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Mycosynthesis of Silver Nanoparticles by The Endophytic Fungus *Alternaria tenuissima* AUMC 13621 and Evaluation of their Antimicrobial, Antioxidant Effect

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GREEN synthetic strategies have been receiving a great interest for metal nanoparticles synthesis. In the current study, extracellular synthesis of silver nanoparticles (AgNPs) using the cell filtrate of the endophytic fungus, *Alternaria tenuissima* AUMC 13621 isolated from healthy leaves of *Ruta graveolens* plant, was achieved. The biosynthesized AgNPs were characterized by UV–visible spectroscopy, transmission electron microscopy (TEM), dynamic light scattering analysis (DLS), and fourier transform infra-red (FT-IR), The UV–visible spectrum shows a maximum absorption peak at 416nm. TEM photography showed the spherical shape of AgNPs with an average size 9.8nm. FT-IR indicates bounding of silver nanoparticles with the supernatant molecules of *A. tenuissima* and provides evidence for the presence of proteins and biomolecules responsible for reduction of Ag^{+} to Ag^{0}, capping and stabilizing the synthesized AgNPs. Parametric optimization for the biosynthesis process showed maximum absorbance of 400–408nm at pH-12, 70°C using 1mM $AgNO_3$ concentration. By studying the effect of gamma irradiation on AgNPs biosynthesis, the results showed that, the obtained peak became more sharper and narrower moreover, the absorbance intensity increased by increasing irradiation dose from 0.5 to 3.0kGy. The synthesized Ag NPs have a potent antimicrobial activity to some tested pathogenic bacterial strains and *Candida albicans* ATCC1023. Additionally, they had excellent free radical scavenging activity. The AgNPs antioxidant activity at 100µg/ml was of 65.99% inhibition that was close to the result obtained for ascorbic acid (67.39%).

Keywords: Silver Nanoparticles, Antimicrobial, Endophytes, Antioxidant.

Introduction

Nanotechnology is an attractive research field that interested in synthesis and characterization of particles with very small size ranging from 1-100 nm. Due to the ultra- fine size of nanomaterials, they have a unique and different physical, chemical, optical and biological properties compared to their macro scale counterparts (Hu et al., 2009). Silver ions and silver related compounds have been used since very ancient ages as antimicrobials towards various infections showing a strong toxicity to a wide range of 116 micro-organisms (Liau et al., 1997) and they have been used for antioxidant, anti-diabetic and anti-hemolytic effects. Also they find various applications in the catalysis field, diagnostic tools, opto-electronics, and photo electrochemical assessments, because of their unique size-related optical, magnetic, and electrical characteristics (Arindam et al., 2015). The most important industrial medical application of AgNPs is topical ointments in order to prevent burns and open wounds infections. Also, they have been applied in a spread range of products of healthcare such as, dressings of burns, water sterilization systems and medical tools (Kim & Kim, 2006; Thomas et al., 2007). Silver nanoparticles have been tested in various biological fields' such as drug delivery and binding of HIV gp 120 protein (Morones et al., 2005).

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Several chemical and physical methods have been used for synthesizing and stabilizing silver nanoparticles such as heating, U.V., microwave and gamma radiation, ultrasonic waves, laser beam, and electrochemical reduction (Klaus et al., 2001; Senapati, 2005). However, these techniques need a high cost and the yield of the synthesized nanoparticles is very low besides a poor morphology (Sau & Rogach, 2010) furthermore; they pollute the environment due to the use of toxic chemicals and elevated temperatures for preparation process (Rai et al., 2008; Birla et al., 2009).

Gamma radiation synthesis is one of the most promising methods to produce AgNPs in a highly pure and stable form (Krkljes et al., 2007). While, green synthesis using plant extracts or microorganisms is a clean process as no toxic chemicals are employed in the production process, also the synthesis process occurs at moderate conditions of pressure and temperature with less cost (Mukherjee et al., 2008). Endophytes refers to fungal or bacterial members live inside plant tissues for short periods or during the whole plant life cycle without affecting the host plant negatively. Green synthesis of AgNPs by endophytes were considered an alternative process (Strobel, 2003). More effort must be applied for biogenic nanoparticles synthesis with a controlled morphology; therefore, this study aims to synthesize AgNPs by the endophytic fungus *Alternaria tenuissima* AUMC13621, optimize different parameters (pH, temperature, $AgNO_3$ concentration) that affecting the biosynthesis process .The effect of gamma radiation on AgNPs biosynthesis had been studied also. Moreover, the antimicrobial activity and the antioxidant activity of AgNPs were also investigated.

Materials and Methods

Chemicals

All chemicals and reagents used in the present investigation were of analytical grade and used without further purification. Chemotherapeutic agents (antibiotic discs) used in this study such as: Amoxicillin discs were obtained from Egyptian International Pharmaceutical Industries Company.

Microorganisms

Bacillus subtilis BW2, *Bacillus cererus* GST4, were obtained from the Microbiology Department, Faculty of Medicine, Zagazig University, Egypt. *Pseudomonas aeruginosa* ATCC 15442 strain, *Escherichia coli* ATCC 11229 and *Slmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC and *Candida albicans* ATCC 10231, were kindly obtained from the National Research Center, Giza, Egypt.

Irradiation Source

The process of irradiation was carried out at Nuclear Research Center, Atomic Energy Authority, Egypt using Co^{60} Gamma rays at a dose rate of 0.68409kGy/h at the time of the experiment.

Isolation of endophytic fungi

Small healthy cuttings of the stem with some leaves of *Ruta graveolens* plant were collected. Entophytic fungi were isolated from plant parts according to the method described by Petrini (1991) with some modifications as follows; each plant sample was surface sterilized by 70% ethanol for 30 seconds, immersed in sodium hypochloride solution for 1 minute, and then rinsed in sterile distilled water for 2 minutes. After proper drying, 4 fragments ($0.25cm^2$) of each plant part were inoculated on plates of Potato Dextrose Agar (PDA), supplemented with antibiotic tetracycline (100µg/ml) then incubated at 28±2°C for 5 to 7 days. Pure fungal colonies were preserved on potato dextrose agar slants at 4°C.

Extracellular synthesis of silver nanoparticles

The isolated endophytic fungi were inoculated in 250ml Erlenmeyer flasks containing 100ml of malt glucose yeast peptone (MGYP) broth media (Karbasian et al., 2008) then, incubated at 28°C on a rotatory shaker at 120rpm for 7 days.

Biosynthesis of silver nanoparticles

The cultures were centrifuged at 6.000rpm for 15min then the obtained supernatants were used for silver nanoparticles synthesis. Also, the mycelial biomass was separated and then extensively washed with sterilized distilled water (SDW). This biomass was taken into flasks containing 100mL SDW, and incubated at the same conditions for 48hr. The cell free filtrate was tested for AgNPs synthesis by adding aqueous solution of 1mM (V/V) silver nitrate and incubated over night at room temperature in dark for reduction of Ag^+ ions to Ag^0 (Bhattacharya & Mukherjee, 2008).

Identification of the endophytic fungus that able to produce silver nanoparticles

The endophytic fungus that was able to produce

AgNPs was identified based on morphological and reproductive characters using standard identification manual (Simmons, 2007), using specific media, PDA, malt extract agar(MEA) and Yeast extract sucrose agar (YESA) (Frisvad & Samson, 2004). After incubation period of 7 days, macroscopic characters (colony diameter; color aspect and mycellial texture) and microscopic characters (somatic and reproductive microstructures) were observed. The completely identified fungal strain, *Alternaria tenuissima* was deposited in Assuit University Mycological Center (AUMC) with accession number of AUMC 13621.

Characterization of silver nanoparticles

UV-visible (UV-Vis) spectrophotometer

Preliminary characterization of AgNPs was carried out using UV–Vis spectroscopy (JASCOJapan- model V- 560). The change in color of the cell free filtrate when mixed with silver nitrate solution was measured at wavelength ranged from 300 to 800nm (Vivek et al., 2012). The fungal supernatant was used as a blank.

Dynamic light scattering (DLS)

Average particle size and size distribution were determined by PSS-NICOMP 380-ZLS particle sizing system. Before measurements, the samples were diluted 10 times with deionized water; 250µl of suspension was transferred to a disposable low volume cuvette. After equilibration to a temperature 25ºC for 2min., five measurements were performed using 12 runs of 10 s each (Jans et al., 2009).

Fourier transform infrared spectrometer (FT-IR)

FT-IR measurements were carried out in order to get information about the effective chemical groups which may be involved in biosynthesis of AgNPs or being responsible for their stabilization. The measurements were carried out using JASCO FT-IR- 3600 infra-red spectrometer by employing potassium bromide (KBr) Pellet technique (Baudot et al., 2010). For sample preparation, 300µl of concentrated colloidal silver nanoparticle solution was mixed with 10mg KBr in clean crucible, until it becomes a fine powder. The sample was prepared, and oven dried to remove the traces of moisture.

Transmission electron microscopy (TEM)

For determining the size and shape of the biosynthesized AgNPs, TEM (Joel 1010, at 80kv) was used. The sonicated sample (5µl drop) was applied on copper grids (400 meshes) coated by conventional carbon, and dried at room temperature for half an hour, the sample was inspected by operating at 80kV (Domsch et al., 1993). Sample images were taken to illustrate the particles composition in a clear representation.

Parametric optimization of AgNPs synthesis by Alternaria tenuissima AUMC 13621

Effect of pH

A different range of pH values from 7.0 to 13.0 with the difference of 2.0, were tested to evaluate the effect of pH on AgNPs biosynthesis by *A. tenuissima*. pH values were adjusted using 0.1N NaOH and 0.1 N KOH solutions (Khan & Jameel, 2016).

Effect of $AgNO_3$ concentration

The production of nanoparticles is also depending on substrate concentration. Accordingly, different $AgNO_3$ concentrations from 0.5 to 3.0mM with a 0.5mM difference, were tested. The optimum $AgNO_3$ concentration for the preparation of nano-silver was estimated by UV-visible absorption. (Khan & Jameel, 2016).

Effect of temperature

In all reactions, temperature presents an important role. Optimization process for AgNPs synthesis with the temperature respect, was studied at a temperature range from 30ºC to 70ºC (Kasture et al., 2008).

Effect of gamma irradiation

For studying the effect of gamma irradiation on AgNPs production, spore suspension (10^6 spores/ml) of *A. tenuissima* was irradiated by Co^{60} gamma rays at different doses of 0.5, 1.0, 1.0, 1.50, 2.0, 2.5 and 3.0kGy at room temperature. The irradiated spore suspensions were kept in darkness overnight at 4ºC. After irradiation process, the irradiated spore suspensions of the fungal strain were inoculated separately in PD broth media. The obtained fungal filtrate was used to synthesize AgNPs and analyzed with UV-visible absorption spectroscopy.

Antimicrobial activity of biosynthesized AgNPs

Antibacterial activity of the biosynthesized AgNPs toward pathogenic microbes included three gram positive bacteria, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* BW2, and

Bacillus cererus GST4 besides three gram-negative bacteria, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 15442 and *Salmonella typhi* ATCC 14028. The antibacterial activity was applied by well diffusion method (Bauer et al., 1966). Firstly, bacterial inoculum with cell density of 1-2*10^7CFU/ml at 600nm according to McFarland calculation was used (Netala et al., 2016). After that, about 100µL of each tested strain was suitably inoculated onto sterile petridishes of Muller-Hinton Agar (MHA). 9mm diameter wells were done by a sterile cork poorer. Silver nanoparticles solution of 100µL at various concentrations (50, 100, 250, and 500µg/ml) had been inoculated separately into each well. The resulted zones of inhibition after incubation for 24hr at 37˚C, were measured. A positive control of amoxicillin, 25µg discs (antibacterial agent), was used while, a negative control of the cell free filtrate of *A. tenuissima* was used. The measurements were estimated in triplicates and the average values± SD were calculated. Also the antifungal activity of AgNPs towards *Candida albicans* ATCC 10231 was done on PDA plates.

Free radical scavenging activity (RSA)

In vitro estimation of free radical scavenging activity of biosynthesized silver-NPs, was applied through 2, 2\-diphenyle picrylhydrazyl (DPPH) radical scavenging methodology (Mittal et al., 2006). For preparation of the stock solution, 4mg of DPPH were dissolved in 100ml of methanol then, kept at 20ºC. Afterthat, 2ml of the prepared solution were transfered to 1ml of tested samples that prepared in methanol at various concentrations (25, 50, 75 and 100µg/ml). Ascorbic acid represented the standered sample. The reaction mixture was kept for 15min in darkness. Radical scavenging activity (RSA) was measured by the optical density at wavelength of 517nm:

RSA (%)= (Ac - As/Ac) x 100

where, Ac refers to the control absorbance, and As is the sample absorbance.

Results and Discussion

Endophytic fungi are a unique source of natural bioactive compounds applicable in medicine and food industry moreover; they were used in synthesis of nanoparticles and nanomaterials (Sunkar & Nachiyar, 2013). This study was started by local isolation of fungal endophytes from twigs, roots and leaves of *Ruta graveolens* plant, the fungi grown out from different plant tissues, were brought into pure culture on PDA.

For screening the potentiality of the isolated fungal endophytes for extracellular biosynthesis of AgNPs, aqueous solution of $AgNO_3$ (1mM) was mixed with their filtrates (V/V). The color of the fungal filtrate was changed from colorless to reddish brown color after mixing with $AgNO_3$ and this observation analysis was a preliminary identification of silver nanoparticles formation (Vivek et al., 2012).

The extracellular biosynthesis is better than the intracellular biosynthesis, nanoparticles that are formed inside the biomass need additional step to release them from the biomass by ultrasound treatment or through using suitable detergents, in extracellular biosynthesis, this step is not necessary**,** furthermore, the extracellular biosynthesis is a cheaper and simpler processing. So much focus has been given to the development of an extracellular process for biosynthesis of metal nanoparticles (Dahan et al., 2003).

Morphological characterization of AgNPs producing isolate

The macroscopic features of the *A. tenuissima* RUT 155 isolate (Fig. 1 a,b) grown on PDA after 7 days at 30ºC, the emerged fungal culture was of a loose, cottony–grayish texture with a black reverse. Figure 1b shows the microscopic criteria of *A. tenuissima* illustrating that conidiophore was pale brown, erect, and simple or branched with one conidial scar or many. Conidia appeared separately or in small chains (3–5 conidia), elliptical or obclavate taperin to a a fine swollen beak, smooth-walled with 2 longitudinal septa besides 4 to 7 transverse septa. This strain was successfully deposited in the culture collection of Mycology Center at Assuit University with accession number AUMC 13621.

This fungus was the most potent fungal strain for synthesis of silver nanoparticles as shown in the UV–Vis absorbance spectrum as in Fig. 2a. The mycellial filtrate of *A. tenuissima* AUMC 13621 contains several bioactive compounds that involved in reduction of Ag^+ to Ag^0 and responsible for synthesis and stabilization of silver nanoparticles.

Fig. 1. The macroscopic and microscopic characteristics of *Alternaria* isolate, (a) Morphology of colonial growth on PDA at 30°C; (b) Microscopic features of growth taken from PDA showing conidiophore and conidia.

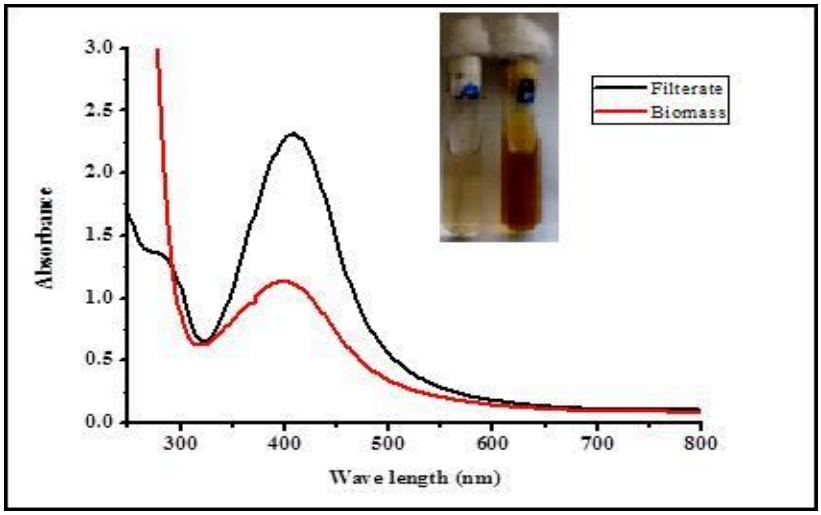

Fig. 2. Absorption spectra of silver nanoparticles produced after mixing fungal extract with 10-3 of $AgNO_3$; (a), (b) Comparison between nanoparticles synthesis by fungal filtrate and the biomass of *A. tenuissima* AUMC13621 [The upper photograph shows the color of extract without $AgNO_3$ (A) and with AgNO3 (B)].

Characterization of silver nanoparticles

Figure 2b shows the UV–Vis absorbance spectrum for AgNPs produced by mycellial filtrate and fungal biomass, after mixing with $AgNO_3$, the intensity of AgNPs that biosynthesized by mycelial filtrate was higher than that obtained by fungal biomass. The upper contrast photograph shows the color difference between the fungal filtrate before and after mixing with 1mM $AgNO_3$. It was found that the color transformed from pale yellow to reddish-brown illustrating the AgNPs formation in solutions. It was hypothesized that the fungus secrets the NADH-dependent nitrate reductase enzyme required for for silver ions reduction in its extracellular environment (Labrenz et al., 2000; Rohm et al., 2001). The presence of NADH-dependent nitrate reductase enzyme in extracellular cell filtrate of the fungus used for nanoparticles synthesis has been confirmed and the mechanism has been studied (Anilkumar et al., 2007; Ingle et al., 2008). Moreover, the proteins that present in fungal extract could most possibly

play a role in forming a covering coat around silver nanoparticles to prevent agglomeration of the particles and stabilizing in the medium (Basavaraja et al., 2008).

The dynamic light scattering (DLS) was used to determine the average size of particles in an aqueous solution and their distribution. Figure 3, indicated the production of very small size of (10.9nm). FT-IR spectrum of AgNPs which synthesized by *A. tenuissima* filtrate (Fig. 4) showed that the spectrum of fungal filtrate–AgNPs had a total higher transmission more than that of the spectrum of fungal filtrate. Table 1 shows the essential function groups which responsible for bio-reduction of Ag^{+} to Ag^{0} and AgNPs stabilization. The peak at 3800 and 3798 cm^{-1} corresponding to O-H stretching of phenols and alcohols (Vivek et al., 2012), the peak at 3440.39 and 3438.46cm^{-1} belonging to N-H stretching of the protein-secondary amide, the peak at 2924.52 and 2926.54 cm^{-1} belonging to C-H stretching of protein-methylene groups (Bozanic et al., 2010), the peak at 2854.13 cm^{-1} belonging to aldehyde C-H group, and the peak at 1641 cm^{-1} belonging to C=O stretching and NH bending in amides (Fasasi et al., 2015).

TEM analysis (Fig. 5, 6) indicated that the biosynthesized AgNPs using *A. tenuissima* AUMC 13621 filterate have the same spherical shape with average size 9.8nm and the particles were well dispersed without aggregation. All these particles have a crystalline nature. The Selected Area Electron Diffraction (SAED) pattern of AgNPs showed circular rings belonging to 111, 200, 220, 222 and 311 planes.

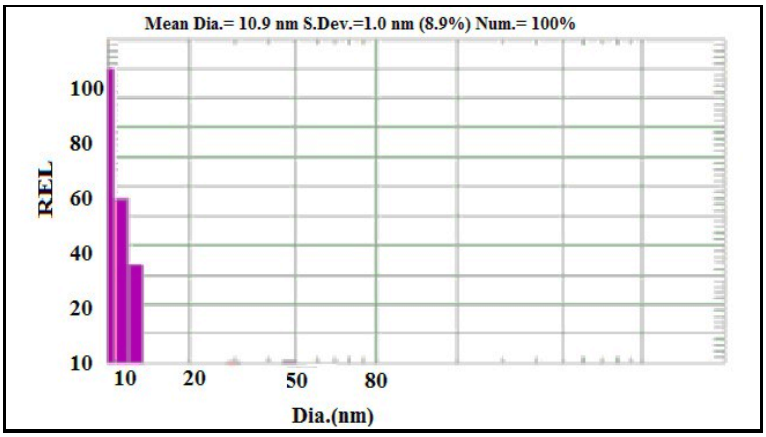

Fig.3. DLS analysis of AgNPs synthesized by *A. tenuissima* AUMC13621.

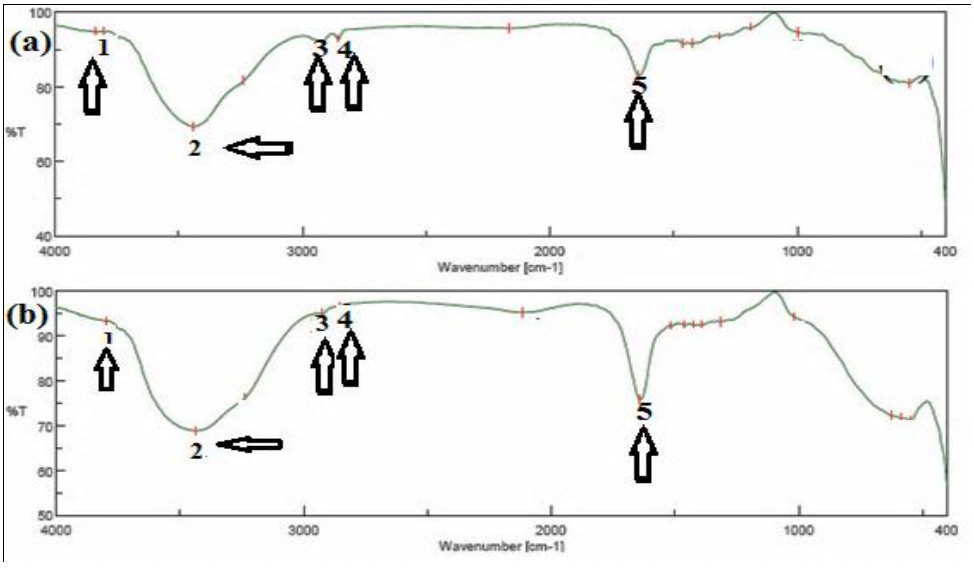

Fig. 4. FTIR of *Alternaria* extract without $AgNO_3$ (a) and FTIR of *Alternaria* extract mixed with $AgNO_3$ (b).

TABLE 1. The essential function groups *in Alternaria tenuissima* AUMC 13621 extract responsible for bioreduction of Ag^{+} to Ag^{0}

No.	Filtrate ʎ (cm^{-1})	Filtrate+ AgNPs ʎ (cm^{-1})	Function group
1	3800	3798	O-H of phenols
2	3440.39	3438.46	N-H stretching of amino group
3	2924.52	2926.54	C-H stretching of alkane amide
4	2854.13	2855.1	C-H of alkans
5	1641.13	1641.13	C = O stretching of amide

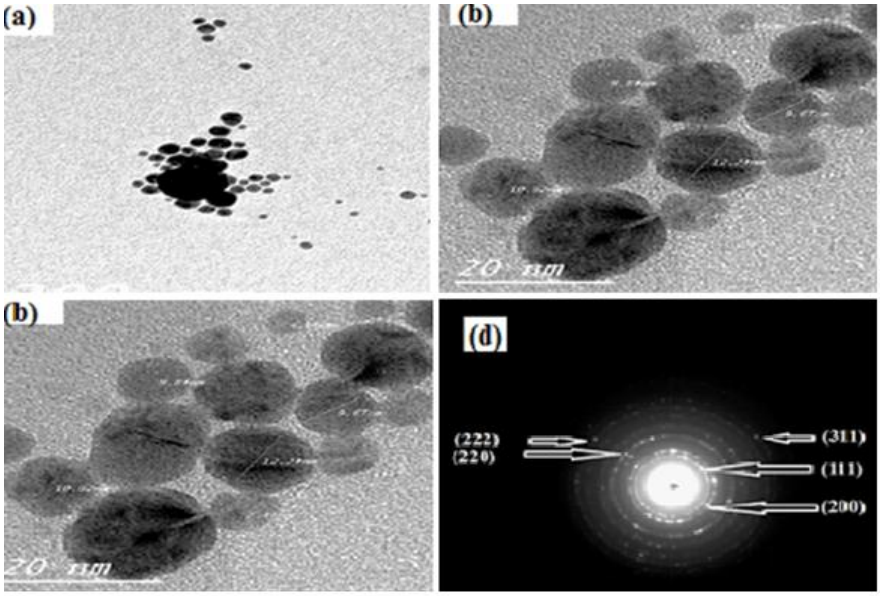

Fig. 5. TEM images of Ag NPs formed by *A. tenuissima* AUMC13621 at (a) 200nm, (b) 20nm, (c) Finger print of AgNPs, (d) Corresponding SAED pattern showed five diffraction rings.

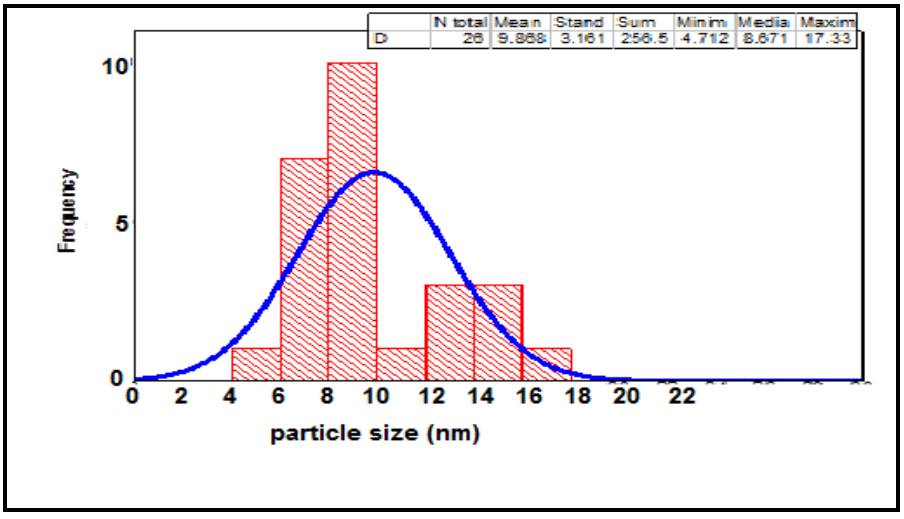

Fig. 6. Nanoparticle size distribution histograms showin Polydisperse silver nanoparticles synthesized by cell free extract of *A. tenuissima.*

Effect of pH variations on biosynthesis

It was clear that pH is an important factor that affects nanoparticles characters, like the size, shape, and stability of the synthesized AgNPs (Kumar et al., 2012). It was observed that, by increasing pH value, there was a change in color. The absorbance was increased due to Surface Plasmon Resonance (SPR) which revealed by UV-visible spectra (Fig. 7-a). A reddish-brown color was seen in the reaction solution and a remarkable sharp peak was obtained at high pH values (11.0 and 13.0) which indicates the presence of AgNPs, while a broad peak was observed at low pH value of 9.0. On the contrary, the decrease in pH to 7.0 did not show any peak.

pH could alter the biomolecules charge, which in a result influencing their capping properties and their stability. By increasing pH over than 11.0, there was a slide spectral shift in λ_{max}. This peak wavelength shifting determines that, the particles size is increasing with the solution pH increasing over 11. The energy required for excitation of surface plasmon electrons decreases by increasing the particle diameter resulting in shifting the maximum absorption towards the region of longer wavelength. Additionally, at very high pH (pH ~ 13.0), there was another peak at 620nm because the particles became unstable and agglomerated. Therefore, pH 11.0 was the most favorable for AgNPs production and this result is in agree with Vivek et al. (2012). In accordance to above reported results, Oza et al. (2012) used pH variations from 2.0 to 10.0 noting a remarkable sharp peak at alkaline pH 10.0, while a broad peaks were seen in the remaining pH values. In contrast to the above result, Ghorbani et al. (2011) reported a different result as the rate of silver ions reduction decreased by increasing pH.

Effect of $AgNO_3$ concentration on AgNPs biosynthesis

Biosynthesis of AgNPs using different $AgNO_3$ concentrations ranging from (0.5mM to 3mM) was studied. Figure 7-b shows that, AgNPs formation increased by increasing $AgNO_3$ concentration. The results also showed that, there was a slight shift in λ_{max} at concentration over than 1mM reflecting that, substrate concentration of 1mM was the optimum as confirmed by UV-visible spectra. When the $AgNO_3$ concentration raised to 2.5mM, an increase in the particle size was noticed because of the accumulation of large silver-NPs (Rao & Trivedi, 2005). Our notices are in agreement with Banu et al. (2011), who used 1.0mM $AgNO_3$ for AgNPs myco-synthesis by Rhizopus stolonifer.

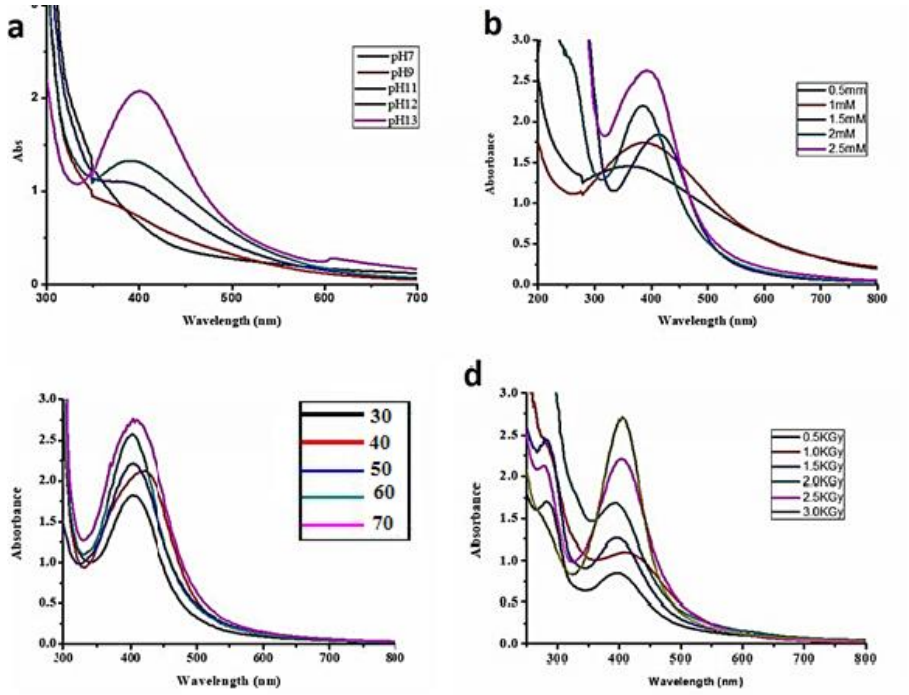

Fig. 7. (a) Effect of pH on AgNPs production, (b) Effect of $AgNO_3$ concentration on AgNPs production, (c) Effect of temperature and (d) Effect of gamma irradiation on AgNPs production by *Alternaria tenuissima* AUMC13621.

Effect of temperature on Ag NPs biosynthesis

Temperature is an effective factor affecting AgNPs production (Singh et al., 2013). The effect of varying temperatures on AgNPs production by *A. tenuissima* AUMC13621 was studied. Figure 7-c shows the biosynthesized AgNPs-absorption spectra at various temperature degrees ranging from 30°C to 70°C. The rate of silver salt reduction was enhanced by increasing the temperature of the reaction, as illustrated by quick change in the solution color, and increasing of absorbance intensity giving the maximum peak at 404nm. Because of the rapid reaction rate at elevated temperature, an increase in the kinetic energy of the molecules is resulted, and the Ag-ions receive energy faster resulting in less capability for particle size growth. Hence, the elevated temperature results in formation of smaller and uniform size distributed particles. The above data are in accordance with that mentioned by Kasture et al. (2008) used temperature variation from 30°C to 90°C, and noted that, as the temperature raises, Surface Plasmon peak starts to appear giving a well determined sharp peak at 90°C. Similarly, Oza et al. (2012) reported that, a high ionic strength coupled with a high temperature (100°C) might enhance the synthesis of silver nanoparticles. In contrast to the above results, Ghorbani et al. (2011) reported that, at temperature of 5°C, more number of nanoparticles was produced than that obtained at 90°C, because by increasing temperature, size of particles become larger and their dispersion become wider.

Effect of gamma irradiation on AgNPs production by A.tenuissima AUMC13621

Gamma radiation is a highly reactive ionizing radiation with a high reduction potential for metal NPs synthesis, thus it is a simple and efficient method for silver nanoparticles synthesis at room temperature and ambient pressure (Li et al., 2012). Because of its capability to fine-tune, it might present a better control in size and distribution of the synthesized nanoparticles (Abdelghany et al., 2017).

As shown in Fig. 7-d, the irradiation of AUMC13621 strain resulted in increasing AgNPs production. When the irradiation dose increases up to 2.5kGy, the intensity of AgNPs absorbance increases represented with a highly pointed peak. This might be occurred by two mechanisms, enhancement of reductase enzyme that reduce Ag^{+} to Ag^{0} or transient radicals creation from H_2O radiolysis of aqueous solution. These radicals have oxidizing and reducing species that cause acceleration of the reduction process, and stabilize the formed AgNPs by preventing their aggregation through "capping" with the hydroxyl anion resulted from water radiolysis. Moreover, the yield of zero-valent nuclei could be controlled by changing the irradiation dose (Eisa et al., 2011; Marignier et al., 1985).

Antimicrobial activity of biosynthesized AgNPs

The antibacterial activity of different AgNPs concentrations (500, 250, 100 and 50µg/ml) against some tested G+ve bacteria (*Staphylococcus aureus, Bacillus subtilis*, and *Bacillus cereus*) and some tested G-ve bacteria (*Salmonella typhi, Pseudomonas aeruginosa*, and *Escherichia coli*), is shown Table 2. It is shown from the table that by increasing AgNPs concentrations, their inhibitory effect increased. The inhibitory effect of AgNPs on bacteria may be due to attachment of AgNPs firstly to the cell membrane of the bacterial strain and then going through the bacterial cell. Consecuently, they reacting with sulphur and phosphorus containing compounds like proteins and DNA causing cell damage leading to bacterial death. This explanation runs parallel with that reported by Baker et al. (2005). Also, it has been assumed that, ionic silver reacts strongly with thiol (-SH) groups of vital enzymes resulting in enzyme inactivation. Hence, the progression of the cellular metabolisms, stop resulting in cell death (Netala et al., 2015 b).

On the other hand, all concentrations of AgNPs had antifungal activity against Candida albicans. The concentration of 50 µg/ml showed inhibition zone of 17.2mm. Netala et al. (2016) studied antifungal activity of AgNPs produced by endophyte A. versicolor against *Candida albicans*, they found that the concentration of 1mg/ml showed inhibition zone of 12.2mm. Kim et al. (2009) reported some information about the mode of action of nano-silver on fungal species. Additionally, transmission electron microscopy analysis confirmed that the interacting effect between the *C. albicans's* membrane structure and nano-silver during exposure to nano-silver causes important membrane changes in the *C. albicans* as "pits" formation on the membrane surfaces, pores formation and consequently, cell death.

Free radical scavenging activity (RSA)

The AgNPs antioxidant activity as the DPPH

inhibition percentage is presented in Table 3. By comparing with the standard ascorbic acid, the AgNPs were efficient free radical scavenger. The AgNPs antioxidant activity at 100µg/ml was of 65. 99% inhibition while, that obtained from ascorbic acid, was 67.39% inhibition. The DPPH assay principle is depend on the diphenyle picrylhydrazyl reduction to diphenyl picrylhy drazine in the presence of a hydrogen-donating antioxidant (AgNPs) as a result the DPPH color was altered from violet to yellow. Bunghez et al. (2012) reported that antioxidant properties of silver phyto-nanoparticles was higher than that obtained by plant extract alone as the antioxidant itself undergoes oxidation to neutralize the free radicals.

Conclusion

Endophytes were considered as an effective biological tools for the extracellular biosynthesis of stable AgNPs. *Alternaria tenuissima* AUMC 13621 fungal filtrate was used to synthesize mono-dispersed AgNPs that have spherical shape with average size of 10.9±1.0 nm *via* characterization by UV-VIS, DLS and FT-IR and TEM spectra. This biological method has enormous advantages over other chemical and physical methods they are eco- friendly, synthesize large amounts of more stable AgNPs with low cost. However our results showed that pH, metal ion concentration and temperature have a significant role in Ag NPs formation and size control. The biosynthesized Ag NPs exhibit a potent antimicrobial activity towards both tested Gram+ve and Gram-ve multi-resistant bacterial species. The bactericidal analysis has confirmed that, AgNPs at low concentrations could kill the bacterial cells, which do not reveal acute toxic effects on human cell. The AgNPs were efficient free radical scavengers by comparing with the standard ascorbic acid.

TABLE 2. The antibacterial activity rebresented by inhibition zone (mm) of the synthesized AgNPs against different strains.

Microbial pathogens	AgNPs conc. (µg/ml)				
	50	100	250	500	Amoxicillin25/ Nystatin 30 (µg)
	Inhibition zone (mm)				
B. cereus GST4	23.7±0.38^{d}	25.3±0.58^{c}	27.0±1.0^{b}	29.0±1.0^{a}	25±1.0 ^{a}
B. subtilis BW2	22.7±0.58^{d}	24.3±0.38^{c}	25.33±0.58^{b}	29.77±0.38^{a}	29±1.0 ^{c}
S. aureus ATCC 6538	13.5±0.5^{d}	20.7±0.58^{c}	24.0±1.0^{b}	27.3±0.58^{a}	19±0.5 ^{a}
S. typhi ATCC 14028	21.5±0.5^{d}	26.67±0.58^{c}	29.5±0.5^{b}	31.0±1.0^{a}	15.5±0.5 ^{c}
E. coli ATCC 11229	17.0±1.0^{c}	23.7±0.58^{b}	25.3±0.58^{b}	30.0±1.0^{a}	14.5±0.5^{d}
P. aeruginosa ATCC 15442	18.5±0.5^{d}	21.0±1.0^{c}	24.5±0.5^{b}	26.5±0.5^{a}	14.5±0.5 ^{c}
C. albicans ATCC 10231	17.0±1.0^{d}	17.7±0.38^{c}	19.0±1.0^{b}	19.7±0.38^{a}	14.3^{d}

Calculated mean is for triplicate measurements from two independent experiments ± SD, $^{a-e}$: Means with different superscripts in the same column are considered statistically different (LSD test, P ≤ 0.05).

TABLE 3. DPPH radical scavenging activities of AgNPs compared with standard ascorbic acid.

Tested sample	Concentration(µg/ml)			
	25	50	75	100
Ascorbic acid	47.75±0.92^{d}	58.02±0.18^{c}	63.2±0.74^{b}	67.39 ± 0.79^{a}
AgNPs	46.48± 0.28^{d}	55.39± 0.19^{e}	61.47± 0.23^{b}	65.99± 0.28^{a}

Calculated mean is for triplicate measurements from two independent experiments ± SD, $^{a-e}$: Means with different superscripts in the same column are considered statistically different (LSD test, P ≤ 0.05).

References

Abdelghany, A.M., Abdelrazek, E.M., Badr, S.I., Abdel-Aziz, M.S., Morsi, M.A. (2017) Effect of gamma-irradiation on biosynthesized gold nanoparticles using *Chenopodium murale* leaf extract. *Journal of Saudi Chemical Society,* **21**(5), 528-537.

Anilkumar, S., Abyaneh, M.K., Gosavi, S.W., Kulkarni, S.K., Pasricha, R., Ahmad, A. (2007)Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$. *Biotechnology Letter,* **29,** 439-45.

Arindam, D., Abhirup, D., Vijay, K., Aakriti, T., Anita, K.V. (2015) Evaluation of the antibacterial efficacy of polyvinylpyrrolidone (PVP) and tri-sodium citrate (TSC) silver nanoparticles. *International Nano Letters,* **5**(4), 223-230.

Baker,C., Pradhan, L., Pakstis, D.J., Shah, Pochan S.I. (2005) Synthesis and antibacterial properties of silver nanoparticles, *Journal of Nanosci.Nanotechnol,* **5**(2), 249-255.

Banu, A., Rathod, V., Ranganath, E. (2011) Silver nanoparticle production by *Rhizopus stolonifer* and its antibacterial activity against extended spectrum -lactamase producing (ESBL) strains of Enterobacteriaceae. *Materials Research Bulletin,* **46**(9), 1417-1423.

Basavaraja, S.; Balaji, S.D., Lagashetty, A., Rajasab, A.H., Venkataraman, A. (2008) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. *Material Research,* **43,** 1164-70.

Baudot, C., Tan, C.M., Kong, J.C. (2010) FTIR spectroscopy as a tool for nanomaterial characterization. I*nfrared Physics & Technology,* **53**(6), 434-438.

Bauer, A.W., Kirby, M.M., Sherris, J.C., Truck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clin. Pathol.* **45,** 493-496.

Bhattacharya, R., Mukherjee, P. (2008) Biological properties of "naked" metal nanoparticles. *Advanced Drug Delivery Reviews,* **60,** 1289-1306.

Birla, S., Tiwari, V.V., Gade, A.K., Ingle, A.P., Yadav, A.P., Rai, M.K. (2009) Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Letters in Applied Microbiology,* **48**(2), 173-179.

Bozanic, D.K., Trandafilovic, L.V., Luty, A.S., Djokovic,V. (2010) Green synthesis and optical properties of silver-chitosan complexes and nanochomposites. *Reactive and Functional Polymers,* **70**(11), 869-873.

Bunghez, I.R., Barbinta, M.E., Badea, N. (2012) Antioxidant silver nanoparticles green synthesized using ornamental plants. *Journal of Optoelectronics and Advanced Materials*, **14**(11), 1016-1022.

Dahan, M., Lévi, S., Luccardini, C., Rostaing, P., Riveau, B., Triller, A. (2003) Diffusion dynamics of glycine receptors revealed by single-quantum dot Trackin. *Science,* **302,** 442-445.

Domsch, K.H., Gams, W., Anderson, T.H. (1993) "*Compendim of Soil Fungi*". Academic Press, London. 860p.

Eisa, W.H., Abdel-Moneam, Y.K., Shaaban, Y., Abdel-Fattah, A.A., Zeid, A.M.A. (2011)Gamma-irradiation assisted seeded growth of Ag nanoparticles within PVA matrix. *Materials Chemistry and Physics,* **128,** 109-113.

Fasasi, A.Y., Mirjankar, N., Fasasi, A. (2015) Fourier transform infrared spectroscopic analysis of protein secondary structures found in Egusi. *American Journal of Applied Chemestry,* **1**(1), 1-4.

Frisvad, J.C., Samson, R.A. (2004) Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide for identification food and airborne terverticillate penicillia and their mycotoxins. In: Samson, R.A. and Frisvad, J.C. (Eds.) *Penicillium* subgenus *Penicillium*: New taxonomic schemes, mycotoxins and other extrolites. *Studies in Mycology,* **49,** 1-174.

Ghorbani, H.R., Attar, H., Safekordi, A.A., Sorkhabadi, S.M. R. (2011) Optimization of silver nanoparticles production by *E. coli* (DH5 [alpha]) and the study of reaction kinetics. *Asian Journal of Chemistry,* **23**(11), 5111-5118.

Hu, R., Yong, K.,-T. Roy, I., Ding, H., He, S., Prasad, P.N. (2009) Metallic nanostructures as localized plasmon resonance enhanced scattering probes for multiplex dark-field targeted imaging of cancer cells. *Journal of Physical Chemistry*, **113,** 2676-2684.

Ingle, A., Gade, A., Pierrat, S., Sonnichsen, C., Rai, M. (2008) Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. *Current Nanoscience,* **4,** 141-144.

Jans, H., Liu, X., Austin, L., Maes, G., Huo, Q. (2009) Dynamic light scattering as a powerful tool for gold nanoparticle bioconjugation and biomolecular binding studies. *Analytical Chemistry,* **81,** 9425-9432.

Karbasian, M., Atyabi, S.M., Siadat, S.D., Momen, S. B., Norouzian, D. (2008) Optimizing nano-silver formation by *Fusarium oxysporum* PTCC 5115 employing response surface methodology. *The American Journal of Agricultural and Biological Science,* **3**(1), 433-437.

Kasture, M., Patel, P., Prabhune, A.A., Ramana,C.V., Kulkarni, A.A., Prasad, B.L.V. (2008) Synthesis of silver nanoparticles by sophorolipids: Effects of temperature and sophorolipid structure in the size of particles. *Journal of Chemical Sciences,* **120**(6), 515-520.

Khan, N.T., Jameel, J. (2016) Optimization of reaction parameters for silver nanoparticles synthesis from Fusarium oxysporum and determination of silver nanoparticles concentration. *Journal of Electrochemistry Science,* **10,** 7905-7916.

Kim, S., Kim, H.J. (2006) Anti-bacterial performance of colloidal silver-treated laminate wood flooring. *International Biodeterioration and Biodegradation*, **57**(3), 155-62.

Kim, K.J., Sung, W.S., Suh, B.K., Moon, S.K., Choi, J.S., Kim, J.G., Lee, D.G. (2009) Antifungal activity and mode of action of silver nanoparticles on *Candida albicans*. *Biometals,* **22,** 235-242.

Klaus-Joerger, T., Joerger, R., Olsson, E., Granqvist, C.G. (2001) Bacteria as workers in the living factory: Metal-accumulating bacteria and their potential for materials science. *Trends in Biotechnology*, **19,** 1-15.

Krkljes˘s, A.N., Marinovic´-Cincovic´, M.T., Kacarevic-Popovic, Z.M., Nedelj-kovic, J.M. (2007) Radiolytic synthesis and characterization of Ag-PVA nanocomposites. *European Polymer Journal,* **43,** 217-2176.

Kumar, C.G., Mamidyala, S.K., Reddy, M.N., Reddy, B.V.S. (2012) Silver glyconanoparticles functionalized with sugars of sweet sorghum syrup as an antimicrobial agent Process. *Biochemical*. **47** 1488-1495.

Labrenz, M., Druschel, G.K., Thomsen, E.T., Gilbert, B., Welch, S.A., Kemner, K.M. (2000) Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate reducing bacteria. *Science,* **290**, 1744-7.

Li, J., Kang, B., Chang, S., Dai, Y. (2012) Gamma radiation synthesis of plasmatic nanoparticles for dark field cell imaging. *Radiation Physics and Chemistry,* **76**, 290-1194.

Liau, S.Y., Read, D.C., Pugh, W.J., Furr, J.R., Russell, A.D. (1997) Interaction of silver nitrate with readilyidentifiable groups: Relationship to the antibacterial action of silver ions. *Letters in Applied Microbiology,* 279-238.

Marignier, J.L., Belloni, J., Delcourt, M.O., Chevalier, J.P. (1985) Microaggregates of non-noble metals and bimetallic alloys prepared by radiation-induced reduction. *Nature,* **317,** 344-345.

Mittal, R., Dong, H., Bozkurttas, M., Lauder, G., Madden, P. (2006) Locomotion with flexible propulsors: II.Computational modeling of pectoral fin swimming in sunfish Bioinsp. *Biomim.* **1**, S35–S41.

Morones, J.R., Elechiguerra, L.J., Camacho, A., Holt, K., Kouri, B.J, Ramirez, T.J., Yocaman, J.M. (2005) The bactericidal effect of silver nanoparticles. *Nanotechnology,* **16,** 2346-2353.

Mukherjee, P., Roy, M., Mandal, B.P., Dey, G.K., Mukherjee, P.K., Ghatak, J., Tyagi, A.K., Kale, S.P. (2008) Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. *Nanotechnology,* **19,** 103-110.

Netala, V.R., Bobbu, P.L., Ghosh, S.B., Tartte, V. (2015) Endophytic fungal assisted synthesis of silver nanoparticles, characterization, and antimicrobial activity. *Asian Journal of Pharmaceutical and Clinical Research,* **8,** 113-116.

Netala, V.R. , Kotakadi, V.S., Gaddam, S.A., Tartte, V. (2016) Endophytic fungal isolate mediated biosynthesis of silver nanoparticles and their free

radical scavenging activity and anti-microbial studies. *Biotechnology Journal,* **6,** 1324-9.

Oza, G., Pandey, S., Shah, R., Sharon, M., (2012) Extracellular fabrication of silver nanoparticles using Pseudomonas aeruginosa and its antimicrobial assay.*Advances in Applied Science Research,* **3**(3), 1776-1783.

Petrini, O. (1991) Fungal endophytes of tree leaves. In: "*Microbial Ecology of Leaves*", Andrews, J.H., Hirano, S.S. (Eds.)*,* pp. 179-197. New York: Spring Verlag.

Rai, M., Yadav, A., Gade, A., (2008) Current trends in phytosynthesis of metal nanoparticles. *Critical Reviews in Biotechnology,* **28**(4), 277-284.

Rao, C.R.K., Trivedi, D.C. (2005) Synthesis and characterization of fatty acids passivated silver nanoparticles—their interaction with PPy. *Synthetic Metals*, **155**(2), 324-327.

Rohm, Y., Lauf, R.J., Mcmillan, A.D., Zhang, C., Rawn, C.J., Bai, J., Phelps, T.J. (2001) Microbial synthesis of metal- substituted magnetites. *Solid State Communications,* **118**(10), 529-34.

Sau, T.K., Rogach, A.L. (2010) Nonspherical noble metal nanoparticles: Colloid-chemical synthesis and morphology control. *Adv Mater.* **22**(16), 1781-1804.

Senapati, S. (2005) Biosynthesis and immobilization of nanoparticles and their applications. *Ph.D. Thesis,* University of Pune, India.

Simmons, E.G. (2007) *Alternaria*: An identification manual (CBS Biodiversing Series 6, Utrecht, the Netherlands (775 pages).

Singh, D., Rathod, V., Ninganagouda, S., Herimath, J., Kulkarni, P. (2013) Biosynthesis of silver nanoparticle by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (turmeric) and its antibacterial activity against pathogenic gram negative bacteria. *Journal of Pharmacy Research,* **7**(5), 448-453.

Strobel, G.A. (2003) Endophytes as sources of bioactive products. *Microbes and Infection,* **5**(6), 535-544.

Sunkar, S., Nachiyar, V. (2013) Endophytes as potential nanofactories. *Journal of Biological and Chemical Sciences,* **1**, 488-491.

Thomas, V., Yallapu, MM., Sreedhar, B., Bajpai, SK. (2007) A versatile strategy to fabricate hydrogel-silver nanocomposites and investigation of their antimicrobial activity. *Journal of Colloid Interface Sciences,* **315**, 389-95.

Vivek, R., Muthuchelian, K., Gunasekaran, P., Kaveri, K., Kannan, S. (2012) Green biosynthesis of silver nanoparticles from Annona sequamosa leaf extract and its *in vitro* cytotoxic effect on MCF-7cells. *Process Biochemistry,* **47,** 2405-2410.

تخليق جسيمات الفضه النانويه بإستخدام فطر *Alternaria tenuissima* AUMC 13621المستنبت داخليا وتقييم تأثيرها المضاد للميكروبات والمضاد للأكسده

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حاذت تقنيات التخليق الحيوى لجسيمات المعدن النانويه على أهمية بالغه لذا تم فى هذه الدراسة إنتاج جسيمات الفضه النانويه بإستخدام مستخلص فطر الألترناريا المعزول من أوراق نبات السذب وتم توصيف الجسيمات الناتجه بإستخدام تقنيات التحليل الطيفى(UV-Vis spectoscopy) حيث كانت أعلى كثافة ضوئية عند λ =416 نانومتر وعن طريق قياس جهاز ديناميكية تشتت الضوء (DLS) و فحص المجهر الإلكترونى (TEM) الذى أثبت تكون جسيمات فضه نانويه كروية الشكل بمتوسط حجم 9.8 نانومتر.كما أثبت تحليل FTIR وجود مركبات حيويه فعاله فى راشح الفطر والمسؤله عن تكوين جسيمات الفضه النانويه وبقاءها معلقة فى المحلول. وبدراسة تأثيرظروف التفاعل على إنتاج النانوفضه وجد أنها تحقق أعلى تركيز فى الأوساط القلويه(pH 12) لراشح الفطر بإستخدام المحلول المائى لنترات الفضه بتركيز 1 مللى مول عند درجة حرارة 70 °م . و بدراسة تأثير أشعة جاما على العزله الفطرية محل الدراسة فقد وُجد أن أقصى إنتاجية تتحقق بالتشعيع عند الجرعة 3.0 كيلو جراي وكانت جسيمات الفضه النانويه الناتجه ذات كفاءة عالية ضد أنواع مختلفة من البكتيريا الممرضه والكانديدا. كما ثبت أنها ذات كفاءة عالية كمضادات للأكسدة بنسبة 65.99% مقارنة بحمض الأسكوربيك(67.39%).