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Original Research

# Green synthesis of silver nanoparticles: The reasons for and against *Aspergillus parasiticus*

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## Abstract

***Objective(s):*** The enzymatic activity of fungi has recently inspired the scientists with re-explore the fungi as potential biofactories rather than the causing agents of humans and plants infections. In very recent years, fungi are considered as worthy, applicable and available candidates for synthesis of smaller gold, silver and other nano-sized particles.
***Materials and Methods:*** A standard strain of *Aspergillus parasiticus* was grown on a liquid medium containing mineral salt. The cell-free filtrate of the culture was then obtained and subjected to synthesize SNPs while expose with 1mM of $AgNO_3$. Further characterization of synthesized SNPs was performed afterward. In addition, antifungal activity of synthesized SNPs was evaluated against a standard strain of *Candida albicans*. The reduction of $Ag^+$ ions to metal nanoparticles was investigated virtually by tracing the color of the solution which turned into reddish-brown after 72 h.
***Results:*** The UV-vis spectra demonstrated a broad peak centering at 400 nm which corresponds to the particle size much less than 70 nm. The results of TEM demonstrated that the particles were formed fairly uniform, spherical, and small in size with almost 90% in 5-30 nm range. The zeta potential of silver nanoparticles was negative and equal to 15.0 which meets the quality and suggested that there was not much aggression. Silver nanoparticles synthesized by *A. parasiticus* showed antifungal activity against yeast strain tested and exhibited MIC value of 4 µg/mL.
***Conclusion:*** The filamentous fungus, *A. parasiticus* has successfully demonstrated potential for extra cellular synthesis of fairly monodispersed, tiny silver nanoparticles.

**Keywords:** *Aspergillus parasiticus*, Biosynthesise, Extracellular, Silver nanoparticles

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## Introduction

The need to evolve eco-friendly, non-toxic approaches for biosynthesis of nano materials and nanostructures has been newly stands out in the world of modern nanoscience which toxic synthesis protocols being used to meet the need. To pursue the objective, both multicellular and unicellular organisms have been recently regarded as well-known apparatus to produce inorganic materials either intra or extra cellular (1-3). However, in very recent years, there has been an increasing tendency for re-exploring of microorganisms as potential biofactories for synthesis of functional metallic nano-particles such as silver, cadmium, gold and *etc* (4). In fact, heavy metal ions are not biodegradable and persist in nature in such a way that they inhibit microbial growth as a consequence of their toxicity. Nevertheless, microorganisms including fungi play a remarkable role in bioremediation of toxic metals by several kinds of mechanisms including using efflux pumps, reduction or oxidation, biosorption and bioaccumulation (5,6).

The great interest in the synthesis of nanoparticles is due to the unique optical (7), chemical (8), photoelectrochemical (9), and electronic (10), properties they possess. These characteristics lead to beneficial effects on health settings via their wide range of application in combating with microbes (11), treatment of cancer (12), and also biolabling (13). The exhibition of unusual magnetic, optic, catalytic and thermal properties by nanoparticles entirely depends on their size and shape (1). Notably, the smaller size the particles possess, the higher surface area-to-volume ratio is. Hence, the biological effectiveness of nanoparticles increase as their specific surface area is extended on the account of a rise in surface energy (14). Despite the fact that nanoparticles can be synthesized by conventional chemical methods in different desirable size and shape, there is a strong possibility that microorganisms produce particles in more minimized size and more uniform shape. So obviously, the major advantage of biosynthesis of nanoparticles over chemical production is the controlled synthesis of nanoparticles by micro-organisms (15).

Moreover, most of the chemical approaches are considered as highly environmental cost (1). Various metal nanoparticles, Au, Ag, Pt, Pd and an alloy of Au–Ag are synthesized by biological processes. Amongst, the silver nanoparticles (SNPs) have received considerable attention as a consequence of their attractive physic-chemical properties (16). In addition to bacteria, a growing number of various genera of fungi have been under investigation in this field of research. Besides, there are sufficient evidences which support the involvement of the metabolic activity of fungi in making them competent to precipitate of nanoparticles in external environment of the cell (17), and eventually, presents them as worthy, applicable and available candidates for synthesis of gold (18, 19), silver (20), and other nano-sized particles (3).

Although SNPs are synthesized both intra as well as extracellulary, ease of control over the environment, large-scale synthesis and straightforward processing steps (21) represent the extracellular method of biosynthesis more advantageous rather than the other one. A vast number of microorganisms are capable to synthesize SNPs extra-cellularly, among which *Fusarium oxysporum* (22), *Bacillus licheniformis* (23), *Aspergillus fumigatus* (5), *Aspergillus niger* (24), *Aspergillus clavatus* (25), *Penicillium brevicompactum* (26), *Escherichia coli* (14), *Cladosporium cladosporioides* (17), *Fuserium semitectum* (27), and *Klebsiella pneumoniae* (28), have been well described extensively.

Apart from the significant negative impact of members of the genus *Aspergillus* on public health as potent pathogens to induce life-threatening systemic infections, the

highly useful utilization of the genus should not be ignored in industrial applications and also other science such as nanotechnology. In view of nanoscience, members of the genus *Aspergillus* are newly considered to bring up a simple, straightforward and eco-friendly system to synthesise SNPs. Considering the published data, *Aspergillus parasiticus* is not regarded as highly virulent as *Aspergillus fumigates*. This species is not even included in the five-member group of the most infectious species of Aspergilli (29). As a matter of fact, the frequency of reports of invasive aspergillosis caused by *A. fumigates* has been gradually rising and is relatively higher than *A. parasiticus* (30). Furthermore, it is well-established that the species *A. parasiticus* is competent to produce aflatoxin, the most highly toxin carcinogen with immunosuppressive properties (31). The above reasons led us to investigate the other possible beneficial properties of this fairly safe species, rather than its negative impact on public health. Accordingly, in the current study, we apply *A. parasiticus* so as to biosynthesis the SNPs extracellulary.

## Materials and Methods

### *Fungal strain*

A standard strain of *A. parasiticus* (ATCC 15517) was used for this investigation. The fungus was preserved on Potato Dextrose Agar medium (PDA) (Sigma, USA) until use.

### *Preparation of cell-free fungal filtrate*

The liquid medium containing mineral salts was used in order to high efficiency in fungal growth and establishing a biomass competent for silver nanoparticles biosy-nthesis. The medium contains: $KH_2PO_4$ 7.0 (g/l), $K_2HPO_4$ 2.0 (g/l), $MgSO_4.7H_2O$ 0.1 (g/l), $(NH_4)_2SO_4$ 1.0 (g/l), yeast extract 0.6 (g/l), and glucose 10.0 (g/l). The constituents were mixed completely by stirring on a magnetic stirrer and then the solution was boiled to digest the undissolved substances and filtered through filter paper. Erlenmeyer flasks containing 200 ml of the mentioned medium were inoculated with *A. parasiticus* spores and incubated aerobically on orbital shaker at 25°C agitating at 150 rpm for 72 h. Afterward, the biomass was extensively washed out twice with distilled sterile water and then harvested by sieving through a plastic sieve, followed by extensive washing to remove all medium ingredients from the biomass.

Fifteen mg of fresh and clean biomass was then taken into a new flask containing 150 ml of Milli-Q deionized water and was agitated at the same condition as described above. After 72 h of incubation, cell-free filtrate was obtained by utilizing Watman filter paper No.1.

### *Synthesis of silver nanoparticles*

Eventually the filtrate was applied to biosynthesis of silver nanoparticles. To achieve the aim, $AgNO_3$ was added to 100 ml of cell-free filtrate in such a way that a solution with 1 mM of $AgNO_3$ in final concentration was made.

The flask was agitated at 150 rpm in dark. A negative control, (only filtrate, without the silver ion) was also run along with the experimental flask.

### *Characterization of synthesized SNPs*

Aliquots of 1ml of the reaction solution were removed after 24 and 72 h and consequently subjected to UV-vis spect-roscopy (LABOMED. Inc, USA). The absorbance was measured at a resolution of 1nm. The shape and also estimated size of the silver nanoparticles was determined by transmission electron microscopy (TEM) as well. The TEM image of the sample was obtained using transmission electron micro-scope (Philips 30ML20) at a voltage of 100 kv. In addition, particle sizing experiments as well as the zeta potential measurements were carried out by means of a Zetasizer Nano ZS (Malvern Instruments, South-borough, UK).

### *Evaluation of antifungal activity of synthesized SNPs*

In order to assess the antimicrobial effect of synthesized silver nanoparticles, one standard (*Candida albicans* ATCC 10261) and 12 Fluconazole-resistant *Candida albicans* strains were applied. The isolates was kept at -80° C as 20% glycerol stocks and were sub-cultured, as required, on SDA plates at 30°C.

Microdilution antifungal susceptibility testing of the *C. albicans* isolate was performed by the broth microdilution method described in CLSI (Clinical and Laboratory Standards Institute) document M27-A3 (32) for SNPs.

Fluconazole was used as a reference antifungal agent. RPMI 1640 medium with L-glutamine and phenol red without bicarbonate was used. The medium was buffered to pH 7.0 with 0.165 mol/L MOPS (3-(N-morpholino) propanesulfonic acid). Fluconazole was dissolved in sterile diluted water to 64µg/L, and then diluted to the final concentration of 0.0125-16 µg/L with the medium according to the standard in the CLSI reference method. After growing on SDA (Merk, Germany) at 35°C for 24 h, the yeasts were re-suspended in culture medium to prepare a working solution at a concentration of $2.5\times10^3$ cells/mL. The prepared yeast solution was exposed to diluted fluconazole (0.0125-64 µg/L) and finally incubated at 35°C for 48 h. All tests were carried out in duplicate. The interpretive criteria for susceptibility to fluconazole were published by the CLSI (32), and are as follows: (i) susceptible $\leq$8 µg/mL; susceptible dose- dependent (S-DD) 16 to 32µg/mL; and resistant ≥64 µg/mL.

For silver nanoparticles, a solution of 16µg/mL was prepared and diluted to the final concentration of 0.0125–16 µg/L with the medium.

The same inoculum size was applies for SNPs susceptibility test and finally, the 96-well plate was incubated at 35° C for 48 h.

## Results

### *Silver reduction*

After addition of $Ag^+$ ions into the filtered cell-free culture in the dark, samples changed in colour gradually from nearly colorless to reddish-brown, with intensity increasing during the time of incubation. After 72 h of incubation, the process was stopped and the particles were subjected to further analysis comprising of transmission electron microscopy, size determination and zeta potential measurements.

The reduction of the $Ag^+$ ions into metal nanoparticles was investigated virtually, so that, the color of the solution changed into intense reddish-brown after 72 h of incubation, which has been indicated in Figure 1a. However, control sample (without silver ions) showed no change in color of the cell filtrates when incubated in the same environmental conditions (Figure 1b). The appearance of a reddish-brown color in the reaction suggested the formation of silver nanoparticles (33). The solution stayed on as hydrosol and no precipitation was observed even after 72h of incubation.

### *UV–visible spectroscopy*

Formation of colloidal silver particles can be easily followed by changes of UV-Vis absorption (Figure 2).

The UV spectroscopy method can be applied for size measurement of silver nanoparticles based on localized surface plasmon resonance band exhibiting at different wavelength.

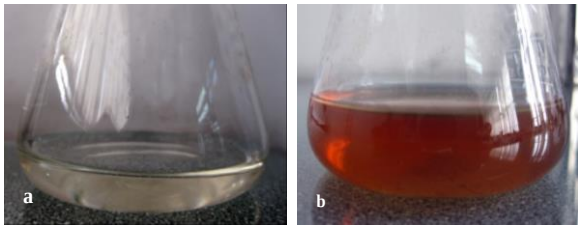


**Figure 1.** Cell filtrate of *A. parasiticus* with silver ion (1 mM): (a) at the beginning of the reaction (b) after 72h of reaction.

The light absorption pattern of the cell filtrate was continuously monitored after 24 and 72 h in the range of 200-800 nm by using UV-visible spectrophotometer.
As illustrated in Figure 2, after 24 h of incubation, a very wide peak located between 350 and 442 nm was obtained. The average wavelength at which the peak occurred was around 420 nm. After 72 h of starting of the reaction, a strong, broad peak located between 362 and 417 nm, centering at around 400 nm, was observed for the silver nanoparticles. Observation of this peak which assign to a surface plasmon, is well-documented for silver metal nanoparticles with sizes much less than 70nm (34). As a result, the fact that silver nanoparticles broad peak remains close to the range between 370 and 420 nm even after 72 h of incubation indicates that the particles were more likely to be well dispersed in the solution.

***Electron microscopy, size and zeta potential of SNPs***

TEM analysis was employed to determine the morphology and shape of silver nanoparticles. The representative TEM image is indicated in Figure 3. Accordingly, the majorities of the SNPs are relatively uniform in diameter and present in spherical shape. Additionally, TEM images depicted that the particles are predominantly formed at even less than 50 nm. The size histogram of SNPs determined from Zetasizer Nano ZS was shown in figure 4.

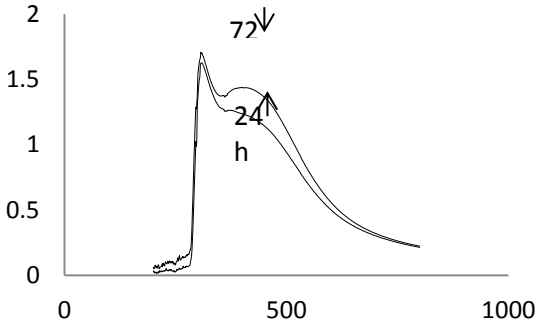


**Figure 2.** UV–visible spectrum of liquid medium containing cell filtrate and silver ion (1 mM).

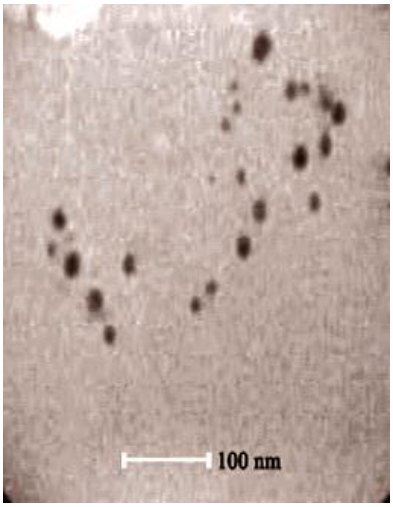


**Figure 3.** TEM micrograph of silver particles synthesized by *A. parasiticus* (scale bar: 100 nm).

It is well-demonstrated that the particles were produced in various different sizes, though less than 50 nm. From this histogram, it is depicted that there is variation in the particle sizes with more than 90% of the particles in 5-30 nm range and 4% of the particles were formed in 30-40 nm. Only 1.5% of the particles were larger than 40 nm.
The zeta potential of silver nanoparticles was also investigated (Figure 5). At natural conditions (pH close to 8), the zeta potential was negative and equal to -15.0. Obviously, the obtained result meets the quality and indicated the small amount of aggression.

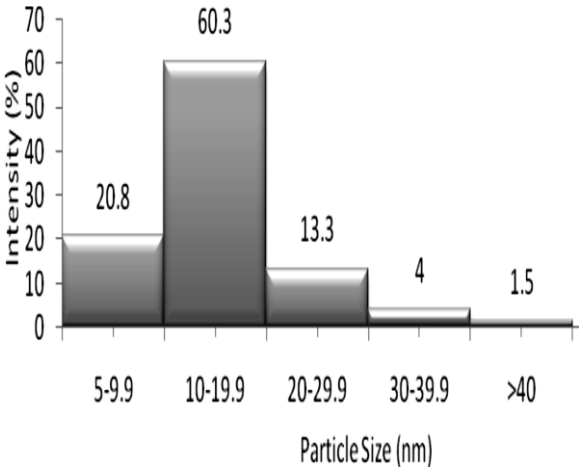


**Figure 4.** The particle size distribution histogram of silver nanoparticles synthesized by *A. parasiticus*.

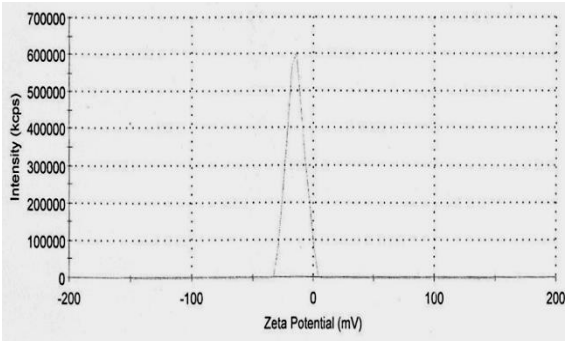


**Figure 5.** Zeta potentioal of silver nanoparticles synthesized by *A. parasiticus*.

### *Evaluation of antifungal activity of synthesized SNPs*

In this study, fluconazole, an antifungal agent which is widely used to treat *Candida*-
associated infections, was used as a positive control to compare with SNPs.

According to CLSI guideline, the standard strain was susceptible dose-dependent (S-DD) against fluconazole, showing MIC values of 16 μg/mL toward the fungal strain tested.

Silver nanoparticles synthesized by *A. parasiticus* showed antifungal activity against both standard and clinical fluconazole-resistant strains tested and exhibited MIC value of 4 μg/mL. Apparently, it was a notable finding for fluconazole-resistant *C. albicans* strains even for non-resistant ones.

## Discussion

The field of nanoscience has emerged over the last two decades and the need for nanotechnology will only expand rapidly while miniaturization becomes more impor-tant in areas such as biomedical applications (34).

The synthesis of nanoparticles, however, is a fairly established field so that they have been unintentionally synthesized for centuries.

Several methods for the synthesis of Ag nanoparticles have been reported in the literature and can be arbitrarily divided into chemical and biological categories.

In spite of successful employment of chemical and physical methods, interestingly, nature has provided exciting possibilities of utilizing biological systems to achieve this aim. This capacity owes to the fact that microorganisms can reduce the ions into metallic particles while interacting with heavy metal ions such as $Ag^{+}$, $Au^{+}$, $Se^{+}$, and $Ca^{2+}$.

Regarding this wonderful capacity, extracellular biosynthesis of SNPs was investigated in this study by using *A. parasiticus* as a biological model.

It was observed that upon addition of the silver ion (1 mM) into the flask containing the cell filtrate, the color of the medium turn into reddish-brown (Figure 1b). The intensity of color increased due to excitation of surface plasmon vibrations in the metal nanoparticles.

This significant observation indicates that the reduction of the $Ag^{+}$ ions takes place extracellulary.

The production and stability of the reduced SNPs in the colloidal solution was investigated by using UV–vis spectral analysis.

The unique optical properties of metal nanoparticles originate from the collective oscillations of conduction electrons, which, when excited by electromagnetic (35) rad-iation, are termed surface plasmon polariton resonances (SPPR) (34). This characteristics of nanoparticles are well-determined by using a UV-vis spectrophotometer. The shape of SPPR spectrum and the wavelength at which the maximum absorbance is occurred is strongly related to the particle size and relative dimensions. In the present study, the UV–visible spectra demonstrated a broad spectrum with a peak at 400 nm after 72 h of incubation. As the size increases, the field across the particle becomes nonuniform, so the dipole resonance will broadens and excites higher multipole resonances, such as the quadrupole, octupole and etc (34). Respecting above and also according to the fact that longer maximum wavelength (450 nm) represents the particle size beyond 70 nm, the SNPs produced with cell-free filtrate of *A. parasiticus* are

supposed to be spherical in shape, fairly uniform and even very smaller than 70nm. Apparently, the size and uniformity of our results of TEM and Zetasizer studies confirmed the mentioned findings. Another benefit which motivates the scientists to make use of small-sized particles is catalytic activities including the antimicrobial properties which are a result of increasing the surface area-to-volume ratio. Our results for evaluation the antimicrobial properties of synthesized silver nanoparticles were accurately proven. Moreover, it was well demonstrated that the inhibitory effect of synthesized SNPs was even more effective than the common anti-*Candida* antifungal drug, fluconazole. However, there is still work to do on the control of particle size and shape uniformity by modifying the temperature, pH or $Ag^+$ concentration. In the present study, a biological protocol was applied for the synthesis of SNPs by using extracellulary mycelia-free filtrate of the species *A. parasiticus*. It is strongly suggestive that the proteins, polysaccharides and more important, the secreting hydrolytic and photolytic enzymes of the fungus (particularly *nitrate reductase*) are in charge of biosynthesis of silver nanoparticles (11).

Nelson Duran et al.(11) have proven the presence of NADH dependent nitrate reductase as the corresponding agent for reduction of $Ag^+$ ions in case of *Fusarium oxysporium* strains.

Anil Kumar et al. (22) have also demonstrated that an enzymatic route using α-NADPH dependent nitrate reductase along with phytochelatin is required for the synthesis of silver nanoparticles.

Moreover, Ahmad et al.

(27) have reported that certain NADH dependent nitrate reductase was involved in reduction of silver ions in case of *F. oxysporum*.

Generally, utilization of specific enzymes released by organisms such as fungi is so interesting as to be applied in emerging the genetically engineering microbes in order to overexpress specific reducing molecules and capping agents and thereby, control the size and shape of the biogenic nanoparticles.

The rapid synthesis of nanoparticles would be proper for developing a biological process for mass scale production. Furthermore, the extracellular synthesis would make the process simpler and easier for following processes. Moreover, extracellular biosynthesis of SNPs offers a great advantage over an intracellular process in the aspect of application. Since the nanoparticles formed inside the biomass would have required additional step of processing for release of the nanoparticles from the biomass by ultrasound treatment or by reaction with suitable detergents.

## Conclusion

In conclusion, the filamentous fungus *A. parasiticus* has successfully demonstrated potential for extracellular synthesis of fairly monodispersed, SNPs in the range of 2-50 nm.

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## References

1. Mohammed Fayaz A, Balaji K, Kalaichelvan PT, Venkatesan R. Fungal based synthesis of silver nanoparticles--an effect of temperature on the size of particles. Colloids Surf B Biointerfaces. 2009; 74(1): 123-126.
2. Mandal D, Bolander ME, Mukhopadhyay D, Sarkar G, Mukherjee P. The use of microorganisms for the formation of metal nanoparticles and their application. Appl Microbiol Biotechnol. 2006; 69(5): 485-492.
3. Mohanpuria P, Rana N, Yadav S. Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res. 2008; 10(3): 507-517.
4. Sastry M, Ahmad A, Khan M, Kumar R. Biosynthesis of metal nanoparticles using

fungi and actinomycete. Curr Sci. 2003; 85(2): 162-170.
5. Bhainsa KC, D'Souza SF. Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. Colloids Surf B Biointerfaces. 2006; 47(2): 160-4.
6. Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. Ecotoxicol Environ Saf. 2000; 45(3): 198-207.
7. Krolikowska A, Kudelski A, Michota A, Bukowska J. SERS studies on the structure of thioglycolic acid monolayers on silver and gold. Surf Sci. 2003; 532-535: 227-232.
8. Kumar A, Mandal S, Selvakannan P, Pasricha R, Mandale A, Sastry M. Investigation into the interaction between surface-bound alkylamines and gold nanoparticles. Langmuir. 2003; 19(15): 6277-6282.
9. Chandrasekharan N, Kamat P. Improving the Photoelectrochemical Performance of Nanostructured TiO2 Films by Adsorption of Gold Nanoparticles. J Phys Chem B. 2000; 104(46): 10851-10857.
10. Peto G, Molnar G, Paszti Z, Geszti O, Beck A, Guczi L. Electronic structure of gold nanoparticles deposited on SiOx/Si(100). Mater Sci Eng C Mater Biol Appl. 2002; 19(1-2): 95-99.
11. Duran N, Marcato PD, Alves OL, Souza GI, Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. J Nanobiotechnology. 2005; 3: 1-7.
12. Klaus T, Joerger R, Olsson E, Granqvist CG. Silver-based crystalline nanoparticles, microbially fabricated. Proc Natl Acad Sci U S A. 1999; 96(24): 13611-13614.
13. Arora S, Jain J, Rajwade JM, Paknikar KM. Cellular responses induced by silver nanoparticles: In vitro studies. Toxicol Lett. 2008; 179(2): 93-100.
14. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, Muniyandi J, et al. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids Surf B Biointerfaces. 2009; 74(1): 328-335.
15. Krumov N, Oder S, Perner-Nochta I, Angelov A, Posten C. Accumulation of CdS nanoparticles by yeasts in a fed-batch bioprocess. J Biotechnol. 2007; 132(4): 481-486.
16. Burda C, Chen X, Narayanan R, El-Sayed MA. Chemistry and properties of nanocrystals of different shapes. Chem Rev. 2005; 105(4): 1025-1102.
17. Balaji DS, Basavaraja S, Deshpande R, Mahesh DB, Prabhakar BK, Venkataraman A. Extracellular biosynthesis of functionalized silver nanoparticles by strains of Cladosporium cladosporioides fungus. Colloids Surf B Biointerfaces. 2009 ; 68(1): 88-92.
18. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, et al. Bioreduction of AuCl(4)(-) Ions by the Fungus, Verticillium sp. and Surface Trapping of the Gold Nanoparticles Formed. Angew Chem Int Ed Engl. 2001; 40(19): 3585-8.
19. Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, et al. Extracellular synthesis of gold nanoparticles by the fungus Fusarium oxysporum. Chembiochem. 2002; 3(5): 461-463.
20. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, et al. Fungus-Mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. Nano Lett. 2001;1(10): 515-519.
21. Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK, et al. Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnology. 2003; 14: 95-100.
22. Anil Kumar S, Abyaneh MK, Gosavi SW, Kulkarni SK, Pasricha R, Ahmad A, et al. Nitrate reductase-mediated synthesis of silver nanoparticles from AgNO3. Biotechnol Lett. 2007 ; 29(3): 439-445.
23. Kalimuthu K, Suresh Babu R, Venkataraman D, Bilal M, Gurunathan S. Biosynthesis of silver nanocrystals by Bacillus licheniformis. Colloids Surf B Biointerfaces. 2008; 65(1): 150-153.
24. Gade AK, Bonde P, Ingle AP, Marcato PD, Durán N, Rai MK. Exploitation of Aspergillus niger for Synthesis of Silver Nanoparticles. Journal of Biobased Materials and Bioenergy. 2008; 2(3): 1-5.
25. Verma VC, Kharwar RN, Gange AC. Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus Aspergillus clavatus. Nanomedicine (Lond). 2010; 5(1): 33-40.
26. Shaligram NS, Bule M, Bhambure R, Singhal RS, Singh SK, Szakacs G, et al. Biosynthesis of silver nanoparticles using aqueous extract from the compactin

producing fungal strain. Process Biochemistry. 2009; 44(8): 939-943.
27. Basavaraja S, Balaji SD, Lagashetty A, Rajasab AH, Venkataraman A. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. Mater Res Bull. 2008; 43(5): 1164-1170.
28. Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi AA. Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach. Process Biochemistry. 2007; 42(5): 919-923.
29. Dagenais TR, Keller NP. Pathogenesis of Aspergillus fumigatus in Invasive Aspergillosis. Clin Microbiol Rev. 2009; 22(3): 447-465.
30. Lasker BA. Evaluation of Performance of Four Genotypic Methods for Studying the Genetic Epidemiology of Aspergillus fumigatus Isolates. J Clin Microbiol. 2002; 40(8): 2886-2892.
31. Wild CP. Aflatoxin exposure in developing countries: the critical interface of agriculture and health. Food Nutr Bull. 2007; 28(2 Suppl): S372-380.
32. CLSI. Reference method for broth dilution antifungal susceptibility testing of yeasts. CLSI document M27-A3. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Instituts. 2008.
33. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan M, Kumar R, et al. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids Surf B Biointerfaces. 2003; 28(4): 313-318.
34. Evanoff DD Jr, Chumanov G. Synthesis and optical properties of silver nanoparticles and arrays. Chemphyschem. 2005 ; 6(7): 1221-1231.
35. Schmoll HJ, Kollmannsberger C, Metzner B, Hartmann JT, Schleucher N, Schoffski P, et al. Long-term results of first-line sequential high-dose etoposide, ifosfamide, and cisplatin chemotherapy plus autologous stem cell support for patients with advanced metastatic germ cell cancer: an extended phase I/II study of the German Testicular Cancer Study Group. J Clin Oncol. 2003; 21(22): 4083-4091.