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Biosynthesis, Characterization, Antibacterial and Synergistic Effect of Silver Nanoparticles using *Fusarium oxysporum*

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Biosynthesis of silver nanoparticles was achieved using cell filtrate from submerged fermentation of *Fusarium oxysporum*. It was found that $AgNO_3$ reduced to Ag nanoparticles when exposed to the cell filtrate and the colour of solution was dark brown with absorbance peak at 430 nm wavelength. TEM micrograph showed spherical AgNPs with range 10-25 nm in dimension and was well dispersed. AgNPs show high stability in solution due to biological stabilizing and capping agents released from fungus, and have negative charge -25mv.Biosynthesized AgNPs have high potential antibacterial and antifungal activity, highest inhibitory zone was (27) mm against *Candida albicans*. The synergistic effect of AgNPs gave highest fold increase (10) against *E. coli*, followed by (5) fold against Staphylococcus auras using Azithromycin and levofloxacin as standard antibiotics respectively.

Keywords: Biosynthesis, silver nanoparticles, antibacterial, synergic.

Microbial synthesis of nanomaterial is grown fast due to their chemical, optical, electrochemical and electronic properties[1]. Fabrication of metal nanoparticles using fungi is reliable, ecofriendly and low cost. The green synthesis of nanoparticles can be achieved via the selection of an ecofriendly solvent with environmentally accepted reducing and stabilizing agents[2]. Biosynthesis of AgNPs has more economic advantages than physico-chemical methods which need complex and hi-tech instrumentation facilities, harsh chemicals also nanoparticles for biomedical application should be characterized by lower toxicity and higher their safe usage and this is available in biosynthesis with biocompatible chemicals[3] .Several researches have successfully synthesize AgNPs using fungi as reducing agents fungus ''*Fusarium semitectum*'' used for the extracellular synthesis of silver nanoparticles from silver nitrate solution[4]. Silver nanoparticles (AgNPs) have received great attention due to their interesting and significant antimicrobial properties. Unlike commercial antibiotics, AgNPs reveal their effects via more inhibitor mechanism not in a single specific way. Combinations of mechanisms such as damage and change of cellular morphology, disturbing vital cellular enzymes and proteins, depressing the activity of respiratory chain enzymes and finally leading to cell apoptosis[5,6]

The fabricated AgNPs were characterized by UV, TEM, FTIR and Zeta sizer&potential.

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Finally, the fabricated AgNPs were applied in the field of antibacterial and synergistic studies in comparison with standard antibiotics.

MATERIAL AND METHODS

Chemicals and cultures

In the present study the chemicals used are Silver nitrate, nutrient broth , potato dextrose agar purchased from Himedia (P) Ltd., Mumbai, as starting materials without further purification. Sterile milliQ water was used throughout the experiment. Microoganism used in the experiment are *Fusarium oxysporum* was kindly provided by The Regional Center For Mycology and Biotechnology, Azhar University, Cairo Egypt. All chemicals and media used were of analytical grade.

Table 1. Zone of Inhibition (mm) of AgNPs synthesized by *F. oxysporum* against selected bacterial pathogens

Bacterial pathogen	Zone of inhibition (mm) Mean ± SD
B. subtilis	11.48 ± 0.51
Staph. aureus	14.04±0.48
E. coli	18.46 ± 0.42
Candida albicans	27.02±0.80

Synthesis of nanoparticles

For the synthesis of silver nanoparticles, the biomass of fungus F. oxprosum was prepared by growing the fungus aerobically in a liquid medium MYPG contain 0.3 gram (g.) malt extract, 0.3 g. yeast extract, 0.5 g. peptone and 1 g. glucose in 100 ml deionized water The flasks were inoculated and then incubated on orbital shaker at 25 ± 2 °C and agitated at 120 rpm for 96 h. cell filtrate was obtained by passing it through Whattman filter paper No.1. 150 ml $AgNo_3$ 1mM was added to 20 ml free cell filtrate of F. oxprosum and incubated for 48 hours at 30°C with agitation 120 rpm. Separately the cell filtrate and $AgNO_3$ solution were kept under the same conditions figure (fig.) 1.

Characterization of silver nanoparticles

The formation of AgNPs was observed by change in color from pale yellow to brown confirmed by using Uv- visible Spectrophotometer (Shimadzu) operated with 1 nm resolution. TEM images were obtained by JEOL-JEM 2100 (Japan) with an acceleration voltage of 200 KV ,analysis were prepared by coating aqueous AgNPs drops on carbon coated copper grid. Size and potential of AgNPs were measured using Zetasizer Nano-ZS90 (Malvern, UK) by applied diluted sample of

Table 2. Synergistic effect of antibiotics in combination with or without AgNPs against selected human bacterial pathogens

Pathogens	Antibiotics (µg/disc)	Zone of inhibition (mm) disk only	Zone of inhibition (mm)+ Ag	Increased zone size (mm)
Bacillus subtilis	Levofloxacin (5 µg)	28	30	2
	Azithromycin(15 µg)	27	28	1
	Ciprofloxacin(5 µg)	26	29	3
	Amoxicillin(25 µg)	18	20	2
Staph. aurus	Levofloxacin(5 µg)	25	30	5
	Azithromycin(15 µg)	26	28	2
	Ciprofloxacin(5 µg)	25	29	4
	Amoxicillin(25 µg)	24	27	3
E.coli	Levofloxacin(5 µg)	35	37	2
	Azithromycin(15 µg)	25	35	10
	Ciprofloxacin(5 µg)	38	39	1
	Amoxicillin(25 µg)	17	18	1
Candida albican	Levofloxacin(5 µg)	0	19	19
	Azithromycin(15 µg)	0	18	18
	Ciprofloxacin(5 µg)	0	20	20
	Amoxicillin(25 µg)	0	13	13

AgNPs. Further characterization involved Fourier Transform Infrared Spectroscopy (FTIR) (Perkin–Elmer) analysis of drop of silver nanoparticles by scanning the spectrum in the range 450–4,000 cm^{-1} at a resolution of 4 cm^{-1}.

Antibacterial activity of AgNPs against pathogenic bacteria

Antibacterial assay

The antibacterial activity of synthesized AgNPs was evaluated using agar well diffusion method[7]. Pure cultures of selected human pathogenic bacteria were subcultured individually in nutrient broth for 12hrs at 37°C. A 20ml volume of sterile Mueller Hinton Agar medium was poured into each petriplate and each bacterial strain was swabbed uniformly into plates using sterile cotton swabs. Wells of 5 mm diameter were made onto each bacterium inoculated agar plate using sterile gel puncture. 100µl of AgNPs suspension was introduced into the corresponding wells. The bactericidal activity was determined by a clear inhibition zone around the sample loaded well after incubation of plates over night at 37°C.

Synergistic effect of AgNPs

The synergistic effect of AgNPs was carried out by disc diffusion method. To determine the synergistic effect, four standard antibiotic discs such as Amoxicillin, Ciprofloxacin, Azithromycin and levofloxacin were impregnated individually with 100µl each of freshly prepared AgNPs and were placed onto the Mueller Hilton Agar medium inoculated with individual test organisms. Standard antibiotic discs alone were used as positive controls. These plates were incubated overnight at 37°C. After incubation, the result was recorded by measuring the inhibitory zone diameter (mm).

RESULT AND DISCUSSION

The color change of $AgNO_3$ solution from pale yellow to dark brownish yellow indicated the formation of AgNPs. The color change is due to the excitation of surface plasmon vibration in the NPs[8]. The active molecules (proteins and Enzymes) present in the *Fusarium oxysporum* filtrate reduced the silver metal ions into AgNPs. The formation of AgNPs was confirmed by intense absorption peak at wavelength 435 nm, which are typical absorption bands of spherical AgNPs due to their

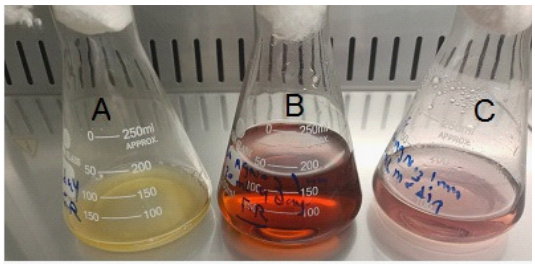

Fig. 1. A- cell filtrate B- Cell filtrate with $AgNO_3$ C- $AgNO_3$ only

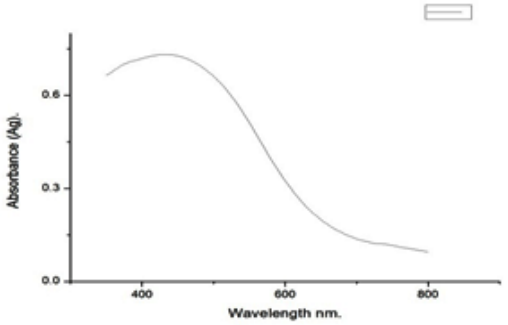

Fig. 2. Absorbance of Biosynthesized AgNPs

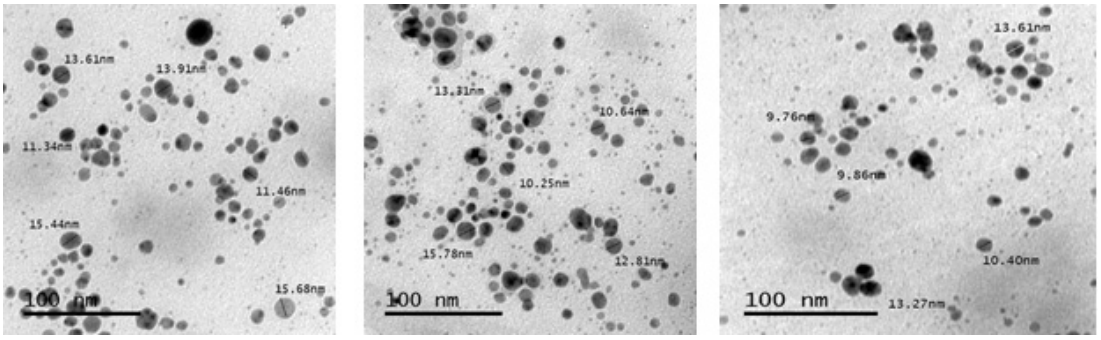

Fig. 3. TEM micrograph showed different size of AgNPs with spherical shape

surface plasmon resonance (Fig. 2). Results were compatible with *Fusarium oxysporum* cell filtrate produce AgNPs solution yielded a maximum absorbance at 436 nm[9]. Similar absorption peaks were observed in AgNPs formation using *Fusarium* species cell filtrate with a maximum absorption band at 420 nm[4,8]. TEM technique was employed to visualize the size and shape of the silver nanoparticles formed. Fig. 3 shows single spherical silver nanoparticles in shape with range 10-25 nm in size and almost polydispersed. AgNPs showed moderate stability with negatively charged -25mv

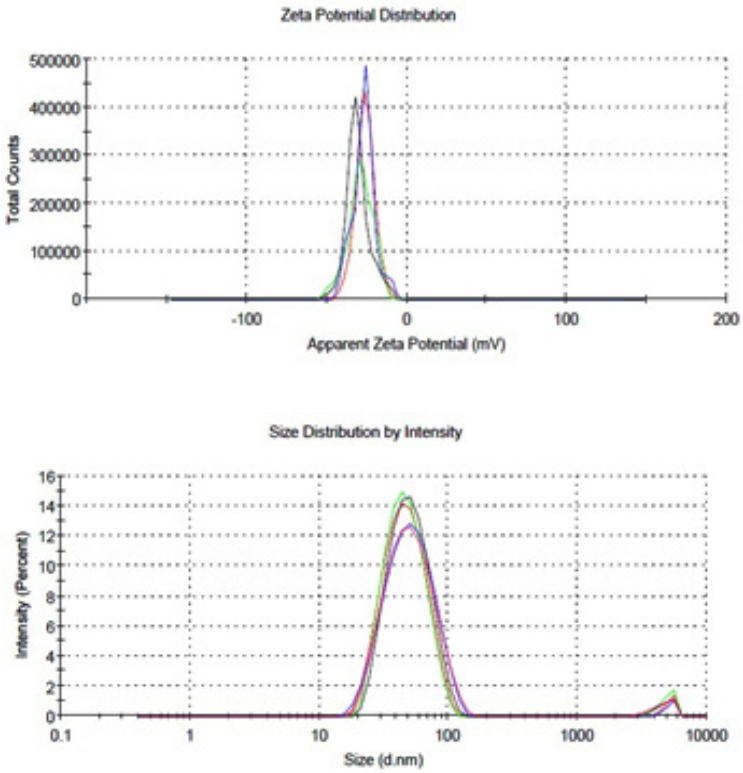

Fig. 4. Zeta sizer and zeta potential

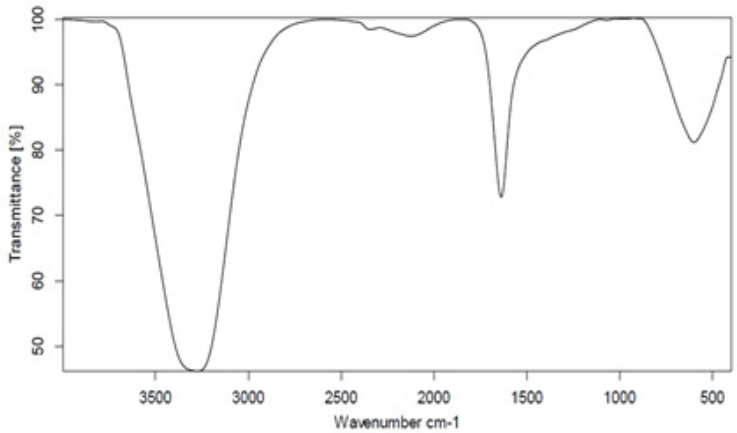

Fig. 5. FTIR of biosynthesized silver nanoparticles

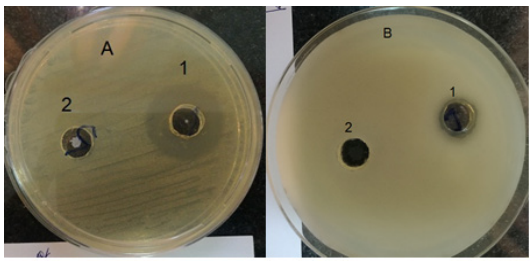

Fig. 6. A) *Candida albicans* (1 AgNPs, 2 cell filtrate); B) *B. subtilis* (1 AgNPs , 2 Cell filtrate).

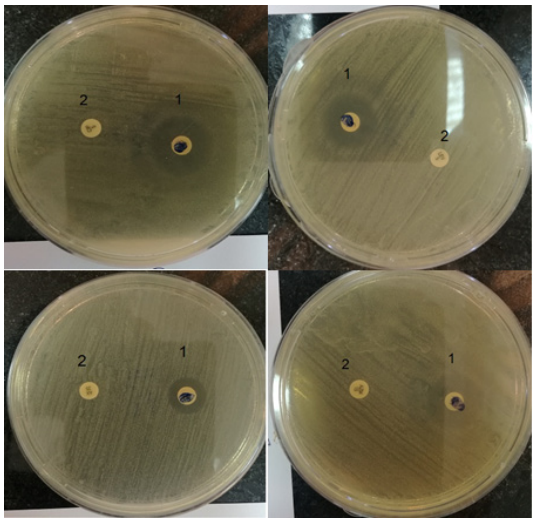

Fig. 8. Shows 1- Antibiotic with AgNPs 2- Antibiotic only

fig. 4 due to proteins moiety attached to AgNPs as bio coating agents, also fig. 4 shows good size distribution intensity of particles using zeta seizer measurements. Previous reports confirmed that the prepared nanoparticles with zeta potential value greater than +25 mV or less than - 25 mV typically have high degree of stability[11]. zeta potential value -12.02 mV for green AgNPs and -10.4 mV for chemical AgNPs[3].

Further FTIR show band at 1637 which can be attributed to carbonyl stretch of amides and thereby could be related to proteins that may be encapsulate[11] fig. 5.

Antibacterial activity

The antibacterial activity of AgNPS was investigated against the human bacterial pathogens such as *B.subtilis*, *S. aureus*, *E.coli* and *candida albican* and the result on the inhibitory zone (mm) is represented in Table 1. AgNPS gave the highest

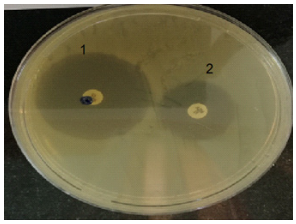

Fig. 7. Azithromycin with AgNPs 2. Azithromycin only *against E.coli*

zone of inhibition (27.02 mm) against Candida albican , whereas the lowest zone of inhibition (11.48mm) was recorded against Bacillus subtilis fig 6. Similarly, an effective antimicrobial activity against higher antibacterial activity against *S. typhi* than than *B. subtilis* a using AgNPs[12] Antibacterial activity against E. coli and S. aureus showed good results showing maximum zone of inhibition of 17mm and 16 mm, respectively[13].

The result on synergistic effect of biosynthesized AgNPs is given in Table 2. It revealed that the distinct difference was observed between the inhibitory zones by antibiotics with and without AgNPs, similarly Gold nanoparticles showed synergistic effect against different pathogenic bacteria[2].The enhanced zone of inhibition was observed and it was increased from 25 to 35 mm when the AgNPs were incorporated with Azithromycin antibiotics against *E.coli*. figure 7. In contrast, inhibition zone was zero with all tested antibiotics against *candida albican* then show increase of inhibition zone with combination with AgNPs but still lower than AgNPs only Fig. 8. Ciprofloxacin was subsequently shown to be greater against Staph and bacillus than against *E.coli*.

CONCLUSION

Biosynthesis of AgNPs has many advantages such as economic viability and easy scale up. Applications of nanoparticles in medical and other fields make this method potentially use for the large-scale synthesis of other inorganic nanomaterials. Narrow size distribution and small nanosize AgNPs also offer advantages for self-assembled monolayer formation and enhanced surface area. Silver colloidal solution is biologically well suited and has the potential

Antibacterial activity also can be used with other pharmaceutical compound to enhance their activity.

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