CHAPTER - 3

ANTIMICROBIAL ACTIVITIES OF MYCOSYNTHESIZED SILVER AND GOLD NANOPARTICLES
3.1. Introduction

In the last decade, synthesis and characterization of nanoparticles has received considerable research interest across the globe. Nanomaterials have extensively been synthesized using physical, chemical and biological methods; among them, the bio-based protocols have now turned into promising alternative to the conventional methods of nanoparticle preparation (Krishnaraj et al., 2014).

Silver has long been known for its potential activity against a wide range of microbes including several bacteria and fungi. Several silver based compounds such as AgNPs have been used as antimicrobial agents to inhibit the growth of various human and plant pathogens (Nomiya et al., 2004). Narayanan and Sakthivel (2010) and Thakkar et al. (2010) have made a comprehensive review on biological synthesis of nanoparticles using microorganisms such as algae, bacteria, actinomycetes, fungi, yeasts and viruses. Of these, fungi are considered as a better resource for the extracellular synthesis of metal nanoparticles (Du et al., 2011; Muhsin et al., 2014). In recent years, the size and shape controlled synthesis methods are found to be more useful in boosting the chemico-physical properties of synthesized nanomaterials. To achieve good monodispersity, greater particle stability and excellent biocompatibility, it is imperative to optimize the parameters employed in the nanoparticle synthesis protocol (Krishnaraj et al., 2012; Singh et al., 2014).

Mounting evidences have clearly revealed the broad spectrum antibacterial activity of mycosynthesized AgNPs against multi drug resistant bacterial strains (Singh et al., 2014). Owing to the increase of fungal disease in various plants caused by different pathogens that are becoming more resistant to currently available drugs, it is very important that novel antifungal agents should be identified and developed. Qian et al. (2013) have demonstrated the antifungal activity of Epicoccum nigrum derived AgNPs against some dreadful clinical isolates including Candida spp., Cryptococcus neoformans, Aspergillus fumigatus, A. flavus, Fusarium solani and Sporothrix schenckii. Gajbhiye et al. (2009) have reported the synergistic antifungal activity of Alternaria alternata synthesized AgNPs in combination with fluconazole. In an interesting study, Pereira et al. (2014) have compared the antifungal activity of fungal-derived AgNPs with that of chemically derived nanoparticles against Trichophyton rubrum. However, the effects of AgNPs against plant pathogens have received only marginal attention and most of these
studies have mainly focused chemically made nanoparticles (Kim et al., 2008; Kim et al., 2012). Some researchers have shown the antibacterial activity of plant extract synthesized AgNPs against few phytopathogenic bacterial strains (Paulkumar et al., 2014; Velmurugan et al., 2013). In a similar way, Krishnaraj et al. (2012) have shown the antifungal activity of Acalypha indica synthesized AgNPs against six different plant pathogenic fungal species. The application of nano-sized silver particles as antimicrobial agents has become very common as the technological advances make their production even more economical. One of the potential applications of silver in the field of agricultural biotechnology is to manage plant diseases (Clement and Jarrett, 1994).

Due to the emergence and increase of microbial organisms resistant to various antibiotics and the continuing emphasis on health-care costs, several researchers are tried to come across new and potent antimicrobial agents with reduced resistance and cost. Such kind of problems and their needs have resulted in resurgence in the application of silver and silver based nanostructures which were known to exhibit broad spectrum activity and also reducing their susceptibility to bring upon microbial resistance than the commercial antibiotics (Jones et al., 2004). This chapter deals with the antibacterial, anticandidal and antifungal activities of A. terreus synthesized AgNPs and AuNPs. Further, the mode of action of AgNPs was carried out using membrane leakage studies and electron microscopic characterization.
3.2. Materials and methods

3.2.1. Pathogenic test strains

In this study, a total of 10 different human pathogenic strains (7 bacterial and 3 yeast pathogens) *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 2719 (Gram negative), *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 6633, methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus* ATCC 29736 (Gram positive), *Candida albicans* ATCC 2091, *Candida parapsilosis* MTCC 1744 and *Candida glabrata* MTCC 3019 were used as test organisms. The above test strains were procured from the American Type Culture Collection centre (ATCC, Manassas, VA, USA) and Microbial Type Culture Collection centre (MTCC, Chandigarh, India). To study the antifungal activity of nanoparticles, the plant pathogenic fungi such as *Curvularia lunata*, *Colletotrichum* sp., *Aspergillus flavus* and *Penicillium* sp. were obtained from the Fungal Culture Collection Centre, Centre for Advanced Studies in Botany, University of Madras, Chennai, India.

3.2.2. Antimicrobial activity of myco-derived silver and gold nanoparticles

3.2.2.1. Antibacterial activity of silver and gold nanoparticles

**Mueller Hinton Broth (Himedia) Composition (g/ L)**

- Beef extract - 300 g
- Casein acid hydrolysate - 17.50 g
- Starch - 1.50 g
- pH - 7.4 ± 0.1

**Mueller Hinton Agar (Himedia) Composition (g/ L)**

- Beef extract - 300 g
- Casein acid hydrolysate - 17.50 g
- Starch - 1.50 g
- Agar - 17.00 g
- pH - 7.4 ± 0.1

In this study, seven different human pathogenic strains such as *Escherichia coli* ATCC 8739, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, *B. subtilis*, methicillin-resistant *Staphylococcus aureus* and *S. aureus* were used as test organisms and were grown in
Mueller-Hinton agar (MHA) medium. Agar well diffusion assay was performed to determine the antimicrobial activity of mycosynthesized AgNPs and AuNPs.

3.2.2.1.1. Preparation of McFarland turbidity standard (0.5)

Prepare 1.175% (w/v) of BaCl₂ using double distilled water. In a volumetric flask, 85 mL of conc. H₂SO₄ was taken and 0.5 mL of BaCl₂ (1.175%, w/v) was added drop wise and the final volume was made up to 100 mL using conc. H₂SO₄. The preparation was mixed properly and verified by measuring the absorbance at 625 nm using UV-vis spectrophotometer. The preparation was sealed and maintained until further use.

3.2.2.1.2. Preparation of inoculum

The test strains were inoculated in sterile Mueller Hinton broth (MHB) separately and incubated at 37 °C for overnight. For the antimicrobial evaluation of nanoparticles, an inoculum density of approximately 10⁶ cell/ mL was prepared which is equivalent to 0.5 McFarland standard.

3.2.2.1.3. Well diffusion method

The freshly prepared inoculum of the test strains was used for the preparation of lawn culture using sterile swab on MHA plates and then four wells of 4 mm in diameter were made using sterile cork borer. Then, 25 µL of AgNPs (1 mg/ mL), AuNPs (1 mg/ mL) and freshly prepared silver nitrate solution (1 mM) and gold chloride solution (1 mM) were loaded into the respective well of the Petri plates. Mycelial free extract was used to compare the antimicrobial activity of synthesized nanoparticles; also, streptomycin (1 mg/ mL) was used as positive control. After inoculation, the plates were incubated at 37 °C for 24 h and then zone of inhibition (ZOI) was measured in terms of millimeter. These assays were carried out in triplicate.

3.2.2.1.4. Minimum inhibitory concentration (MIC) of silver nanoparticles against bacterial pathogens

The minimum inhibitory concentration (MIC) of AgNPs was determined by broth microdilution method given by the Clinical Laboratory Standards Institute (CLSI) using 96-well microtitre plates (CLSI, 2007). Two-fold serial dilutions of known concentrations of AgNPs (100, 50, 25, 12.5, 6.25, 3.12, 1.5, 0.78, 0.39 and 0.19 µg/ mL) and antibiotics were made using Mueller-Hinton broth with appropriate control. To each well, overnight
fresh culture of 5 μL inoculum (≈5 × 10^5 CFU/ mL) was added and the plates were incubated at 37 °C for 24 h. The lowest concentration which completely inhibited the growth of microbes was recorded as MIC. From the above assay, a loopful of inoculum was taken from each well showing no visual growth after incubation and spotted onto MHA plates to validate the MIC assay. All the experiments were performed in triplicate.

3.2.2.2. Anticandidal activity of myco-derived silver and gold nanoparticles

3.2.2.2.1. Well diffusion assay

The anticandidal activity of AgNPs and AuNPs was carried out using the modified Kirby-Bauer method (Bauer et al., 1966) against C. albicans, C. parapsilosis and C. glabrata. Freshly prepared overnight culture suspension (approximately 10^5 cells per mL) was used for the preparation of lawn culture using sterile swab on MHA plate. Four wells of 4 mm in diameter were made using sterile cork borer. Then, 25 µL of AgNPs (1 mg/ mL), AuNPs (1 mg/ mL) and freshly prepared silver nitrate solution (1 mM) and gold chloride solution (1 mM) were loaded into the respective well of the Petri plates. Mycelial free extract was used to compare the anticandidal activity of synthesized nanoparticles; also, fluconazole (1 mg/ mL) was used as positive control. After inoculation, the plates were incubated at 37 °C for 24 h and then ZOI was measured in millimeter. This experiment was repeated thrice for reproducibility.

3.2.2.2.2. MIC of silver nanoparticles against Candida spp.

Anticandidal activity of mycosynthesized AgNPs was examined by determining the MIC in accordance with CLSI methodology (2008) using Yeast Nitrogen Base Glucose (YNBG) medium. Two-fold serial dilutions of known concentrations of AgNPs (100, 50, 25, 12.5, 6.25, 3.12, 1.5, 0.78, 0.39 and 0.19 µg/ mL) were prepared using sterile YNBG medium and added to the respective wells of the 96 well plate with appropriate controls. Earlier, pathogenic strains of Candida spp. were cultured into YNBG broth before 24 h and an inoculum density of approximately 1.5 ± 1.0 × 10^3 cell/mL was prepared which is equivalent to 0.5 McFarland standard. Then 100 μL suspension of each strain of Candida spp. was added into individual well and incubated at 35 °C for 24 h. The MIC of AgNPs was determined visually as the lowest concentration that caused significant growth diminution levels (CLSI, 2008a and b). All the experiments were performed in triplicate.
3.2.2.3. Mode of action of silver nanoparticles against bacterial and yeast pathogens

3.2.2.3.1. Protein leakage (Kim et al., 2011)

The protein leakage from bacterial cells during the treatment of AgNPs was detected using Bradford’s protein assay (Bradford, 1976). Briefly, the test strains were inoculated aseptically in sterile nutrient broth and incubated at 37 °C for overnight. Five mL of overnight suspension of each test strain (10^5 cells per mL) was treated with mycosynthesized AgNPs at MIC and incubated for 6 h at 37 °C. At the end of incubation, 1 mL of the culture sample was collected and centrifuged at 6000 rpm for 15 min at 4 °C. The supernatant was used for the determination of protein with Bradford’s assay reagent and the optical density was measured at 595 nm. For each sample, 200 µL of the supernatant was mixed with 800 µL of Bradford reagent and then incubated for 10 min at room temperature. The optical density was measured at 595 nm using Hitachi-U 2900 spectrophotometer to determine the concentration of protein in the respective sample. Bovine serum albumin (BSA) was used as a standard protein and the experiments were done in triplicate with appropriate controls.

**Bradford reagent**

<table>
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<th>Component</th>
<th>Concentration</th>
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<tr>
<td>Coomassie Brilliant Blue G-250</td>
<td>- 100 mg</td>
</tr>
<tr>
<td>Ethanol</td>
<td>- 95%</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>- 85% (w/v)</td>
</tr>
</tbody>
</table>

One hundred milligrams of Coomassie Brilliant Blue G-250 was mixed with 50 mL of 95% ethanol. Then 100 mL of 85% (w/v) phosphoric acid was added and the final volume was made up to 1 liter; the preparation was filtered through Whatman No. 1 filter paper before use. The prepared reagent was stored in brown color bottle.

3.2.2.3.2. Nucleic acid leakage assay (Tiwari et al., 2008)

For the evaluation of nucleic acid leakage, AgNPs treated cell suspension was studied at different time intervals. Briefly, bacterial inoculum size of 10^5 cells/ mL was prepared using 24 h old culture, treated with AgNPs of MIC and incubated at 37 °C for 6 h. Five mL of cell suspension was collected at different incubation time and the nucleic acid leakage was determined by measuring the absorbance at 260 nm (A_{260}) using UV–visible spectrophotometer. Similarly, the above mentioned method was also used to
determine nucleic acid leakage from *Candida* spp. and the experiments were done in triplicate with appropriate controls.

### 3.2.2.3.3. Potassium leakage analysis (Tiwari *et al.*, 2008)

For potassium (K⁺) leakage analysis, 10 mL of overnight grown bacterial test strains (10⁵ cells/ mL) were treated with mycosynthesized AgNPs at MIC and incubated at 37 °C for 6 h. Five mL of sample was withdrawn from each test mixture at the end of the incubation and centrifuged at 5000 rpm for 15 min. Then the resulting supernatant was used for the determination of K⁺ ions by atomic absorption spectroscopy and the experiments were done in triplicate with appropriate controls.

### 3.2.2.3.4. Scanning Electron Microscopic (SEM) studies

To understand the mode of action of AgNPs on human pathogenic microorganisms, SEM analysis was carried out using the method of Ma *et al.* (2011) with slight modifications. For this study, the strains *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, *B. subtilis*, Methicillin-resistant *S. aureus*, *S. aureus* and *C. albicans* were selected based on their susceptibility against mycosynthesized AgNPs. Five 5 mL of overnight grown pathogenic strain (10⁵ cells/ mL) was treated with mycosynthesized AgNPs at MIC and incubated for 6 h at 37 °C. After incubation, 1.0 mL of each test pathogen treated with AgNPs was withdrawn, centrifuged and washed thrice with PBS buffer. The microbial cells were then fixed with 2.5% glutaraldehyde for 4 h and washed twice with PBS. After fixation, the cells were concentrated by centrifugation at 4000 rpm for 10 min, followed by washing twice with PBS buffer. Subsequently, the cells were gradually dehydrated with increasing concentrations of ethanol 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% for 5 min each. After drying, the cells were observed under SEM (Ma *et al.*, 2011).

**Potato Dextrose Agar** (Himedia) Composition (g/ L)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
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<tbody>
<tr>
<td>Potatoes, infusion</td>
<td>200 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>pH</td>
<td>5.6 ± 0.2</td>
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3.2.2.4. Antifungal activity of myco-derived silver and gold nanoparticles (Aguilar-Méndez et al., 2011)

The antifungal activity of mycosynthesized AgNPs and AuNPs was evaluated using the method of Aguilar-Méndez et al. (2011). Briefly, fungal strains such as Curvularia lunata, Colletotrichum sp., Aspergillus flavus and Penicillium sp. were cultured in PDA medium and incubated at 27 °C for 7 days. Different concentrations of (10, 20, 30, 40 and 50 µg AgNPs per mL of PDA) A. terreus synthesized AgNPs in colloidal solution were mixed to the sterilized PDA plates along with appropriate control. Using sterile cork borer, mycelial plugs of 3 mm diameter of each fungus was punched out and spotted on the center of the PDA plates amended with AgNPs. The plates were then incubated for 6 days at 27 °C and the measurements of the colony was performed for both the control and AgNPs treated plates. The antifungal index (AI) was determined at the end of the experiment using the following equation,

\[
\text{AI} (%) = [1-(D_1/D_2)] \times 100
\]

Where,

- AI is the antifungal index (%),
- \(D_1\) is the diameter of colony observed in the test plate amended with AgNPs (cm),
- \(D_2\) is the diameter of colony observed in the control plate without AgNPs (cm).

3.2.3. Statistical analysis

All the experiments were performed in triplicate and the data were expressed as means ± standard deviation.
3.3. Results and discussion

3.3.1. Antimicrobial activity of silver and gold nanoparticles synthesized from A. terreus

3.3.1.1. Antibacterial activity of mycosynthesized nanoparticles

Antibiotic resistance by various pathogenic bacteria is of major concern and hence, the researchers are focusing on the development of new antibacterial agents. In the present scenario, AgNPs as antibacterial agents have come up as a promising candidate in the field of medicine (Duran et al., 2007). The extremely small size of nanoparticles exhibits enhanced or different properties when compared with the bulk material. The metal particles have a distinctive advantage compared to the conventional chemical antimicrobial agents that are the greatest concern of causing multidrug resistance (Hamouda et al., 2001). Silver ions and silver based components are well known for their potential antimicrobial properties (Furno et al., 2004).

In the preliminary screening assay, AgNPs synthesized from A. terreus showed excellent antimicrobial activity than the corresponding AuNPs. Consequently, AgNPs alone were used for further studies. The mycosynthesized AgNPs showed superior antimicrobial activity against all the tested human pathogens and the data were presented in Figs. 3.1a and b and Table 3.1. The AgNPs synthesized from A. terreus exhibited potent inhibitory activity against the pathogenic bacterium, E. coli with a clear inhibition zone of 22.47 mm at the concentration of 1 mg/ mL. Similarly, AgNPs significantly inhibited the growth of E. faecalis (19.47 mm), P. aeruginosa (17.13 mm), B. subtilis (16.43 mm), methicillin-resistant S. aureus (16.15 mm), K. pneumoniae (16.13 mm) and S. aureus (14.27 mm) at a test concentration of 1 mg/ mL. Conversely, mycelial free extract did not show any inhibition zone against all the tested pathogens. Although silver nitrate showed a moderate activity, but due to its strong cytotoxic nature on human cells, its effect was found to be insignificant.

3.3.1.2. Minimum Inhibitory Concentration (MIC) of silver nanoparticles

MIC was recorded as the lowest concentration at which no visible growth of test pathogens was observed (Table 3.2). In this present study, AgNPs showed appreciable antimicrobial activity against E. coli, E. faecalis and methicillin-resistant S. aureus exhibiting MIC of 3.12 µg/ mL. Similarly, 6.25 µg/ mL was found to be MIC for
P. aeruginosa, K. pneumoniae and B. subtilis while 12.5 µg/ mL was proved as MIC against S. aureus. Additionally, the obtained results were validated using MHA plates which showed no organisms in it.

Similarly, other soil fungi have also exhibited considerable antimicrobial activity against dreadful human pathogens (Gade et al., 2008; Li et al., 2012; Muhsin and Hachim, 2014). For instance, AgNPs biologically synthesized from the soil fungus A. terreus showed good antimicrobial activity against clinical isolates including Gram positive, Gram negative bacteria and few fungal species (Li et al., 2012). However, the zone of inhibition exhibited by this soil fungus was found to be lower than the present report, which showed superior antimicrobial activity using same fungus isolated from the different environment. The higher activity of AgNPs was probably due to the proteins, enzymes or other biocompatible materials adsorbed on the surface of the synthesized nanoparticles. These biocompatible materials may have augmented the antimicrobial property of synthesized AgNPs to some extent. Presence of amide and amine groups along with the mycosynthesized AgNPs, which evidently suggest that the released extracellular proteins could have assisted the formation and the possible stabilization of AgNPs in the aqueous medium. Therefore, it is apparent that biomolecules especially proteins have functionalized the synthesized nanomaterials and in turn increased the antimicrobial properties of synthesized nanoparticles. Because of their greater biocompatibility, the mycosynthesized AgNPs have displayed pinnacle antimicrobial activity than the standard antibacterial drug, streptomycin (Table 3.1). It is also observed that the mycosynthesized AgNPs have displayed significantly higher antimicrobial activity against Gram negative bacteria than the Gram positive ones. Contrary to the present and earlier reports, Gade et al. (2008) have shown that AgNPs synthesized extracellularly from the soil fungus A. niger exhibited superior antibacterial activity against Gram positive S. aureus than the Gram negative E. coli. This is more likely due to the difference in the cell wall composition between these two groups.

Antibacterial activity of Fusarium acuminatum synthesized AgNPs was investigated against various human pathogens. AgNPs showed excellent antibacterial activity against both multidrug resistant pathogens and other bacterial pathogens. The maximum activity was observed against S. aureus followed by S. epidermidis, S. typhi and E. coli (Ingle et al., 2008).
Maliszewska and Sadowski (2009) have explored the antibacterial property of extracellularly synthesized AgNPs using two *Penicillium* strains against various human pathogens by well diffusion method. AgNPs synthesized from *Penicillium* sp. K1 showed pronounced antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* with an inhibition clear zone of 15, 13, 14 and 16 mm, respectively.

Shahverdi et al. (2007) have studied the combinational effects of *Klebsiella pneumoniae* synthesized AgNPs along with conventional antibiotics against *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity was found to be increased when AgNPs were combined with commercial antibiotics vancomycin, amoxicillin and penicillin G particularly against *S. aureus* than the *E. coli*. The antimicrobial effect of AgNPs against different Gram positive and Gram negative pathogens was investigated by several researchers. In all cases, AgNPs exhibited discrete antibacterial activities against bacterial pathogens (Thirumurugan et al., 2009; Nanda and Saravanan, 2009; Naqvi et al., 2013).

### 3.3.1.3. Anticandidal activity of silver nanoparticles

The fungistatic activity of mycosynthesized AgNPs was performed against pathogenic strains of *C. albicans*, *C. parapsilosis* and *C. glabrata*. The mycosynthesized AgNPs showed superior antimicrobial activity against *C. albicans* compared to the other strains of *Candida* spp. (Fig. 3.2 and Table 3.3). The AgNPs synthesized from *A. terreus* showed an inhibitory activity against the fungal pathogen, *Candida albicans* with a clear inhibition zone of 18.33 mm at the concentration of 1 mg/mL. On the contrary, AgNPs exhibited moderate activity against *C. parapsilosis* (13.27 mm) and *C. glabrata* (14.63 mm) and the zone of imbibitions was found to be lesser than that of the commercially tested antibiotic, fluconazole. Similar to the antibacterial assay, the mycelial free extract did not show any inhibition zone against all the tested pathogens; whereas, silver nitrate showed a moderate activity. The MIC of AgNPs was performed against *Candida* spp. at a test concentration ranging from 100 to 0.19 µg/mL. The obtained results showed that the mycosynthesized AgNPs inhibited *C. albicans* with a lower MIC value of 3.12 µg/mL. The other yeast pathogens *C. parapsilosis* and *C. glabrata* were found to be less sensitive and recorded an MIC of 12.5 µg/mL (Table 3.4).
Kim and co-workers (2009) have investigated the antifungal activity of AgNPs against *Candida albicans* and the MIC was found to be 2 μg/mL, similar to our results. They have also studied the mode of action of AgNPs using transmission electron microscopy, which revealed the formation of pit like structure in the cell wall and also pores in the plasma membrane. Similar work was performed by Monteiro *et al.* (2012) who have demonstrated the size-dependent effects of silver nanoparticle colloidal suspensions against *Candida* spp. They have reportedly synthesized AgNPs with three different average sizes 5, 10 and 60 nm and showed the fungicidal activity at very low concentrations between 0.4 – 3.3 μg/mL.

Few studies have reported the antifungal activities of AgNPs against *C. albicans*. However, the MIC was found to be different from the ones we found in this present work (Kim *et al.*, 2009; Hwang *et al.*, 2012; Nasrollahi *et al.*, 2011; Monteiro *et al.*, 2011).

On the contrary, Panáček *et al.* (2009) have reported an MIC value of 0.21 mg/L of AgNPs against yeast pathogens. They have also enhanced the antifungal activity of AgNPs by stabilizing it with sodium dodecyl sulfate and showed the MIC equal to 0.05 mg/L, which is comparatively better than the previously synthesized nanoparticles.

### 3.3.1.4. Mechanism of action of silver nanoparticles on microbial cells

#### 3.3.1.4.1. Protein leakage

Protein leakage from the membranes of bacterial and yeast cells treated with AgNPs was almost the same as that from cells in the control group at the beginning of the experiment (0 h). However, at 3 h after treatment, the treated cells leaked nearly 35 to 43 μg/mL of protein while the untreated cells maintained the same level of leakage.

After 6 h of incubation, the protein leakage from AgNPs treated cells considerably increased but there was no change in the amount of protein leakage from the untreated cells. Protein leakage from AgNPs treated cells was found to be significantly higher than that of cells in the positive control group treated with streptomycin. Interestingly, *E. faecalis* cells showed highest amount of protein leakage (99 μg/mL) followed by *P. aeruginosa* and *B. subtilis* (89 μg/mL), MRSA (86 μg/mL), *K. pneumoniae* (85 μg/mL), *S. aureus* (84 μg/mL), *E. coli* (80 μg/mL), *C. albicans* (79 μg/mL), *C. parapsilosis* (76 μg/mL) and *C. glabrata* (75 μg/mL) at 6 h after treatment with AgNPs (Figs. 3.3a and b). Similarly, the pathogens treated with conventional antibiotic
also showed increased protein leakage with increasing incubation time. The maximum amount of leakage was observed in *C. albicans* (63 µg/ mL) in case of yeast pathogen when treated with fluconazole and in case of bacteria, *P. aeruginosa* (66 µg/ mL) when treated with streptomycin. However, this was found to be lesser than the AgNPs treated group.

### 3.3.1.4.2. Nucleic acid leakage

The leakage of intracellular material after exposure to AgNPs at different time interval was assessed by measuring the optical density of AgNPs treated cell suspension at 260 nm (OD<sub>260</sub>). Initially, the nucleic acid leakage from nanoparticles treated cells and the control cells was almost the same. The bacterial cells exposed to AgNPs treatment gave rise to the increased release of nucleic acids, which was more marked with *B. subtilis* followed by MRSA and *E. faecalis* at 3 and 6 h after treatment with AgNPs (Figs. 3.4a and b). Similarly, maximum release of nucleic acid was found in AgNPs treated against *C. albicans* compared to the other two yeast pathogens, *C. parapsilosis* and *C. glabrata*. However, the untreated cells and the antibiotic treated bacterial and yeast cells showed lesser OD values than the AgNPs treated group.

### 3.3.1.4.3. Potassium leakage

The potassium (K<sup>+</sup>) leakage analysis determines the leakage of intracellular K<sup>+</sup> ions responsible for the maintenance of membrane potential of microbial cells, which in turn maintains turgor pressure (Bouhdid *et al.*, 2009; Lambert and Hammond, 1973). The results showed that there was an increase in the loss of intracellular K<sup>+</sup> ion with increasing incubation time. The maximum K<sup>+</sup> ion leakage was observed in *K. pneumoniae* (25.4 µg/ mL) followed by *E. coli* (22.4 µg/ mL) and *C. albicans* (21.1 µg/ mL) at 6 h after treatment with AgNPs (Figs. 3.5a and b). Similarly, the other tested pathogenic strains showed intracellular K<sup>+</sup> leakage between 15.8 and 19.6 µg/ mL compared to the antibiotic treated cells (12.3 to 20.1 µg/ mL).

Although the bactericidal effects of AgNPs have extensively been studied, the mechanism of action is not yet fully understood. However, fewer hypotheses have been proposed to give some insights on the antibacterial action of nano-sized silver particles. The bactericidal activity of AgNPs is relied much on the size of the particles (Morones *et al.*, 2005). Metallic nanoparticles have exhibited increased activity due to their large
surface to volume ratios and crystallographic surface structures. It is well-established that AgNPs of less than 20 nm diameter easily get attached to the sulfur-containing proteins of bacterial cell membranes, thus leading to greater permeability of the membrane (Gogoi et al., 2006; Morones et al., 2005; Sondi and Salopek-Sondi, 2004). In this present study, the ability of AgNPs to damage the bacterial membrane was evaluated by measuring the release of intracellular contents. The AgNPs treated bacterial cells showed considerably increased amount of protein leakage compared to the control group (Figs. 3.3a and b); this indicates that AgNPs could increase permeability (Kim et al., 2011) and affect membrane transport (Sondi and Salopek-Sondi, 2004) due to the serious damage of cell membrane structure. This result is found to be consistent with the nucleic acid leakage assay, which showed increased OD values in the AgNPs treated bacterial cells than the control cells (Figs. 3.4a and b). The increase in OD260 indicates the leakage of intracellular nucleic acids and consequently, reflects a loss in membrane integrity (Sampathkumar et al., 2003; Álvarez-Ordóñez and Prieto, 2010). This leakage of intracellular components also suggests that AgNPs may cause formation of pores in the cytoplasmic membrane (Álvarez-Ordóñez et al., 2013). The amount of protein and nucleic acid released after treatment with AgNPs was found to be increased with increasing incubation time and was dependent on the AgNPs concentration and bacterial strains. Similar to our results, Sondi and Salopek-Sondi (2004) have observed the formation of “pits” in the bacterial cell wall, membrane damage and increased permeability, when E. coli was challenged with AgNPs. Amro et al. (2000) have also reported a progressive release of LPS molecules and membrane proteins following increased membrane permeability.

The present results showed an increase in the release of K+ ions after treatment with AgNPs, suggesting that there was an early damage in the cytoplasmic membrane of bacterial and yeast strains. This study also confirms the cell membrane damage and correlates with the earlier observation such as protein and nucleic acid leakage.

Several researchers have proposed that the silver ions may bind to the functional groups of enzymes which in turn cause the release of K+ ions from the microbial cells. Thus, it is clear that cytoplasmic associated enzymes would be the major targeted site for silver ions (Rayman and Sanwal, 1972; Schreurs and Rosenberg, 1982). The current results were found to be consistent with the earlier reports and AgNPs interaction with microbial cells changed the membrane permeability that lead to the release of cytoplasmic cations like K+ (Kim et al., 2011; Rastogi et al., 2014; Jung et al., 2008).
Some researchers have also proved that, in addition to their role in enzymatic effect, silver ions were deposited in the cell wall granules and vacuoles during their action against microbes. Silver ions damaged the cell envelope and target the DNA bases rather than the phosphate groups of nucleic acids, however, the exact lethal of AgNPs remains elusive (Thurman and Gerba, 1989; Rahn and Landry, 1973).

3.3.1.5. SEM analysis

SEM analysis was performed to understand the mode of action of AgNPs on human pathogenic microorganisms. This study revealed the accumulation of AgNPs over the cell wall, suggesting that nanoparticles entered through pores present on the cell membranes. In addition, cell wall breakage was also observed due to the action of AgNPs (Figs. 3.6a-h).

It has also been reported that AgNPs have the ability to enter inside the bacterial cell and therefore they react with sulfur-containing proteins in the interior of the cell, as well as with phosphorus-containing compounds such as DNA (Morones et al., 2005). Sondi and Salopek-Sondi (2004) have observed the AgNPs accumulation in E. coli bacterial membrane. In another study, Morones et al. (2005) have clearly demonstrated the presence of AgNPs on the surface of bacterial cell membrane and also inside the bacteria. Similarly, our SEM studies revealed the accumulation of AgNPs over the cell wall, suggesting that nanoparticles entered through pores present on the cell membrane; in addition, cell wall breakage was also observed (Figs. 3.6a-h). This implies that AgNPs could penetrate inside the bacteria and cause further damage by interacting with DNA as reported by Morones et al. (2005). The changes occurred in the microbial cells after treatment with AgNPs have led to the increased permeability, which eventually resulted in cell death (Ma et al., 2011; Pal et al., 2007). On the basis of our findings, it is here speculated that AgNPs have a dual mode of action. Firstly, they exert a physical effect on bacterial membranes with the formation of pores, which subsequently results in the increased release of intracellular components such as protein, nucleic acid and K+ ions.

3.3.1.6. Antifungal activity of mycosynthesized silver nanoparticles

Earlier reports have proved that AgNPs had inhibitory effects against several filamentous fungi (Xia et al., 2014; Jo et al., 2009). The effect of mycosynthesized AgNPs against different plant pathogenic fungi (Curvularia lunata, Colletotrichum sp.,
Aspergillus flavus and Penicillium sp.) was investigated by amending AgNPs into the fungal growth medium. The results (Figs. 3.7a-d and Table 3.5) showed that the maximum antifungal indices of AgNPs was recorded against Penicillium sp. of 60.16%, followed by Aspergillus flavus (49.91%), Curvularia lunata (47.24%) and Colletotrichum sp. (39.98%) when concentration was maintained at 50 µg/mL. Among the four different plant pathogen tested, the mycosynthesized AgNPs exhibited significant growth inhibition at different concentration (10, 20, 30, 40 and 50 µg/mL) against Penicillium sp. followed by Curvularia lunata, Aspergillus flavus and Colletotrichum sp. The result clearly showed that increase in the AgNPs concentration increased the antifungal index of tested plant pathogens.

Similar investigations were also performed by Krishnaraj et al. (2012) who have synthesized AgNPs using leaf extracts of Acalypha indica. They have studied the antifungal activity of AgNPs against 6 different plant pathogenic fungi, Alternaria alternata, Sclerotinia sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, Botrytis cinerea and Curvularia lunata and reported that 15 mg concentration of AgNPs showed good inhibitory activity against all the tested fungal pathogens. Qian et al. (2013) have also investigated the extracellular biosynthesis of AgNPs using Epicoccum nigrum and studied the antifungal activity of AgNPs against pathogenic fungi.

The antifungal activity of AgNPs against sclerotium-forming pathogens such as Sclerotinia sclerotiorum, S. minor and Rhizoctonia solani was investigated by Min and his co-workers (Min et al., 2009). The maximum antifungal activity of AgNPs was observed against R. solani followed by S. sclerotiorum and S. minor. This was further confirmed by microscopic studies, which showed damaged and collapsed hypae. Due to their unique physic-chemical properties and large surface area, AgNPs have effectively interacted with microorganisms and damaged the cell membrane structure leading to cell death (Rai et al., 2009).

These results were highly correlated with the earlier studies and confirmed the antifungal effects of AgNPs against various fungal pathogens (Gajbhiye et al., 2009; Aguilar-Méndez et al., 2011; Thenmozhi et al., 2013; Senthil Kumar and Sudha, 2013).
3.4. Summary

1. The biologically synthesized AgNPs and AuNPs were investigated for their potential antibacterial, anticandidal and antifungal activities.

2. In the preliminary screening assay, AgNPs synthesized from *A. terreus* showed excellent antimicrobial activity than the corresponding AuNPs. Consequently, AgNPs alone were used for further studies.

3. The mycosynthesized AgNPs exhibited superior antibacterial activity against all the tested pathogens. The maximum activity was found in *E. coli* with a clear inhibition zone of 22.47 mm, followed by *E. faecalis* (19.47 mm) and *P. aeruginosa* (17.13 mm) at a test concentration of 1 mg/ mL.

4. The MIC of AgNPs was found to be 3.12 µg/ mL against *E. coli, E. faecalis* and methicillin-resistant *S. aureus*. For *P. aeruginosa, K. pneumoniae* and *B. subtilis*, it was 6.25 µg/ mL.

5. Similarly, the mycosynthesized AgNPs also showed good anticandidal activity against all the tested pathogens. The maximum activity was observed against *C. albicans* with a clear zone of inhibition of 18.33 mm followed by *C. parapsilosis* (13.27 mm) and *C. glabrata* (14.63 mm) at a concentration of 1 mg/ mL.

6. The MIC of AgNPs was found to be 3.12 µg/ mL against *C. albicans*. In case of *C. parapsilosis* and *C. glabrata*, it was 12.5 µg/ mL.

7. The mechanism of action of AgNPs on microbial cells was studied using membrane leakage studies (protein, nucleic acid and potassium leakage) and electron microscopic characterization.

8. The maximum protein leakage was found in AgNPs treated *E. faecalis* cells (99 µg/ mL) followed by *P. aeruginosa, B. subtilis* (89 µg/ mL) and MRSA (86 µg/ mL) at 6 h after treatment. In case of *Candida* spp., the maximum protein leakage was observed in *C. albicans* (79 µg/ mL) followed by *C. parapsilosis* (76 µg/ mL) and *C. glabrata* (75 µg/ mL) at 6 h after treatment.

9. The maximum nucleic acid leakage was recorded in AgNPs treated *B. subtilis* cells followed by MRSA and *E. faecalis* at 3 and 6 h after incubation. In case of *Candida* spp., the maximum release of nucleic acid was found in *C. albicans* compared to other tested pathogens.
10. The maximum release of K\(^+\) ions was recorded in AgNPs treated *K. pneumoniae* (25.4 µg/ mL) followed by *E. coli* (22.4 µg/ mL) and *C. albicans* (21.1 µg/ mL) at 6 h after treatment.

11. The SEM analysis of AgNPs treated bacterial as well as candidal cells clearly revealed the accumulation of AgNPs over the cell wall. In addition, cell wall breakage was also observed.

12. The mycosynthesized AgNPs showed excellent antifungal activity against all the tested plant pathogenic fungi. The maximum antifungal index was recorded in *Penicillium* sp. with 60.16% followed by *C. lunata* (47.24%), *Colletotrichum* sp. (39.98%) and *A. flavus* (49.91%) at 50 µg/ mL of AgNPs.
3.5. References


Fig. 3.1a: Antibacterial activity of mycosynthesized AgNPs

Antibacterial activity of *A. terreus* synthesized silver nanoparticles against human pathogenic organisms by well diffusion assay; (a) *E. coli*; (b) *K. pneumoniae*; (c) *P. aeruginosa*; (d) *B. subtilis*. Each well was treated with (i) Mycelial free extract (25 µL), (ii) Silver nitrate (1 mM), (iii) Silver nanoparticles (1 mg/ mL) and (iv) Streptomycin (1 mg/mL).
Antibacterial activity of A. terreus synthesized silver nanoparticles against human pathogenic organisms by well diffusion assay; (e) E. faecalis; (f) S. aureus; (g) MRSA (Methicillin-resistant S. aureus). Each well was treated with (i) Mycelial free extract (25 µL), (ii) Silver nitrate (1 mM), (iii) Silver nanoparticles (1 mg/mL) and (iv) Streptomycin (1 mg/mL).
Fig. 3.2: Anticandidal activity of mycosynthesized AgNPs

Anticandidal activity of *A. terreus* synthesized silver nanoparticles against human pathogenic organisms by well diffusion assay; (a) *C. albicans*; (b) *C. glabrata*; (c) *C. parapsilosis*. Each well was treated with (i) Mycelial free extract (25 µL), (ii) Silver nitrate (1 mM), (iii) Silver nanoparticles (1 mg/mL) and (iv) fluconazole (1 mg/mL).
Fig. 3.3a: Protein leakage analysis of AgNPs treated bacterial strains

Measurement of protein leakage of AgNPs treated bacterial strains; the bacterial strains were treated with mycosynthesized AgNPs at MIC; the values represent mean ± standard deviation of three independent experiments; the different colors of the bars represent the bacterial strains treated with mycosynthesized AgNPs and Streptomycin at different time intervals; untreated cells were also shown in the figure.

Fig. 3.3b: Protein leakage analysis of AgNPs treated Candida spp.

Measurement of protein leakage of AgNPs treated Candida spp.; the Candida spp. were treated with mycosynthesized AgNPs at MIC; the values represent mean ± standard deviation of three independent experiments; the different colors of the bars represent the bacterial strains treated with mycosynthesized AgNPs and Fluconazole at different time intervals; untreated cells were also shown in the figure.
Fig. 3.4a: Nucleic acid leakage analysis of AgNPs treated bacterial strains

Measurement of nucleic acid leakage of AgNPs treated bacterial strains; the bacterial strains were treated with mycosynthesized AgNPs at MIC; the values represent mean ± standard deviation of three independent experiments; the different colors of the bars represent the bacterial strains treated with mycosynthesized AgNPs and Streptomycin at different time intervals; untreated cells were also shown in the figure.

Fig. 3.4b: Nucleic acid leakage analysis of AgNPs treated Candida spp.

Measurement of nucleic acid leakage of AgNPs treated Candida spp.; the Candida spp. were treated with mycosynthesized AgNPs at MIC; the values represent mean ± standard deviation of three independent experiments; the different colors of the bars represent the bacterial strains treated with mycosynthesized AgNPs and Fluconazole at different time intervals; untreated cells were also shown in the figure.
Fig 3.5a: Potassium leakage analysis of AgNPs treated bacterial strains

Measurement of potassium leakage of AgNPs treated bacterial strains; the bacterial strains were treated with mycosynthesized AgNPs at MIC; the values represent mean ± standard deviation of three independent experiments; the different colors of the bars represent the bacterial strains treated with mycosynthesized AgNPs and Streptomycin at different time intervals; untreated cells were also shown in the figure.

Fig. 3.5b: Potassium leakage analysis of AgNPs treated Candida spp.

Measurement of potassium leakage of AgNPs treated Candida spp.; the Candida spp. were treated with mycosynthesized AgNPs at MIC; the values represent mean ± standard deviation of three independent experiments; the different colors of the bars represent the bacterial strains treated with mycosynthesized AgNPs and Fluconazole at different time intervals; untreated cells were also shown in the figure.
Mode of action of silver nanoparticles against pathogenic strain - *E. coli*

(i) Untreated (control) cell image
(ii & iii) Images of AgNPs treated cells.

Arrow indicates the accumulation of nanoparticles on the cell membrane and the breakage of cell wall after treatment with silver nanoparticles for about 6 h.
Fig. 3.6b: Electron microscopic studies of AgNPs treated
*Klebsiella pneumoniae*

(i) Untreated (control) cell image

(ii & iii) Images of AgNPs treated cells.

Arrow indicates the accumulation of nanoparticles on the cell membrane and the breakage of cell wall after treatment with silver nanoparticles for about 6 h.
Fig. 3.6c: Electron microscopic studies of AgNPs treated *Pseudomonas aeruginosa*

Mode of action of silver nanoparticles against pathogenic strain – *P. aeruginosa*

(i) Untreated (control) cell image
(ii & iii) Images of AgNPs treated cells.

Arrow indicates the accumulation of nanoparticles on the cell membrane and the breakage of cell wall after treatment with silver nanoparticles for about 6 h.
Fig. 3.6d: Electron microscopic studies of AgNPs treated *Bacillus subtilis*

(i) Untreated control cell image

(ii & iii) Images of AgNPs treated cells.

Arrow indicates the accumulation of nanoparticles on the cell membrane and the breakage of cell wall after treatment with silver nanoparticles for about 6 h
Fig. 3.6e: Electron microscopic studies of AgNPs treated
*Enterobacter faecalis*

(i) Untreated control cell image
(ii & iii) Images of AgNPs treated cells.

Arrow indicates the accumulation of nanoparticles on the cell membrane and the breakage of cell wall after treatment with silver nanoparticles for about 6 h.
Fig. 3.6f: Electron microscopic studies of AgNPs treated *Staphylococcus aureus*

(i) Untreated (control) cell image

(ii & iii) Images of AgNPs treated cells.

Arrow indicates the accumulation of nanoparticles on the cell membrane and the breakage of cell wall after treatment with silver nanoparticles for about 6 h.
Fig. 3.6g: Electron microscopic studies of AgNPs treated MRSA

Mode of action of silver nanoparticles against pathogenic strain – MRSA

(i) Untreated (control) cell image

(ii & iii) Images of AgNPs treated cells.

Arrow indicates the accumulation of nanoparticles on the cell membrane and the breakage of cell wall after treatment with silver nanoparticles for about 6 h.
Fig. 3.6h: Electron microscopic studies of AgNPs treated *Candida albicans*

(i) Untreated (control) cell image
(ii & iii) Images of AgNPs treated cells.

Arrow indicates the accumulation of nanoparticles on the cell membrane and the breakage of cell wall after treatment with silver nanoparticles for about 6 h.

Mode of action of silver nanoparticles against pathogenic strain - *Candida albicans*
Fig. 3.7a: Antifungal activity of mycosynthesized AgNPs against plant pathogenic fungi - *Aspergillus flavus*

Aspergillus flavus

Antifungal activity of *A. terreus* synthesized silver nanoparticles against *Aspergillus flavus*; PDA plates were amended with different concentrations of AgNPs (i) Control (ii) 10 μg/mL of AgNPs; (iii) 20 μg/mL of AgNPs; (iv) 30 μg/mL of AgNPs; (v) 40 μg/mL of AgNPs; (vi) 50 μg/mL of AgNPs.
Fig. 3.7b: Antifungal activity of mycosynthesized AgNPs against plant pathogenic fungi - *Colletotrichum* sp.

*Colletotrichum* sp.

Antifungal activity of *A. terreus* synthesized silver nanoparticles against *Colletotrichum* sp.; PDA plates were amended with different concentrations of AgNPs (i) Control (ii) 10 µg/mL of AgNPs; (iii) 20 µg/mL of AgNPs; (iv) 30 µg/mL of AgNPs; (v) 40 µg/mL of AgNPs; (vi) 50 µg/mL of AgNPs.
Fig. 3.7c: Antifungal activity of mycosynthesized AgNPs against plant pathogenic fungi - *Curvularia lunata*

Antifungal activity of *A. terreus* synthesized silver nanoparticles against *Curvularia lunata*; PDA plates were amended with different concentrations of AgNPs (i) Control (ii) 10 µg/mL of AgNPs; (iii) 20 µg/mL of AgNPs; (iv) 30 µg/mL of AgNPs; (v) 40 µg/mL of AgNPs; (vi) 50 µg/mL of AgNPs.
Fig. 3.7d: Antifungal activity of mycosynthesized AgNPs against plant pathogenic fungi - *Penicillium sp.*

Antifungal activity of *A. terreus* synthesized silver nanoparticles against *Penicillium sp.*; PDA plates were amended with different concentrations of AgNPs (i) Control (ii) 10 µg/mL of AgNPs; (iii) 20 µg/mL of AgNPs; (iv) 30 µg/mL of AgNPs; (v) 40 µg/mL of AgNPs; (vi) 50 µg/mL of AgNPs.
Table 3.1: Antibacterial activity of *A. terreus* derived silver nanoparticles against different bacterial pathogens

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Mycelial free extract (25 µL)</th>
<th>Silver nitrate (1 mM)</th>
<th>Silver nanoparticles (1 mg/mL)</th>
<th>Streptomycin (1 mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean zone of inhibition (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>-</td>
<td>12.73 ± 0.40</td>
<td>16.43 ± 0.21</td>
<td>24.37 ± 0.23</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>-</td>
<td>16.47 ± 0.50</td>
<td>19.47 ± 0.06</td>
<td>17.53 ± 0.21</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
<td>13.03 ± 0.06</td>
<td>14.27 ± 0.12</td>
<td>25.33 ± 0.15</td>
</tr>
<tr>
<td>MRSA</td>
<td>-</td>
<td>8.83 ± 0.11</td>
<td>16.15 ± 0.07</td>
<td>5.97 ± 0.15</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
<td>12.67 ± 0.15</td>
<td>22.47 ± 0.06</td>
<td>14.27 ± 0.15</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>-</td>
<td>12.23 ± 0.15</td>
<td>16.13 ± 0.06</td>
<td>5.67 ± 0.25</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>15.67 ± 0.76</td>
<td>17.13 ± 0.15</td>
<td>13.33 ± 0.15</td>
</tr>
</tbody>
</table>

Streptomycin was used as a positive control for bacterial strains
Values are mean ± Standard deviation of three independent experiments; (-) No activity

Table 3.2: Minimum inhibitory concentration (MIC) of *A. terreus* derived silver nanoparticles against different bacterial pathogens

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>6.25</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>3.12</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>12.5</td>
</tr>
<tr>
<td>Methicillin-resistant <em>S. aureus</em></td>
<td>3.12</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>3.12</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>6.25</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>6.25</td>
</tr>
</tbody>
</table>

Values are mean of three independent experiments
**Table 3.3:** Anticandidal activity of *A. terreus* derived silver nanoparticles against different *Candida* spp.

<table>
<thead>
<tr>
<th>Yeast strain</th>
<th>Mycelial free extract (25 µL)</th>
<th>Silver nitrate (1 mM)</th>
<th>Silver nanoparticles (1 mg/mL)</th>
<th>Fluconazole (1 mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>-</td>
<td>11.38 ± 0.17</td>
<td>18.13 ± 0.02</td>
<td>10.12 ± 0.10</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>-</td>
<td>13.37 ± 0.15</td>
<td>14.63 ± 0.15</td>
<td>21.13 ± 0.06</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>-</td>
<td>11.73 ± 0.06</td>
<td>13.27 ± 0.21</td>
<td>24.63 ± 0.21</td>
</tr>
</tbody>
</table>

Mean zone of inhibition (mm)

Fluconazole was used as a positive control for *Candida* spp. Values are mean ± Standard deviation of three independent experiments; (-) No activity.

**Table 3.4:** Minimum inhibitory concentration (MIC) of *A. terreus* derived silver nanoparticles against different *Candida* spp.

<table>
<thead>
<tr>
<th>Yeast strain</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>3.12</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>12.5</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>12.5</td>
</tr>
</tbody>
</table>

Values are mean of three independent experiments.
### Table 3.5: Antifungal activity of *A. terreus* derived silver nanoparticles against different plant pathogens

<table>
<thead>
<tr>
<th>Fungal plant pathogen</th>
<th>Antifungal index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment (µg/mL of PDA)</td>
</tr>
<tr>
<td></td>
<td>10 µg/mL</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.28 ± 1.98</td>
</tr>
<tr>
<td><em>Colletotrichum</em> sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.89 ± 1.32</td>
</tr>
<tr>
<td><em>C. lunata</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.69 ± 1.53</td>
</tr>
<tr>
<td><em>Penicillium</em> sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43.43 ± 1.85</td>
</tr>
</tbody>
</table>

Values are mean ± Standard deviation of three independent experiments.
PDA – Potato dextrose agar