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Original Paper

Antibacterial effect of mycological synthesized silver nanoparticles using *Aspergillus oryzae* and *Fusarium solani* filtrates

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Abstract

In this study, two novel marine fungal isolates (*Aspergillus oryzae* and *Fsarium solani*) were used for generating silver nanoparticles (SNPs). The antibacterial effect of mycological synthesized SNPs (12.31±0.26 and 22.6 ±1.2nm for *A. oryzae* and *F. solani*, respectively) was tested against Gram-positive (*Bacillus cereus* SBTBC, *Enterococcus faecalis* 8J, *Lesteria monocytogenes* 10403S and *Staphylococcus aureus* 7A) and Gram-negative bacteria (*E. coli* and *Salmonilla* sp.). Silver NPs exhibited higher bactericidal effect against Gram-negative strains compared to the Gram-positive ones. The maximal inhibitory concentration for *Bacillus cereus* SBTBC, *Enterococcus faecalis* 8J, *Escherichia coli* and *Salmonilla* sp. was 1.97, 1.64, 0.26 and 0.25 mg/mL, respectively, for synthesized SNPs by *A. oryzae* filtrate. Whilst it was, 9.31, 6.12, 2.61 and 0.921 mg/mL respectively, for synthesized SNPs by *F. solani* filtrate against the same bacterial strains. Our results show that mycological synthesized SNPs are promising compounds as antibacterial agents, however the exact mechanism of their bactericidal effect needs to be elucidated, and the safety of using them commercially still need to be more confirmed.

Keywords: Antibacterial; *Aspergillus oryzae*; *Fusarium solani*; silver nanoparticles.

Introduction:

Nanotechnology is the application of science and technology to control matter at the atomic or the molecular level (Vahabi *et al.*, 2011). This technology provides great opportunity and beneficial impact on many areas such as energy, medicine, electronics, and space industries (Zhang *et al.*, 2011). Biological approaches (higher plant's extraction, fungi, or bacteria) for synthesis of nanoparticles (NPs) are considered to be inexpensive, clean, non-toxic and eco-friendly (Ingle *et al.*, 2008). Fungi have the advantage of easy handling during downstream processing and large-scale production, so in this work since the fact that fungal filtrates can be used as a source of reductant, a new source of fungal isolates (from algal and higher plant's leave samples from Red Sea Resort, Hurghada, Egypt) will be used to identify strains with improved SNPs activity.

One of the most important applications of silver nanoparticles (SNPs) is the disinfection of water-filtering systems (nanofilters) due to their antimicrobial activity (Revina and Egorova, 1998). Also, SNPs are used in topical ointments and creams to prevent infection of burns and open wounds (Becker, 1999).

Silver NPs possess effective antimicrobial properties due to their extremely large surface area and small size that facilitates their penetration through the bacterial cell membranes allowing them to affect intracellular processes (Mahendra *et al.*,2009). The inhibitory effect of SNPs was reported against 650 species of microbes and against antibiotic resistant bacterial strains (Mahendra *et al.*, 2009). Although the exact mechanism of

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SNP antimicrobial effect is not clearly addressed there are theories about how SNPs can act on bacteria via multiple mechanisms (Feng, 2000). Silver NPs can easily attach to bacterial cell membranes by electrostatic interaction, then penetrate inside the cell causing structural changes such as permeability of the cell membrane. Another possible mechanism is the formation of free radicals (a free radical is a molecule that can exist indecently and contains unpaired electron in an atomic orbital; Lobo *et al*., 2010) by SNPs, where free radicals are unstable and highly reactive; they attack mainly lipids, nucleic acids and proteins leading to cell death (Lobo *et al*., 2010). It was also proposed that SNPs may release Ag^{+} and these ions have the tendency to act as a soft acid (Morones *et al*., 2005) which interact with sulphur-containing proteins and phosphorus containing compounds like DNA, which would act as a soft base (P & S) (Hatchett and White 1996). This can have a lethal impact on the cell and it is ability to replicate.

The antimicrobial effect of SNPs on viruses, bacteria and fungi have been extensively explored (Yadav *et al*., 2015). Naqvi *et al*. (2013) confirmed the potential effect of SNPs compared to commercial antibiotics against *Bacillus* sp., *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Other studies have reported the effectiveness of SNPs against Gram positive and negative bacteria as well (Mahendra *et al*., 2009). Morones *et al*. (2005) described the importance of the size of SNPs as antimicrobial agent as a size-dependent activity. In that study, SNPs were used against *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholera*; among NPs ranging from 1 to 100 nm, only nanoparticles of size ~1-10 nm were found to be effective. (Taglietti *et al*., 2012). reported the minimal inhibitory concentration (MIC) that was 180 µg/mL for SNP effectiveness against *Staphylococcus aureus* and reported 15 µg/mL with *E. coli*. Kanmani and Lim (2013) investigated the antimicrobial effect of SNPs against *E. coli*, *L. monocytogenes*, *K. pneumonia* and *P. aeruginosa*. In this study, they found that *P. aeruginosa* was more susceptible to SNPs followed by *E. coli* and *K. pneumonia*, while *L. monocytogenes* was less susceptible to the SNPs. In addition, Gram-negative bacterial pathogens were more highly suppressed by SNPs than Gram-positives.

The antifungal effect of SNPs is less well studied compared to their antibacterial effect (Yadav *et al*., 2015), although antifungal activity of SNPs has been reported against *Candida albicans*, *C. glabrate*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *Trichophyton mentagrophytes* (Kim *et al*., 2008). Gajbhiye *et al*. (2009) showed the effectiveness of biosynthesized SNPs against *C. albicans*, *F. semitectum*, *Phoma glomerata*, *P. herbarum*, and *Trichoderma* spp.. Other reports indicate the antifungal activity of SNPs against *A. flavus* and *C. albicans* (Kandile *et al*., 2010), *C. albicans* and *Saccharomyces cerevisiae* (Nasrollahi *et al*., 2011), *A. flavus*, *A. niger*, *Curvularia* spp., *Fusarium* spp. and *Rhizopus* spp. (Savithramma *et al*., 2011), *Alternaria alternata*, *A. flavus*, and *Rhizoctonia solani* (Kaur *et al*., 2012), *A. fumigatus,C. albicans*, and *Trichophyton rubrum* (Arjun and Bholay, 2012), and against keratitic fungi such as *Alternaria*, *Aspergillus* and *Fusarium* (Xu *et al*., 2013), *C. albicans* (Dar *et al*., 2013).

Experimental:

Isolation of fungi from marine samples

The two fungal isolates (*A. oryzae* and *F. solani*) used in this study were isolated from *Padina* (brown algae) samples that was collected from the Sea Gull resort 35 km southern of El Gouna, Hurghada, Egypt. *Padina* sp. Sample was kept submerged in sea water inside several clean plastic bags to avoid water leakage and stored at 4 °C for fungal isolation within a few days. Living thalli were washed with sea water several times, and then thalli segments were cut into equal pieces and were plated on to Potato Dextrose Agar (PDA) obtained from CONDA Pronadisa (Spain). Medium was suspended in 50:50 (v/v) distilled water:sea water of the Red Sea. Plates were inoculated using fine forceps under aseptic conditions.

Morphological identification of fungal isolates

Morphological identification was performed at Assiut University mycological

centre (AUMC), Egypt, using morphological characteristics and appropriate identification books (Barnett 1960; Ellis 1971; Nelson *et al.,* 1983; Pitt 1985; Kitch & Pitt 1992)**.**

Generation of SNP susing fungal filtrates

A new simple-method was employed to save supplies and growth media, where inoculum preparation, harvesting mycelium and treatment with $AgNO_3$ were as follows: liquid cultures were prepared and incubated at 25°C for 96 h on an orbital shaker at 120 rpm. Mycelial mats were washed several times (more than thrice) with sterile ultra-pure water, until a clear filtrate was achieved, to remove all medium components. The mycelial mat was squeezed gently to eliminate water as much as possible, then 5 g (fresh weight) was taken precisely and suspended in 50 mL sterile Milli-Q water, that was already prepared and autoclaved in a sterile 250 mL flask. The re-suspended mycelium was incubated for 24 h at 25°C on an orbital shaker at 120 rpm. Fungal filtrates were obtained by performing two filtration steps, using a sterile muslin cloth followed by a 0.22 µm membrane (Whatman) in a safety cabinet. Freshly prepared 500 mM $AgNO_3$ (10 µL)was added to 4,650 µL fungal filtrate (93%) and the total reaction volume was made up with sterile Milli-Q water (340 µL) to 5,000 µL. Reaction mixtures were then mixed gently in glass test tubes and incubated at 25°C for 48 h in the dark.

Characterization of SNPs by UV-vis spectrophotometer and TEM

Presence of SNPs was detected visually by observing the change in colour of the solution mixture from colourless to pale yellow. The *Spectramax*® 190 microplate spectrophotometer (operated by SoftMax® Pro 7 software version 7.0.2) was used to generate UV-visible spectra, where aliquots of 200 µL of the sample were loaded as technical triplicates into sterile microplates (96 wells, clear, flat bottom, Sterilin™) and scanned from 200 to 800 nm at a resolution of 1 nm.

For transmission electron microscopy (TEM) micrographs, reaction mixture (200 µL) was transferred into sterile microfuge tubes and shaken vigorously after 24 h incubation, and then centrifuged at high speed for 20 min. The supernatant was discarded and the pellet washed thrice with 600 µL Milli-Q water, then centrifuged at high speed for 15 min. The pellet was washed with ethanol 70%, centrifuged for 15 min again and finally the pellet was dried at 55°C overnight. Sterile distilled water (500 µL) was added to re-suspend the SNPs and sonicated, then 5 µL of the suspension were loaded onto carbon-coated copper grids (Agar Scientific) 0.3 mm in diameter and then allowed to dry at room temperature over 24 h. Micrographs were obtained at the Electron Microscopy Laboratory (EMLab), University of Reading, using the CM20 and JEM 2100 plus TEMs operating at 200 kV (0.23 nm resolution).

Size of mycological synthesized SNPs was determined using Scandium M image analysis software.

Antimicrobial effect of generated SNPs

Collected SNPs by centrifugation were 140 and 160 mg for *A. oryzae* and *F. solani*, respectively. Each weight was dissolved in 3 mL, strongly vortexed and sonicated before use each time.

Bacterial growth for microbiological assay

Bacillus cereus SBTBC, *Enterococcus faecalis* 8J, *E. coli*, *Lesteria monocytogenes* 10403S, *Salmonilla* and *Staphylococcus aureus* 7A strains, were obtained from School of Biological Sciences, University of Reading. Bacterial strains were recovered from frozen (-80°C) glycerol (15% *v*/*v*) stocks on Luria Bertani (LB) agar plates at 37°C for 24 h. (Andrews, 2001). protocol for determination of minimum inhibitory concentration (MIC) was followed, where an overnight (16-18 h) cultures (1:10) were prepared by picking single colonies to be inoculated in 9 mL LB broth, 225 rpm, at 37°C. Formed cultures were diluted into 1:100 by sub-cultured 1 mL into 9 mL LB broth, 250 rpm, at 37°C for 2-5 h to exponential phase (OD_{600} 0.4-0.6). Cultures were adjusted to $OD_{600} = 0.3$ and diluted in LB (1:50) prior to use in microbiological assays. In a 96-well flat-bottom transparent plate (Greiner), blank wells were loaded with 200 µL LB and negative control wells were loaded with 200 µL bacterial cells without SNPs, other wells were loaded with 100 µL of SNPs

(stock solution concentration, 46.7 mg/mL; dilution factor 0.5) with various concentrations (23.3, 11.75, 5.82, 2.9, 1.5, 0.72, 0.36, 0.18, 0.09 and 0.05 mg/mL). Prepared bacterial cultures as described earlier were added (100 µL; equivalent to, ~$5x10^5$ bacteria/well) in technical duplicates, with two biological replicates for each strain. Microplates were incubated at 37°C for 16 h or 24 h, and absorbance measurements were taken at 600 nm. The optical density of each individual culture at 16 h or 24 h was plotted in Excel (Microsoft office 2016) and Sigmoidal curves using polynomial order 2 function were used to calculate the LC_{50}. The MIC was determined by observing the lowest concentration that inhibited a visible growth of each bacterium, also by comparing OD_{600} values with the control sample. To determine the minimum bactericidal concentration (MBC), which can be identified as the lowest concentration of SNPs that will prevent the growth of bacteria after sub-culturing on to SNPs-free medium. The MBC concentration is considered the concentration that proceed the MIC concentration, with no apparent bacterial growth. For confirmation 100 µL of inhibited bacterial growth (MBC) was transferred into 100 µL (2.5% peptone water), mixed by pipetting and 100 µL was spread into LB plates and incubated overnight at 37°C.

Results and discussion:

Isolated fungi of SNPs producing activity from marine samples

A total of 53 fungal strains (data not shown) were obtained from *Padina* sp., which included five genera (*Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma*) belonging to the Hyphomycetes group, 16 species and one variety.

Thirty-one out of fifty-three isolates showed good evidence for SNPs production over 48 h (data not shown), based on a change in colour of the SNP reaction mixture from colourless to bright yellow (Petit *et al.*,1993) and the formation of the SNP-dependent absorbance peak between 400-450 nm which is characteristic for metal NPs with sizes ranging from 2 to 100 nm (Henglein, 1993). Among the 31 SNP^+ isolates (Ascomycota and Deuteromycota), *Aspergillus flavus* strains (PAF8 and AtAF) ranked the first place in this reduction activity. **But**, *Aspergillus flavus* is known of causing aspergollosis in immunodeficient individuals (Amaike and Keller, 2011) and **many strains** were reported for **producing** potent **mycotoxins** (Hedayati *et al.*, 2007**)**. For these reasons, *Aspergillus flavus*was was excluded from further studies and *Aspergillus oryzae* (PAO) and *Fusarium solani* (PFS2) were selected for more investigation. Both showed a good evidence of SNPs production, OD= 0.88 and 0.42, respectively.

Aspergillus oryzae (PAO) belongs to the *A. flavus* group (Raper and Fennell, 1965) and considered as a non-pathogenic fungus (Domsch *et al.*, 1980). Also, the US Food and Drug Administration categorize *A. oryzae* as 'generally recognized as safe' (Matsushita-Morita *et al.*, 2010). It is a fast-growing fungus, with high extracellular enzymes activity and high competitiveness against other fungal species (Liang *et al.*, 2009) all these advantages make it ideal for research study. For these reasons, the *Padina A. oryzae* isolate was selected for the experimental analysis of its SNPs forming properties. Silver nanaoparticles generation using *A. oryzae* was reported by other researchers (Binupriya *et al.*, 2009; Phanjom & Ahmed 2015; Bhimba *et al.*, 2015). but the exact reaction mechanism of SNPs formation is yet to be elucidated and optimized conditions for synthesis are yet to be determined. From imperfect fungi (Deuteromycota) isolates, *F. solani* (PFS2) which is an important plant pathogen was selected as only one report about its use in SNPs production was published up to date (Ingle *et al.*, 2009) without presenting details about the antimicrobial effect of synthesized SNPs. This is the first report up to date, about using SNPs synthesized by *F. solani* against bacterial strains that were mentioned previously.

Characterization of SNP by transmission electron microscopy (TEM)

According to the TEM micrograph, the SNPs formed were approximately spherical (Fig. 1 and 2). For size determination,

Scandium image analysis software was used, and measurements of 80 and 168 SNP were determined within the *A. oryzae* and *F. solani* micrographs.

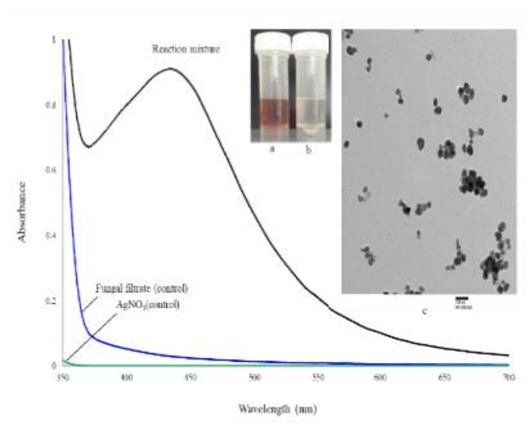

Figure 1: UV-visible spectra of synthesized SNPs using fungal filtrate of *A. oryzae* PAO. Spectrum of reaction mixture measured at 24 h; two controls were used, $AgNO_3$ and fungal filtrate. Inset: shows change in colour of (a) reaction mixture ($AgNO_3$ with fungal filtrate) and (b), fungal filtrate only (control), at 24 h; (c) TEM of synthesized SNPs are shown spherical in shape and with average particles size 12.6 nm. Scale bar 100 nm.

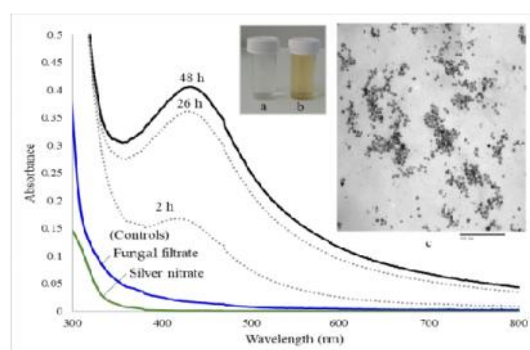

Figure 2: UV-visible spectra of synthesized SNPs using fungal filtrate of *F. solani* PFS2. Two controls were used; silver nitrate and fungal filtrate. Controls were exposed to the same experimental conditions. Inset figure of visual change in colour of reaction mixture; (a), fungal filtrate (control); (b), reaction mixture at 24 h. TEM of synthesized SNPs are shown spherical in shape and with average particles size of 19.06 nm. Scale bar 500 nm.

Antimicrobial effect of SNPs synthesized using A. oryzae PAO filtrate

Different antimicrobial effects of the mycological synthesized SNPs against Gram-positive bacteria including, *Bacillus cereus* SBTBC, *Enterococcus faecalis* 8J, *Lesteria monocytogenes* 10403S and *Staphylococcus aureus* 7A was recorded and the MICs and MBCs value showed that *Enterococcus faecalis* 8J was more susceptible to the inhibitory effect of SNPs than *Bacillus cereus* SBTBC (Table 1). While, SNPs had no inhibitory effect against *Lesteria monocytogenes* 10403S or *Staphylococcusaureus* 7A.This was in contrast with (Binupriya *et al*., 2009) who reported that synthesized SNPs using live and dead cell filtrates of *Aspergillus oryzae* var. *viridis* (5 to 50 nm) had an antibacterial effect against *Staphylococcus aureus* KCCM 12256 strain of MBC= 40 mg/L. That was the only study about the effect of synthesized SNPs by *A. oryzae* against bacterial strains up to date. More studies need to be carried out to clarify the effect of synthesized SNPs by *A. oryzae* against pathogenic bacteria. Silver NPs also showed a high inhibitory effect against Gram-negative bacteria, including *Escherichia coli* and *Salmonilla* with EC50 of 0.26 and 0.25 mg respectively.

Tested bacteria	EC50 Value	EC50 Standard error	EC90 Value	EC90 Standard error
Bacillus cereus SBTBC	1.972	0.245	0.086	0.025
Enterococcus faecalis 8J	1.641	0.258	0.017	0.008
Escherichia coli	0.261	0.030	0.019	0.006
Salmonilla	0.255	0.031	0.016	0.005

Table 1: Measured EC50 and EC90 for tested bacterial strains against synthesized SNPs by *A. oryzae* filtrate.

Silver NPs exhibited higher bactericidal effect on the Gram-negative strains compared to the Gram-positive ones. This is probably due to the different bacterial cell-wall structures between Gram-positive and Gram-negative bacteria, as SNPs are basically

interacting electrostatically (Ciobanu *et al.*, 2013). The Gram-negative bacteria as characterized by a porous outer membrane and a periplasmic membrane that would allow the passage of SNPs inside the bacterial cell and their intracellular accumulation (Ciobanu *et al.*, 2013).

Antimicrobial activity of generated SNPs by F. solani MH005062 filtrate

The MIC, MBC and LC_{50} values were recorded in Table, 2. Results showed that, SNPs had a bactericidal effect against *B. cereus* SBTBC, *Enterococcus faecalis* 8J, *Escherichia coli* and *Salmonilla* with MBC concentrations of 9.21, 6.12, 2.6 and 0.921 mg, respectively. Results showed that Gram-negative bacteria were more susceptible to the inhibitory effect of SNPs than Gram-positive bacteria. While, SNPs had no inhibitory effect against the Gram-positive bacteria, *Lesteria monocytogenes* 10403S and *Staphylococcus aureus* 7A.

Tested bacteria	EC50		EC90	
	Value	Standard error	Value	Standard error
Bacillus cereus SBTBC	9.213	1.827	0.995	0.446
Enterococcus faecalis 8J	6.155	1.139	0.725	0.305
Escherichia coli	2.606	0.564	0.135	0.069
Salmonilla	0.921	0.192	0.054	0.027

Table 2: Measured MC50 and MC90 for tested bacterial strains against synthesized SNPs by *F. solani* filtrate.

Tenover, 2006 explained three possible mechanisms for the antibacterial effect of SNPs. One is due to the high surface area to volume ratio that acquire NPs with better penetration properties than bulk materials and accumulate in the cytoplasmic membrane which disturb permeability and respiration (Murray *et al.*, 1965), ending with the cell death. A second explanation is possibly due to SNPs interaction with sulfur- and phosphorus-compounds such as protein and DNA and eventually damage the cell (Gibbins & Warner, 2005). The third proposed mechanism suggests that SNPs can release Ag^{+} ions into cytoplasmic components that has an essential role in the bactericidal effect (Feng *et al.*, 2000).

Our results show that mycological synthesized SNPs are promising compounds as antibacterial agents, however the exact mechanism of their bactericidal effect needs to be elucidated, and the safety of using them commercially still need to be more confirmed.

Conclusions:

In conclusion, mycological synthesized SNPs at a size of 12.6 and 19.06 nm that are spherical in shape can act effectively against different Gram-positive and Gram-negative bacterial strains. But the exact mechanism of this their bactericidal effect still need to be more studied for each bacterial strain to address whether this effect occurs outside or inside the bacterial plasma membrane and to determine whether this toxic effect refers to the action of silver ions (Ag^{+}) or SNPs. The antimicrobial effect of SNPs when used with medical devices can have some drawbacks because of nanotoxicity. Thus, intensive studies about the mechanism by how NPs can be generated and the mechanism by how NPs affect pathogenic bacteria need to be carried out to address their toxicity concerns toward health and environment.

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References:

Amaike, S., Keller, N.P., *Aspergillus flavus*. Annual review of phytopathology. (2011). Sep 8; 49:107-33.

Andrews, J.M., Determination of minimum inhibitory concentrations. Journal of antimicrobial Chemotherapy. 2001 Jul 1; 48(suppl_1):5-16.

Arjun, T.V., Bholay, A.D., Biosynthesis of silver nanoparticles and its antifungal activities. Journal of Environmental Research and Development. (2012). Jul;7.

Barnett, H.L., Illustrated genera of imperfect fungi. (1960).

Becker, R.O., Silver ions in the treatment of local infections. Metal-based drugs. (1999). 6(4-5):311-4.

Bhimba, B.V., Gurung, S., Nandhini S.U., Silver nanoparticles synthesized from marine fungi *Aspergillus oryzae*. Int J Chem Tech Res. (2015). 7(01):68-72.

Binupriya, A.R., Sathishkumar, M., Yun, S.I., Myco-crystallization of silver ions to nanosized particles by live and dead cell filtrates of *Aspergillus oryzae* var. *viridis* and its bactericidal activity toward *Staphylococcus aureus* KCCM 12256. Industrial & Engineering Chemistry Research. (2009). Nov 20; 49(2):852-8.

Ciobanu, C.S., Iconaru, S.L., Chifiriuc, M.C., Costescu, A., Le Coustumer, P., Predoi, D., Synthesis and antimicrobial activity of silver-doped hydroxyapatite nanoparticles. BioMed Research International. 2013; (2013).

Dar, M.A., Ingle, A., Rai, M., Enhanced antimicrobial activity of silver nanoparticles synthesized by Cryphonectria sp. evaluated singly and in combination with antibiotics. Nanomedicine: Nanotechnology, Biology and Medicine. (2013). Jan 1; 9(1):105-10.

Domsch, K.H., Gams, W., Anderson, T.H., Compendium of soil fungi. Volume 1. Academic Press (London) Ltd.; (1980); 2:2293-5.

Ellis, M.B., Dematiaceous Hyphomycetes. Commonwealth Mycological Institute Kew, Surrey, England; (1971) 464-465 pp.

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O., A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. Journal of biomedical materials research. 2000 Dec 15; 52(4):662-8.

Gajbhiye, M., Kesharwani, J., Ingle, A., Gade, A., Rai, M., Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine: Nanotechnology, Biology and Medicine. (2009). Dec 1; 5(4):382-6.

Gibbins, B., Warner, L., The role of antimicrobial silver nanotechnology. Med Device Diagnostic Indust Mag. (2005). Aug; 1:1-2.

Hatchett, D.W., White, H.S., Electrochemistry of sulfur adlayers on the low-index faces of silver. The Journal of Physical Chemistry. (1996). Jun 6; 100(23):9854-9.

Hedayati, M.T., Pasqualotto, A.C., Warn, P.A., Bowyer, P., Denning, D.W., *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiology. (2007). Jun 1; 153(6):1677-92.

Henglein, A., Physicochemical properties of small metal particles in solution:" microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition. J Phys Chem Biophys. (1993); 97(21):5457-71.

Ingle, A., Gade, A., Pierrat, S., Sonnichsen, C., Rai, M., Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. Current Nanoscience. (2008). May 1; 4(2):141-4.

Ingle, A., Rai, M., Gade, A., Bawaskar, M., *Fusarium solani*: a novel biological agent for the extracellular synthesis of silver nanoparticles. Journal of Nanoparticle Research. (2009). Nov 1; 11(8):2079.

Kandile, N.G., Zaky, H.T., Mohamed, M.I., Mohamed, H.M., Silver nanoparticles effect on antimicrobial and antifungal activity of new heterocycles. Bulletin of the Korean Chemical Society. (2010); 31(12):3530-8.

Kanmani, P., Lim, S.T., Synthesis and structural characterization of silver nanoparticles using bacterial exopolysaccharide and its antimicrobial activity against food and multidrug resistant pathogens. Process

Biochemistry. (2013). Jul 1; 48(7):1099-106.

Kaur, P., Thakur, R., Choudhary, A., An in vitro study of the antifungal activity of silver/chitosan nanoformulations against important seed borne pathogens. International Journal of Scientific & Technology Research. (2012). Aug 25; 1(7):83-6.

Kim, Y.S., Kim, J.S., Cho, H.S., Rha, D.S., Kim, J.M., Park, J.D., Choi, B.S., Lim, R., Chang, H.K., Chung, Y.H., Kwon, I.H., Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhalation toxicology. (2008). Jan 1; 20(6):575-83.

Kitch, M.A., Pitt, J.I., A laboratory guide to the common *Aspergillus* species and their teleomorphs; Common Wealth Scientific and Industrial Research Organization, Division of Food Processing (CSIRO), North Ryde: Australia; (1992).

Liang, Y., Pan, L., Lin, Y., Analysis of extracellular proteins of *Aspergillus oryzae* grown on soy sauce koji. Bioscience, biotechnology, and biochemistry. (2009). Jan 23; 73(1):192-5.

Lobo, V., Patil, A., Phatak, A., Chandra, N., Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews. (2010). Jul;4(8):118.

Mahendra, R, Alka, Y., Bridge P., Aniket, G., Myconanotechnology: a new and emerging science. In: Rai MK, Bridge PD (eds) Applied mycology, CAB International, New York; (2009):258-67.

Matsushita-Morita, M., Furukawa, I., Suzuki, S., Yamagata, Y., Koide, Y., Ishida, H., Takeuchi, M., Kashiwagi, Y., Kusumoto, K.I., Characterization of recombinant prolyl aminopeptidase from *Aspergillus oryzae*. Journal of applied microbiology. (2010). Jul; 109(1):156-65.

Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., Yacaman, M.J., The bactericidal effect of silver nanoparticles. Nanotechnology. (2005). Aug 26; 16(10):2346-53.

Murray, R.G., Steed, P., Elson, H.E., The location of the mucopeptide in sections of the cell wall of *Escherichia coli* and other gram-negative bacteria. Canadian journal of microbiology. (1965). Jun 1; 11(3):547-60.

Naqvi, S.Z., Kiran, U., Ali, M.I., Jamal, A., Hameed, A., Ahmed, S., Ali, N., Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria. International journal of nanomedicine. (2013); 8:3187-95.

Nasrollahi, A., Pourshamsian, K.H., Mansourkiaee, P., Antifungal activity of silver nanoparticles on some of fungi. International Journal of Nano Dimension. (2011). Mar 1; 1(3):233-9.

Nelson, P.E., Toussoun, T.A., Marasas, W.F., *Fusarium* species: an illustrated manual for identification. University Park, PA: Penn State University Press; (1983).

Petit, C., Lixon, P., Pileni, M.P., In situ synthesis of silver nanocluster in AOT reverse micelles. J Phys Chem Biophys. (1993); (49):12974-83.

Phanjom, P., Ahmed, G., Biosynthesis of silver nanoparticles by *Aspergillus oryzae* (MTCC No. 1846) and its characterizations. J. Nanosci. Nanotechnol. (2015); 5(1):14-21.

Pitt, J.I., Laboratory Guide to common Penicillium species CSRIO Division of Food Research, North Ryde. New South Waler, Australia. (1985).

Raper KB, Fennell DI. The genus *Aspergillus*. William & Wilkins Co., Baltimore. (1965).

Revina AA, Egorova EM. Radiation-chemical technology for the synthesis of stable metallic and bimetallic clusters. In International Conference on Advances in Technology in the 21st Century

(ICAT '98) Moscow, (1998). Oct (pp. 411-413).

Savithramma, N., Rao, M.L., Rukmini K, Devi PS. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. International Journal of ChemTech Research. (2011). Jan 1; 3(3):1394-402.

Taglietti, A., Diaz Fernandez, Y.A., Amato, E., Cucca, L., Dacarro, G., Grisoli, P., Necchi, V., Pallavicini, P., Pasotti, L., Patrini, M., Antibacterial activity of glutathione-coated silver nanoparticles against gram positive and gram-negative bacteria. Langmuir. (2012). May 15; 28(21):8140-8.

Tenover, F.C., Mechanisms of antimicrobial resistance in bacteria. American journal of infection control. (2006). Jun 1; 34(5):S3-10.

Vahabi, K., Mansoori, G.A., Karimi, S., Biosynthesis of silver nanoparticles by fungus *Trichoderma reesei* (a route for large-scale production of AgNPs). Insciences J. (2011); 1(1):65-79.

Xu, Y., Gao, C., Li, X., He, Y., Zhou, L., Pang, G., Sun, S., In vitro antifungal activity of silver nanoparticles against ocular pathogenic filamentous fungi. Journal of Ocular Pharmacology and Therapeutics. (2013). Mar 1; 29(2):270-4.

Yadav, A., Kon, K., Kratosova, G., Duran, N., Ingle, A.P., Rai, M., Fungi as an efficient mycosystem for the synthesis of metal nanoparticles: progress and key aspects of research. Biotechnology letters. (2015). Nov 1; 37(11):2099-120.

Zhang, X., Yan, S., Tyagi, R.D., Surampalli, R.Y., Synthesis of nanoparticles by microorganisms and their application in enhancing microbiological reaction rates. Chemosphere. (2011). Jan 1; 82(4):489-94.

الملخص العربي

في هذه الدراسة تمإستخدام معزولتان فطريتان جديداتان من بيئة بحرية (أسبرجلس أوريزي و فيوزاريوم سولاني) لتكوين جزيئات الفضة ( ) النانوية. تم إختبار تأثير هذه الجزيئات (. . نانوميتر لكل من الأسبرجلس اوريزي و الفيوزاريوم سولاني علي التوالي) علي بعض السلالات البكتيرية الممرضة . حيث كانت التركيزات التي تسببت في أقل نمو ظاهري هي . . . . مليجرام لكل مليليتر في حالة الباسيلاس سيريس الإنتيروكوكاس فيكاليس، الإشيريشيا كولاي و السالمونيلا، علي التوالي، لتاثير جزيئات الفضة النانوية المتكونة من فطر الأسبرجلس أوريزي. وكانت التركيزات التي تسببت في اقل نمو ظاهري لنفس الأنواع البكتيرية كانت . . . . لنفس الأنواع البكتيرية علي التوالي، لجزيئات الفضة النانوية المتكونة من فطر الفيوزاريوم سولاني. هذه النتائج توضح أهمية استخدام جزيئات الفضة النانوية المنتجة بواسطة الفطريات لتثبيط نمو بعض السلالات البكتيرية الممرضة، ولكن مازال هناك إحتياج لدراسة مدي خطورة هذه المركبات للإستخدام التجاري و إكتشاف الآلية التي تؤثر بها هذه الجزيئات علي أنواع البكتيريا المختلفة.