, 23.69×10^6, 29.62×10^6 cell /ml) by using haemocytometer slide. Put 50 µl per well of microplates 96 wells, and the number of viability of *Candida* spp. was calculated by the MTT cell proliferation assay (Figure 1-1).

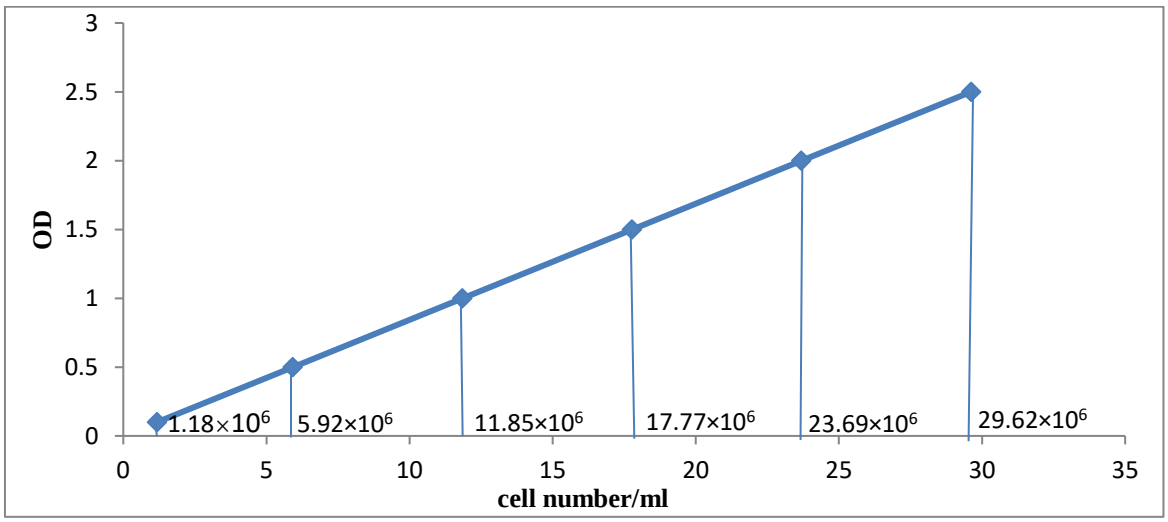

Figure 1-1: Standard curve for MTT assay (BioVision, USA)

RESULTS AND DISCUSSION

Biosynthesis of Ag-NPs by *Fusarium oxysporum:* Synthesis of extremely steady, crystalline Ag-NPs in the answer was detected through rapid descent of the silver ions. Silver Nanoparticles (Ag-NPs) synthesis was noticed by observing the change in color of the fungiform remainder on reacting with silver nitrate solution arrival of dark brown color in fungiform cell filtrate designated the creation of Ag-NPs figure (1-2). The color variations observed can be credited to the surface Plasmon resonance of put Ag-NPs (Hany *et al*., 2014). Several useful characteristics of Silver nanoparticles have made it the vastest applicable nanoparticles in science and the most important characteristic is related to its antimicrobial activity.

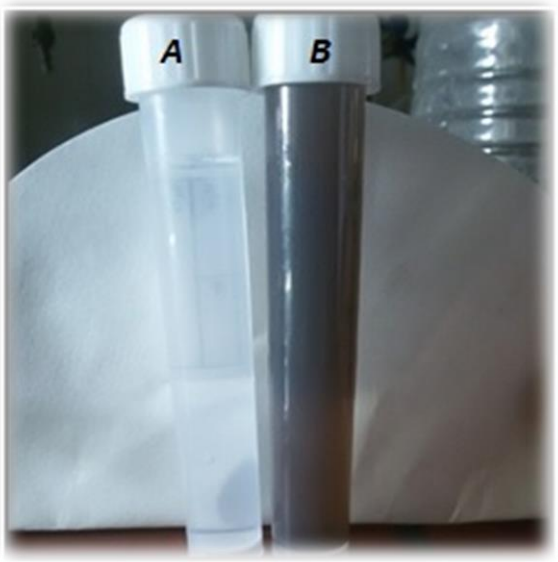

Figure 1-2: Biosynthesis of Ag-NPs color change reaction: tube containing the filtrate of *Fusarium oxysporm* (A), and tube containing the filtrate of the *F. oxysporum* after exposure to $AgNO_3$ solution (B).

Description of Ag-NPs

1. **UV–Visible Spectral:** Mixture of Ag-NPs exhibitions sturdy fascination in the noticeable variety unpaid to the native superficial Plasmon timbre, UV-Visible spectral of the examples remained reported (Figure 1-3). Mixture of Ag-NPs presented absorbance peak about 418 nm, which is exact aimed at the Ag-NPs. Here remained a single peak representative mixture of spherical nanoparticles. It is well recognized that there is a very near connotation amid the UV-Vis absorbance spectrum and scope and figure of Ag-NPs. By the surge in the atom size, the optical absorption spectra of metal nanoparticles that are conquered through surface Plasmon fonts (SPR) shift to longer wavelengths (redshift) (Birla *et al*.,2013).

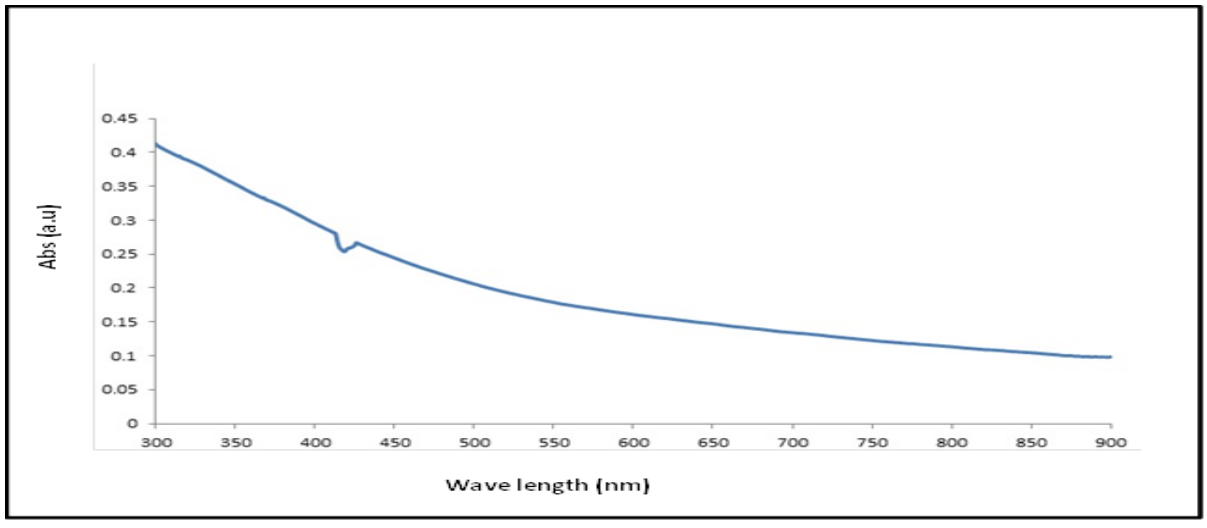

Figure 1-3: Absorptions spectrum of biosynthetic Ag-NPs using *F. oxysporum*

Husseiny *et al*., (2015) Ag-NPs synthesized shown maximum absorbance peak at 420 nm by UV-Visible spectroscopy, Nelson *et al*., (2005) show the strong surface Plasmon resonance centered at 415–420 nm clearly increases in intensity with time.

X-Ray Diffraction: The X-RD pattern of Ag-NPs, Figure (1-4) showed the presence of three peaks. Strong diffraction peaks were 38.19º (111), 48° (200) and 67° (220).

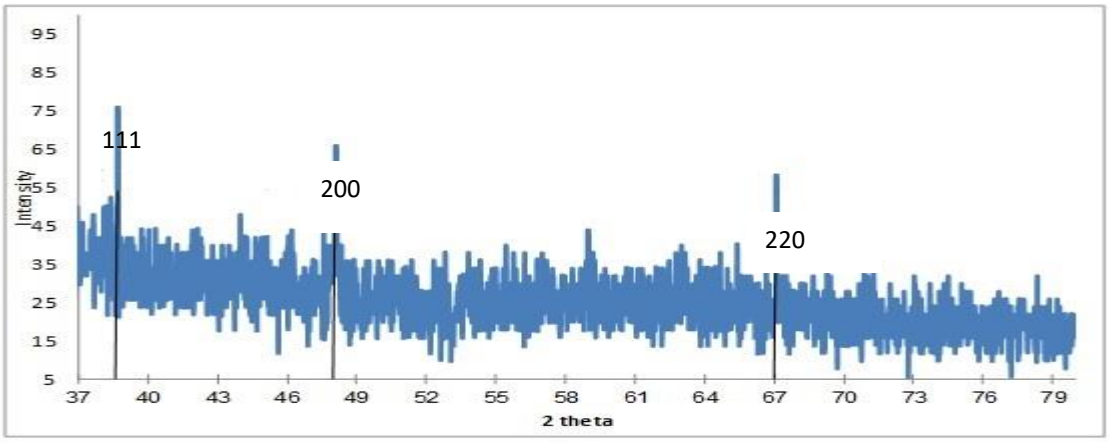

Figure 1-4: X-Ray pattern of SNPs

Husseiny *et al*., (2015) The XRD range presented four distinct deflection peaks on 38.15°, 44.18°, 64.63° and 77.50° consistent lattice plane worth remained indexed at (111), (200), (220) and (311) planes of the face centered cubic (FCC) silver through a lattice parameter of a = 4.08 Å which remained in decent preparation with the position of the FCC structure after a joint group of powder diffraction standard (JCPDS) Card No-087-0720. Cristiane *et al.*, (2017) shows XRD examination, it was likely to notice a well-defined face-centered cubic (FCC) construction of Ag for all materials, at 38 º, 44 º, 64.5 º, 77 º, and 82 º reliable to aircraft (1 1 1), (2 0 0), (2 2 0), (3 1 1), (2 2 2) respectively.

3. Atomic Force Microscopy: The calculated Ag-NPs sizes were measured, using the software of the AFM Figure 1-5A. Size ranges were (55 - 90), (95 - 130) and (135-195) nm with average diameter 73.73 nm, Roughness average (Ra) and Root mean square (Sq) of was 14.7 nm ,18.2 nm, respectively. Figure 1-5 B shows AFM topographic images of biosynthesis Ag-NP.

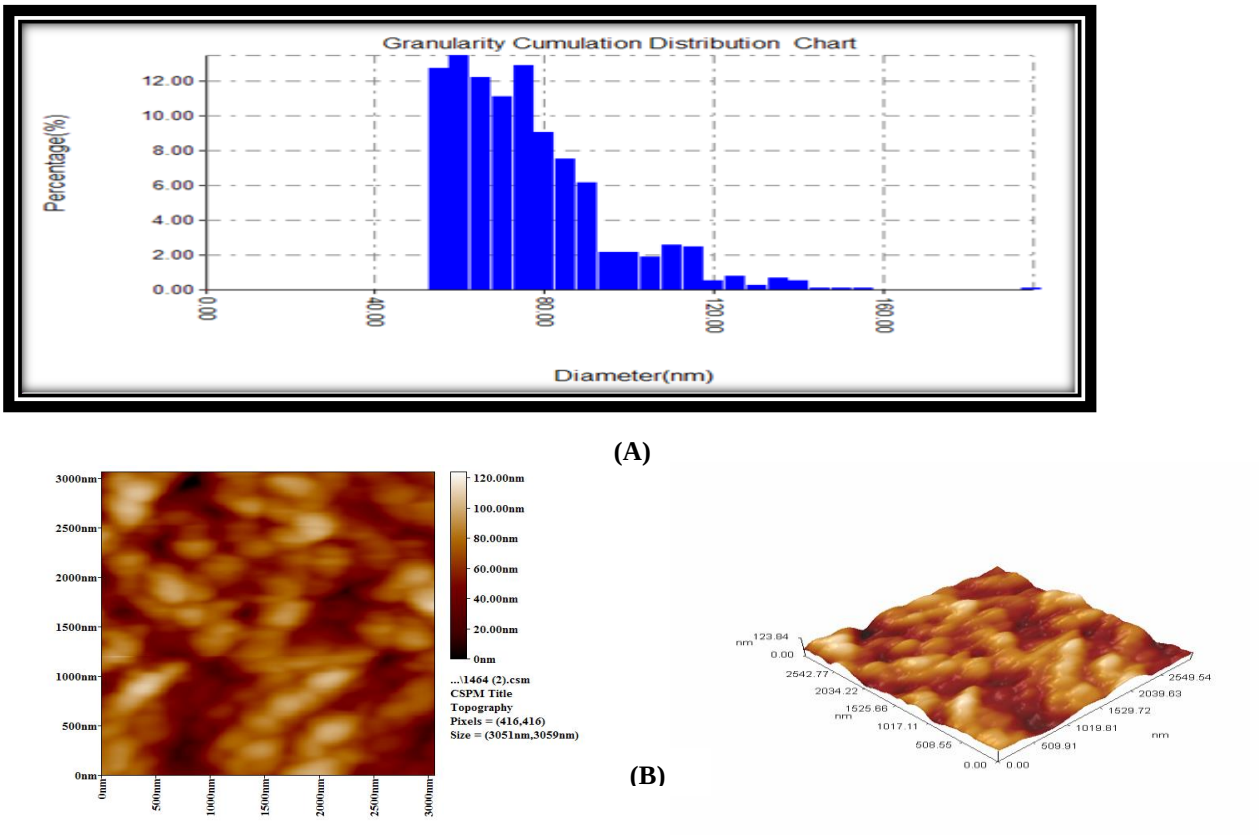

Figure (4-3): (A) Granularity volume d**Figure 1-5:** (A) Granularity volume distribution chart of Ag-NPs synthesis by *F.oxysporum*, (B) the Ag-NPs scope, then complexity remain designated through the color gauge on the lateral of AFM micrograph.

Shafiq *et al*., (2016) described Ag-NPs were biosynthesized by *Fusarium graminaerum* in diverse sizes remained slow through consuming AFM the width, initial from 1 to 95.5 nm then the regular of the NPs width remained 45.5 nm. Ishida *et al*., (2014) display Morphological education through AFM bare two separate mechanical preparations of Ag-NPs: one of 300 ± 57 nm and additional of 77 ± 30 nm.

4. Fourier Transform Infrare Spectroscopy (FTIR): FTIR measurements of the samples of dried powder were carried out to identify the probable interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping material) of Ag-NPs. The FTIR spectrum of biosynthesized Ag-NPs showed six distinct peaks ,3711.87,1699.18, 1108.81,834.71,446.70 and 419.33 cm^{-1} as shown in the Figure (1-6). The peak at3 711.87 cm^{-1} refers to NH stretch vibration of amide of protein, 1699.18 cm^{-1} refers to -C=O is stretching, 1108.81 cm^{-1} refers to C-O stretching,834.71 cm^{-1} assigned to the C-N stretching vibration, 446.70 cm^{-1} and 419.33 cm^{-1} related to Ag-NP binding with oxygen.

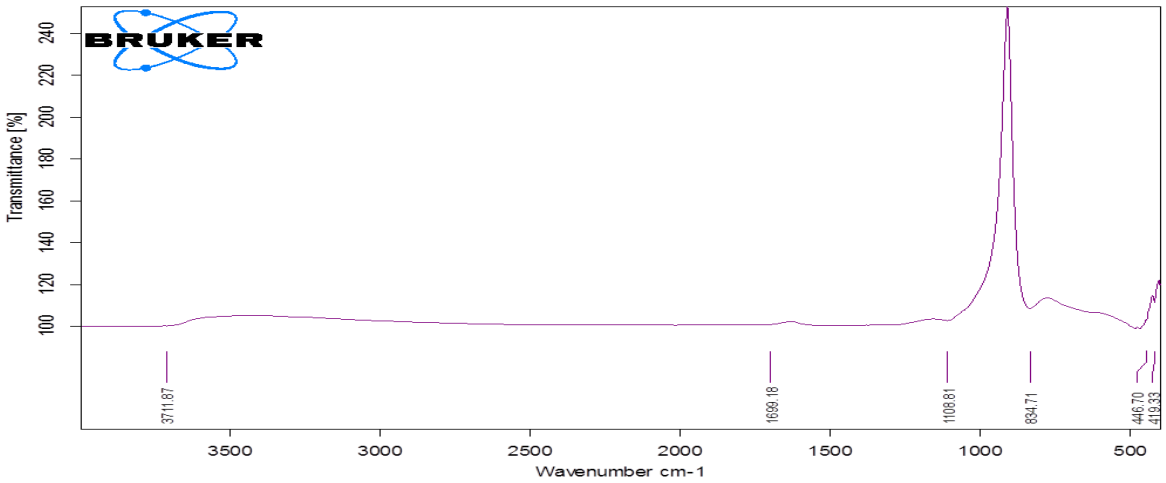

Figure 1-6: FTIR spectra of Ag-NPs synthesis by *F. oxysporum*

The overall observation confirms the presence of protein in samples of silver nanoparticles from *F. oxysporum*. It has also been reported earlier that protein can bind to silver nanoparticles through their free amine groups or cysteine residues (Gole *et al*., 2001, Jeevan *et al*., 2012), or through free amide groups (Bansal *et al*., 2004, Saravanan *et al*., 2013). So that the protein could most possibly form a coat covering around Ag-NPs and it stabilizes the aqueous synthetic medium. This evidence suggests that the biomolecules could possibly perform the function for the formation of stable Ag-NPs in aqueous medium.

Birla *et al*., (2013) in research also confirmed, deprived of a fungal cell filtrate mixture of Ag-NPs fixed not occur in sunlight. In adding, the wild mixture of Ag-NPs remained experiential in the presence of sunlight then at high temperature. Though, the careful device of Ag-NPs in light needs not remained established.

Inhibitory effect of Ag-NPs against *Candida* spp

1. **Inhibitory effect of Ag-NPs against *Candida* spp by agar plate well diffusion assay:** The making Ag-NPs by aqueous excerpts of the fungus *F. oxysporum* is a dormant applicant for little-price, then naturally approachable production of steady and consistently sized Ag-NPs with anticandidal and anticryptococcal actions (Ishida *et al*., 2014) Table 1-1 shows the effect of Ag-NPs synthesis by *F.oxysporum* on different species of *Candida*, the results showed that all *Candida* spp isolate were susceptible to Ag-NPs and the inhibition rate rises with the increase of concentration. In 100µg/ml concentration, the high inhibition effect of Ag-NPs which obtainable in *C.sphaerica* shadowed through *C.tropicalis* with inhibition zone 18mm and 15mm respectively, then *C. glabrata*, *C. lambica* and *C.famata* with inhibition zone 14mm for each. While the low effect of Ag-NPs was on *C. krusei* and *C.albicans* by inhibition zone 12mm. The results, statistical analysis showed significant ($P \leq 0.001$) between *Candida* spp. isolates. While in 50µg/ml concentration, the high inhibition effect of Ag-NPs which obtainable in *C. sphaerica* with inhibition zone 14mm, shadowed through *C.tropicalis*, *C.glabrata* then *C. famata* with inhibition zone 13mm for each, then *C.lambica* with inhibition zone 12mm, While the low effect of Ag-NPs wre on *C.krusei* and *C. albicans* by inhibition zone 11mm. the results of statistical analysis showed significant ($P \leq 0.01$) between *Candida* spp. isolates. But in 25µg/ml concentration, the high inhibition effect of Ag-

NPs which obtainable in *C. tropicalis* and *C. sphaerica* with inhibition zone 12mm for each, while the low effect of Ag-NPs was on *C. famata*, *C. glabrata*, *C. albicans*, *C. lambica* and *C. krusei* by inhibition zone 11mm. the results of statistical analysis showed significant (P≤0.01) between *Candida* spp. isolates.While in 10µg/ml concentration, the inhibition effect of Ag-NPs which presented in *C. sphaerica*, *C. tropicalis*, *C.famata*, *C. glabrata*, *C. lambica*, *C. krusei* and *C. albicans* with inhibition zone 10mm for each.The results of statistical analysis showed non-significant.

Table 1-1: Effect of Ag-NPs synthesis by *F. oxysporum* against *Candida* spp.

***Candida* spp**	**Concentration of naonparticals µg/ml**				**P- value**
	Inhibition zone(mm)				
	100	**50**	**25**	**10**	
C.albicans	12.6±0.5	11±0	10±0	10±0	0.001
C.krusei	12.3±0.3	11±0	10±0	10±0	0.001
C.glabrata	14.7±0.4	13.3±0.3	10±0	10±0	0.004
C.lambica	14.3±0.3	12.3±0.3	10±0	10±0	0.004
C.famata	14.3±0.3	13.3±0.3	10±0	10±0	0.004
C.sphaerica	18.3±0.3	14.7±0.4	12.3±0.3	10±0	0.006
C.tropicalis	15±0	13.3±0.3	12.3±0.3	10±0	0.006
P-value	0.001	0.01	0.01	NS	

Comparable consequences remained before stated through Kaviya *et al*., (2011) and Musarrat *et al*., (2010), who tried, the result of Ag-NPs happening fungi and bacteria, Ishida *et al*., (2014) obtainable that dispersal technique caused in the creation of zones of development, inhibition reaching after 8-15 mm when tested the effect of fungal Ag-NPs on pathogenic yeast.

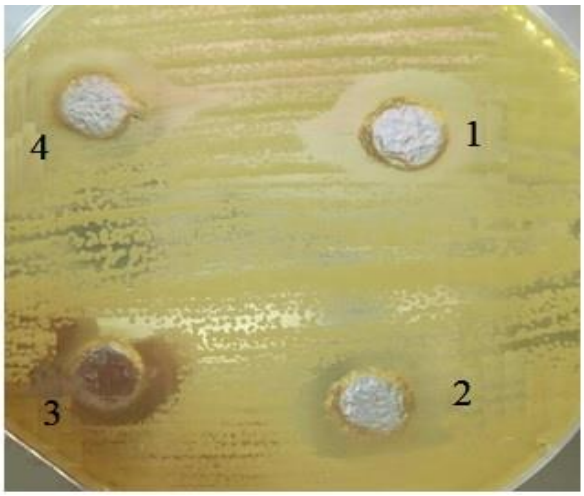

Figure 1-7: Antifungal action of Ag-NPs beside *Candida glabrata* on sabouraud dextrose agar.

2-Inhibitory effect of Ag-NPs against *Candida* spp by MTT assay: Seven types of *Candida* spp. chosen in this experiment for study effect of Silver Nanoparticles against *Candida* spp by MTT assay (Table 1-2). In 100µg/ml concentration, the high inhibition effect of Ag-NPs which presented in *C.krusei* followed by *C.albicans*, then *C.famata* and followed by the equivalent effect in *C. glabrata*, *C.lambica*, While the low effect of Ag-NPs was on *C. tropicalis*, and *C.sphaerica*. While in 50µg/ml concentration, the cell number is low which obtainable in *C. tropicalis*, *C.krusei*, *C. albicans*, and *C.lambica*. Then the equivalent effect in *C.famata*, *C.glabrata*. While the high cell number in *C.sphaerica*. But in 25µg/ml concentration, the high inhibition effect of Ag-NPs which obtainable in *C.lambica* and *C.albicans* then equal result Ag-NPs in *C.krusei*, *C.tropicalis*, *C.famata* and *C.glabrata*. While the low effect in *C. sphaerica*. Although in 10µg/ml concentration, the cell number is low which obtainable in *C. albicans* and *C.lambica* .Then the equal effect on *C. krusei*, and *C.glabrata* .Although the high cell number in *C.famata*, *C. tropicalis* and *C. sphaerica*. In Clotrimazole the cell number is low which presented in *C.glabrata*, *C.krusei*, *C. lambica* and *C.famata*, followed by *C. tropicalis*, whereas the high cell number in *C.sphaerica* and *C.albicans*. Results statistical analysis showed significant difference (P ≤ 0.05) between *Candida* spp isolates and control in all concentration also between *Candida* spp. isolates in all nanoparticles concentration that used in this study.

Table 1-2: Inhibitory effect of Ag-NPs against *Candida* spp by MTT assay

***Candida* spp**	**Mean cell number ×10^6 ± SD**	**P-value**
	Concentration of naonparticals µg/ml	

	100	50	25	10	Clotrimazole	Control	
C.albicans	A 3.11±0.8 b	A4.3±1.4 a	B5.1±0.6 a	B 5.5±0.8 a	C6.6±5.1 d	D19.8±14.8 e	0.06
C.krusei	A0.51±0.04 a	B 4.1±0.8 a	C7.4±1.2 c	D 9.6±1.4 c	B3.4±0.1 a	D11.3±4.9 b	0.001
C.glabrata	A 4.1±0.1 c	B 6.3±0.3 c	C8.1±0.2 c	C 9.5±1.8 c	A4.6±0.6 b	C9.7±2.7 a	0.001
C.lambica	A 4.1±0.6 c	A 4.4±0.4 a	B6.7±0.7 b	B 6.9±0.7 b	A4±0.5 a	C15.1±2.5 d	0.001
C.famata	A 3.9±0.2 c	B 6.2±0.5 c	C7.8±0.9 c	C10.1±1.3 d	A4.8±0.3 b	C11.2±0.7 b	0.001
C.sphaerica	A 6.1±0.6 e	B 7.3±1.7 d	C9.1±**0**.9 d	D11.1±0.4 e	A6.3±0.5 d	D11±3.3 b	0.004
C.tropicalis	A 5.3±0.7 d	A 5.7±0.7 b	B8.2±0.7 c	C10.4±1.5 d	A5.9±1.2 c	D12.9±2.7 c	0.001
P-value	0.03	0.02	0.02	0.01±	0.04	0.01	

Different capital letters into raw mean significant change ($P \leq 0.05$) amid nanoparticles concentration, then control, While Different small letters into Colum mean significant change ($P \leq 0.05$) between *Candida* spp.

The results in table 1-3 show the Percentage of inhibitory effect of Ag-NPs beside *Candida* spp. by MTT assay. In 100µg/ml concentration, the high effect of Ag-NPs which presented in *C.krusei* ,*C.albicans* and *C.lambica* with percentage 95.83% ,84.70% , and 74.01% respectively, followed by *C.famata,C. tropicalis* and *C. glabrata* with percentage 65.26%,59.25%,and 58.53% respectively. While the low effect of Ag-NPs in *C.sphaerica* with percentage 46.31%. While in 50µg/ml concentration, the high effect of Ag-NPs which presented in *C.albicans* and *C.lambica* with percentage 75.29% and 70.07%, respectively, then in *C.krusei* and *C. tropicalis* with percentage 64.58 % and 55.55% respectively. While the low effect of Ag-NPs in *C. sphaerica C.famata* and *C.glabrata* with percentage 44.21%,35.78%, and 35.36% respectively. But in 25µg/ml concentration, the high effect of Ag-NPs which presented in *C.krusei*, *C.albicans* with percentage 74.70%, followed by *C.lambica* with percentage 55.90%.While the low effect of Ag-NPs in *C.krusei*, *C.tropicalis*, *C.sphaerica C.glabrata* and *C.famata*, with percentage 36.11 %, 34.37%, 31.57%, 21.05% and 17.07% respectively. While in 10µg/ml concentration, the media effect of Ag-NPs which presented in *C.albicans* and *C.lambica* with percentage 63.52% and 54.33% respectively.While the low effect of Ag-NPs in *C.tropicalis*, *C.krusei*, *C.famata*, *C. glabrata* and *C.sphaerica* with percentage 19.44 %, 15.62%, 7.36%, 3.65% and 1.05%. Compared with Moreover, in Clotrimazole the high effect of Ag-NPs which presented in *C.albicans* and *C.lambica* with percentage 82.35%, 73.22% then in *C.krusei*, *C.glabrata* and *C.tropicalis* with percentage 69.79%, 52.43% and 54.62% respectively. While the low effect of Ag-NPs in *C. famata, C.sphaerica* with percentage 44.21%, 43.75%. Compared with Clotrimazole the results of inhibitory effect of Ag-NPs with concentration of 100µg/ml shows higher effect more than Clotrimazole against all *Candida* spp. The results of statistical analysis showed significant difference ($P \leq 0.05$) between *Candida* spp isolates and control in all concentration also between *Candida* spp isolates in all nanoparticles concentration that used in this study.

Table 1-3: Percentage of inhibitory effect of silver nanoparticles against *Candida* spp by MTT assay

***Candida* spp**	**Percentage of inhibitory (%)***				
	Concentration of naonparticals(µg/ml)				
	100	50	25	10	Clotrimazole
C.albicans	84.70%	75.29%	74.70%	63.52%	82.35%
C.krusei	95.83%	64.58%	34.37%	15.62%	69.79%
C.glabrata	58.53%	35.36%	17.07%	3.65%	52.43%
C.lambica	74.01%	70.07%	55.90%	54.33%	73.22%
C.famata	65.26%	44.21%	31.57%	7.36%	44.21%
C.sphaerica	46.31%	35.78%	21.05%	1.05%	43.75%
C.tropicalis	59.25%	55.55%	36.11%	19.44%	54.62%

***Percentage of inhibitory**(%) =**Control OD- sample OD/ Control OD (Lalitha *et al*., 2013).**

This results partly agrees with the results were done Ishida *et al*. (2014), That presented Ag-NPs in height antifungal action beside *Candida.* Another study done by Lara *et al*., (2015) show that Ag-NPs remain sturdy inhibitors of *C. albicans* biofilm development. the manner action of Ag-NPs, its competence to dismiss the membrane likely of *C. albicans*, Ag-NPs showed strong antifungal possessions on fungi tried, perhaps done obliteration of membrane honesty; so, it was deci-

ded that Ag-NPs has substantial antifungal action, praiseworthy additional inspection for scientific needs (Nasrollahi *et al*., 2011). Hwang *et al*., (2012) obtainable Ag-NPs possess antifungal belongings done apoptosis. The act persuaded in *C. albicans* yeasts a buildup of ROS, discount in the mitochondrial membrane potential, phosphatidylserine externalization, DNA and nuclear fragmentation and the start of met caspases. Ag-NPs must fungicidal and fungi static belongings on the *Candida* species Instrument of performance Disturbance of cell membrane. Chwalibog *et al*., (2010) likewise recognized that the Ag-NPs container personality-collect and interconnect with *C. albicans* and *S. aureus* cells, foremost to the disintegration of cell wall and cytoplasmic membranes and cytoplasm escape.

Conclusions:

In this study Ag-NPs were prepared by *F. oxysporum* and the Ag-NPs have spherical from with average diameter 73.73nm. Extracellular synthesis of Ag-NPs have potent antifungal activity against *Candida* spp. the concentrated supernatant of Ag-NPs was talented toward decrease feasibility of *Candida* spp comparing with control**.**

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