Introduction

NANOTECHNOLOGY, a highly promising discipline in science and technology, is the emerging and novel trend that will redesign the future of several existing know how which will change every aspect of our lives and leads to the generation of uniqueness in all the streams of technology. Biological synthesis of metal nanoparticles using microbes is gaining momentum due to the ecofriendly nature of the organisms which reduce toxic chemicals. Plant pathogenic fungi produce extracellular degradative enzymes that may be important in pathogenicity. Biosynthesis of silver nanoparticles was investigated using pathogenic Fusarium oxysporum f. sp. lycopersici and nonpathogenic strains of the same fungal species. These silver nanoparticles were characterized by means of UV–vis spectroscopy, transmission electron microscopy (TEM). UV–visible spectrum of the aqueous medium containing silver ion showed a peak at 420 nm corresponding to the Plasmon absorbance of silver nanoparticles. Transmission electron microscopy (TEM) micrograph showed formation of well-dispersed spherical silver nanoparticles with mean sizes 11.20 nm and 15.38 nm for pathogenic and nonpathogenic strains, respectively.

Keywords: Silver nanoparticles, Fusarium oxysporum f. sp. lycopersici, Pathogenic and nonpathogenic strains
because of their ability to secrete large amount of proteins, where, the biological synthesis of SNPs is enzymes catalyzed reaction (Kumar et al., 2007 and Birla et al., 2013).

Also, due to their tolerance and metal bioaccumulation ability, high binding capacity, and intracellular uptake, fungi have been of interest in biological production of the metallic nanoparticles. They secrete large amounts of enzymes which are used to reduce silver ions that induce the formation of the metal nanoparticles (Rauwel et al., 2015).

The fungus *Fusarium oxysporum* can reduce aqueous silver ions extracellularly to generate SNPs. This process likely occurs through the action of both reductase enzymes and electron shuttle quinones. In addition, biological nanoparticle synthesis often yields a more consistent size distribution pattern than other methods due to direct stabilization of the nanoparticles by proteins involved in the synthesis process (Pulit-Prociak & Banach, 2016).

*F. oxysporum f. sp. lycopersici* Snyd. et Hans. (FOL) is a pathogenic fungal species that causes soil-borne vascular wilt disease in the tomato (*Solanum lycopersicum* L.). Non-pathogenic strains of the same species are present in the rhizosphere soil and colonize plant roots, but seem not to invade the vascular system. *F. oxysporum*, can degrade lignin using ligninolytic extracellular peroxidases and laccase (Michielse et al., 2012).

The aim of this study was designed to investigate the differences among the pathogenic and non-pathogenic strains of *Fusarium oxysporum* in production of silver nanoparticles and their size.

**Materials and Methods**

The fungal strain of *Fusarium oxysporum f. sp. lycopersici* (AUMC 1425) and nonpathogenic strain of the same species (AUMC 1422) was kindly obtained from Assiut University Mycological Center (AUMC). The strain was maintained at 4°C on PDA slants.

**Extracellular synthesis of silver nanoparticles by *F. oxysporum***

*Fusarium oxysporum* was grown up in Erlenmeyer flasks containing 100 ml of malt yeast peptone glucose (MYPG) broth with the following composition (3g/l malt extract, 3g/l yeast extract, 5g/l peptone, 10g/l glucose). After 5 days of shaking incubation at 28°C, 150 rpm, the biomass was separated from the medium by filtration and washed three times in sterile deionized water. Ten g of biomass was suspended in 100 ml AgNO₃ aqueous solution of 1 mM final concentration. The mixture was left for 72 h in shaking incubator at 28°C in dark conditions. The control was only metal ions without fungal biomass (Ahmad et al., 2003).

**Characterization of silver nanoparticles**

**UV-visible spectroscopy analysis**

The reduction of silver ions was monitored by measuring the UV-VIS spectrum of the reaction medium after 72 h. The absorbance was recorded at 400-600 nm using UV-VIS spectrophotometer (Beckman DU-20 spectrophotometer USA) (Shahverdi et al., 2007).

**Characterization of SNPs by transmission electronmicroscopy (TEM)**

Transmission electron microscopy technique was used for studying the detailed structure of nanoparticles *i.e.* size and shape. For TEM measurements, a drop of synthesized AgNPs was placed on the carbon coated copper grids and kept to dry. After dryness of sample grid was loaded onto a specimen holder. TEM micrographs of the sample were taken using JEOL–JEM100 TEM, Japan. It is the confirmatory test of AgNPs. The crystalline nature of metallic SNPs was confirmed by selected area diffraction pattern (Ahmad et al., 2003).

**Results and Discussion**

NPs are usually clusters of atoms in the size range of 1–100 nm. The microorganisms take target ions from their environment and by the cell activities through enzymes generated turn the metal ions into the element metal. This process benefits from the development of clean, non-toxic and environmentally acceptable procedures (Li et al., 2012; Honary et al., 2013 and Abdulaziz et al., 2015).

It was found that fungi score more advantages over other biological systems because of their high tolerance towards the heavy metals. It is well known that in different culture media conditions and compositions microbial cell responds differently and secretes different metabolites.
and different kinds of proteins and extracellular enzymes (Thakkar et al., 2010). Also, we know that the biological synthesis of SNPs is enzymes catalyzed reaction (Birla et al., 2013).

When strains of *Fusarium oxysporum* fungal biomass were incubated with silver nitrate salt solution, the colour were exhibited a gradual change to brown color under dark conditions. The colour changed after 72 h of incubation whereas the control (without fungal mycelium) did not exhibit any change in colour. The change in colour is in agreement with the previous studies, which was considered as a visual indication for the production of colloidal suspension (hydrosol) of silver nanoparticles (Ahmad et al., 2003 and Soni & Prakash, 2011).

In biosynthesis of silver nanoparticles, the biomass or biological system used normally contains functional groups with reducing capacity, and it interacts with preformed nanoclusters or nuclei of silver metal present in the system to form silver nanoparticles (Zhang et al., 2016). Fungi should secrete specific enzymes or metabolites which are responsible for reduction of silver ions, high growth rate and low cost requirement for production procedure. (Shabani et al., 2013).

In the present study, MYPG medium might promote the extracellular nitrate reductase secretion and hence enhance the synthesis of SNPs. According to Birla et al. (2013), *Fusarium oxysporum* can reduce aqueous silver ions extracellularly to generate SNPs. This process likely occurs through the action of both reductase enzymes (especially nitrate reductase) and electron shuttle quinones (Kumar et al., 2007 and Ishida et al., 2013).

**Characterization of silver nanoparticles by UV-Visible spectroscopy**

When *F. oxysporum* biomass of different strains were exposed to Ag⁺ ions (AgNO₃, 1 mM), the appearance of dark brown is due to the excitation of surface plasm on resonance in the nanoparticles (Korbekandi et al., 2013). In the present study the formation and stability of the silver nanoparticles in the colloidal solution was monitored by using UV–Vis spectral analysis after completion of reaction for 72 h (Fig. 1).

The UV-vis spectroscopy showed maximum absorption at 420nm (Ravindra. & Rajasab, 2015) for pathogenic and non-pathogenic *Fusarium oxysporum* which is specific for the SNPs and are indicating the synthesis of silver nanoparticles and the maximum color intensity was obtained after three days. Beyond three days of incubation, no further increase in intensity was recorded indicating complete reduction of silver ions by the fungal biomass (Ingle et al., 2009). The reduction of metal ions occurs on the surface by the enzymes presented in the fungal cell wall. The absorptions spectra are due to plasmon excitations of particles and this confirms the extracellular synthesis of silver nanoparticles.

There was a single peak indicating synthesis of spherical nanoparticles. It is well known that there is a very close relationship between the UV-Vis absorbance spectrum and size and shape of SNPs, with the increase in the particle size, the optical absorption spectra of metal nanoparticles that are dominated by surface plasmon resonances (SPR) shift towards longer wavelengths (red shift). Small blue shift or red shift in the wavelength of the absorbance peak could be related to obtaining SNPs in different shape and size (Rai et al., 2009 and Birla et al., 2013).

**TEM analysis of silver nanoparticles**

The direct electron microscopic visualization allows measuring the size and shape of the silver nanoparticles formed. Typical bright-field TEM images of the synthesized silver nanoparticles by non-pathogenic and pathogenic fungus, respectively are shown in Fig. 2(a,b). The silver nanoparticles were predominantly spherical, in shape and single showing a distribution of sizes in the range of 9.19–22.30 nm for nonpathogenic and 7.05–20.23 for pathogenic *Fusarium* strains. The particle size of silver nanoparticles showed that the average size of particles (average diameter) synthesized by non-pathogenic and pathogenic *Fusarium oxysporum* f. sp. *lycopersici* were 15.38 ± 3.86 nm and 11.20 ± 3.84, respectively (Ingle et al., 2009).

![Fig.1. UV-Vis spectra after 72 h of incubation of fungal biomass in silver nitrate solution.](image-url)
The stability of SNPs is likely to be due to capping with proteins secreted by the fungus (Ahmad et al., 2003). The symmetric graph indicates the optimum conversion of silver ions to SNPs which may be due to the secretion of nitrate reductase, an enzyme responsible for the reduction of silver ions. Proteins have multiple effects on the dispersion, including potential screening of the surface charges that help to maintain the repulsion between the particles, or bridging type interactions (Michielse et al., 2012 and Birla et al., 2013).

Conclusion

There are no significant differences between the pathogenic and nonpathogenic Fusarium strains in the production of silver nanoparticles nor sizes of particles formed. Also, the synthesized nanoparticles promising sizes for industrial and medical application.

References


Li, G., He, D., Qian, Y., Guan, B., Gao, S., Cui, Y.,


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The construction of biological copper-based nanoparticles by pathogenic and non-pathogenic strains of Fusarium oxysporum f. sp. lycopersici for diseases of fungi.

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Nanotechnology will redefine the future and change our life. Using microorganisms in the formation of nano is a friendly way to the environment since microorganisms can condense dangerous substances.

This study dealt with the production of copper nanoparticles using pathogenic and non-pathogenic strains of Fusarium oxysporum through a device for measuring the spectrum that confirmed the existence and size of the produced particles using an electronic microscope and the average size of the pathogenic and non-pathogenic strains was studied. nm 15.38 and nm 11.2. These sizes are used to use the produced nanoparticles in antibacterial compounds.