Journal for ReAttach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 September; 6 9s(2): 1827-1834

Biogenic Production Of Silver Nanoparticles And Their Antifungal Potential Against Human Pathogenic Fungal Strains

Dinesh Kumar Sharma[1], Pradip Kumar[2] and Dilshad Ali[3]

[1*,3]Department of Microbiology, C.C.S. University, Meerut, Uttar Pradesh (250 004), India
[2]Department of Biotechnology, C.C.S. University, Meerut, Uttar Pradesh (250 004), India

E-mail of Corresponding authors: dr.dilshad.ali@gmail.com, panwarpradeepp@gmail.com

Abstract

Green synthesis is the best option to opt for the synthesis of nanoparticles and the silver nanoparticles were synthesized by using *Aspergillus niger* biomass. The bio-transformation was routinely monitored visually and by *Uv-Vis* spectrophotometery after different time intervals and spectra was analyzed by Gaussian's function. Colour test of the produced silver nanoparticles was also done by borohydrate as reducing agent. The antifungal assay was done against six human pathogenic fungi by standard disc diffusion method. At the tested concentration of silver nanoparticles solution, the growth of *Cunninghamella* sp. (67.44%), *Aspergillus niger* (59.61%), *Absidia* sp. (50.98%), *Mucor* sp. (46.42%), *Aspergillus flavus* (27.90%) and *Rhizopus* sp. (26.41%) were reduced. The crude silver nanosolution was found toxic to a greater extent against tested potential human pathogenic fungal strains. The biogenic production of silver nanoparticles through fungus were found effective, easy and cheap in comparison to physico-chemical methods.

Keywords: Green synthesis, silver nanoparticles, Gaussian function, disc diffudion method, antifungal acitivity, fungal pathogens.

INTRODUCTION

Nanoparticles of noble metals such as gold, silver and platinum are well recognized and have the significant applications in electronics, magnetic, optoelectronics and information storage. One such important member of the noble metal nanoparticles are the silver nanoparticles (AgNPs). They are also widely applied in shampoos, soaps, detergents, cosmetics, toothpastes and medical and pharmaceutical products and are hence directly encountered by human systems (Banerjee *et al.,* 2014). Nanoparticles can be prepared through different approaches such as chemical and photochemical reactions, thermal decomposition, electrochemical and also by biological methods (Fadel and Al-Mashhedy, 2017). One of the wide challenges of recently nanotechnology is to develop reliable experimental protocols for the nanoparticles synthesis over the range of chemical composition, size and synchronized monodispersity that must be nontoxic, clean and eco-friendly by using the ambient biological sources. Green synthesis of nanoparticles has attracted considerable attention in recent years and eco-friendly synthesis of inorganic nanoparticles is a fast growing research in the branch of nanotechnology. Utilizing green substances has several advantages including low energy consumption and moderate operation conditions (e.g. pressure and temperature) without using any toxic chemicals (Mie *et al.,* 2014). The importance of biological synthesis is being emphasized globally at present because chemical methods are capital intensive, toxic, non eco-friendly and have low productivity (Arun *et al.,* 2013). Therefore, green synthesis techniques using various biological organisms such as yeast, mold, algae and bacteria, and plant extracts have been developed for nanoparticles synthesis (Kaviya *et al.,* 2011). The Silver nanoparticles are already part of our daily life, that are present in our clothes e.g. in socks household and personal care products, mainly due to their antimicrobial potential (Pacioni *et al.,* 2015) and greatest advantage in biomedical areas, such as targeted drug delivery, imaging, sensing and antibacterial activity (Rout *et al.,* 2011). In general, the silver nanoparticles have been used with promising results such as bactericides, antimicotics and anticancer agents (Leon *et al.,* 2013).

Annually, over 150 million severe cases of fungal infections occur worldwide, resulting in approximately 1.7 million deaths per year (Kainz *et al.,* 2020). Fungal diseases are one of the leading causes of death worldwide at present. Moreover, the problem of mycoses is exacerbated by the increase in emerging pathogenic fungi, but also by resistance to the limited antifungal drugs available, which significantly reduces the effectiveness of treatments (Denning *et al.,* 2017). A crucial factor that contributes to the rising number of fungal infections is the drastic increase of the at-risk population that is specifically vulnerable to fungal infections (Kainz *et al.,* 2020), including HIV, diabetes mellitus, burns, pulmonary diseases, malignancies, tuberculosis, silicosis, cirrhosis, cancer, ulcer, use of immunosuppressive agents or transplantation, elderly population, critically ill or severely immunocompromised patients, intravenous cannulation, trauma, occlusive dressing, poor medical practices, careless by caretakers or health workers, unhygienic environment during post-surgery or burn rehabilitation. The growing numbers of cancer, AIDS and transplantation

patients with the concomitant subscription of immune-modulating drugs as well as the excessive antibiotic use compose risk factors for the development of fungal infections (Enoch *et al.,* 2017; Friedman and Schwartz, 2019; Lockhart and Guarner, 2019). Furthermore, the increasing usage of medical devices such as catheters or cardiac valves leads to a higher risk for the formation of biofilms. Biofilms represent an assembly of highly diverse, complex and eminently organized cells embedded in an extracellular matrix that conveys protection from physical and/or chemical insults. Thus, biofilms are often resistant to existing treatments and, in fact, are considered to essentially contribute to the high mortality rates associated with invasive fungal infections (Uppuluri *et al.,* 2009; Pierce *et al.,* 2015). During post surgery or burn rehabilitation, immuno-compromised individuals often develop fungal infections primarly due to contamination through spores inhalation, unhygenic occlusive clothing/ dressing, semi occlusive clothing/ dressing and unhygenic non-occlusive clothing. Mucormycosis and aspergillosis are the most frequent fungal infections caused by ubiquitous filamentous fungi (Boroujeni *et al.,* 2020). *Aspergillus* species is the most ubiquitous fungi seen in soil, water and decaying vegetations. It affects the lungs, central nervous system, naso-orbital area, skin and sometimes, it may be disseminated. Aspergillosis is an uncommon opportunistic fungal infection caused by a different species such as *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger* are the common ones (John and Shadomy, 1987). Person *et al.* (2010) mentioned that *Aspergillus niger* is a mould that is rarely reported as a cause of pneumonia. Walsh *et al.* (2008) mentioned that *Aspergillus flavus* is more common in the air for unclear reasons and causes aggressive and invasive aspergillosis. Chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections, and osteomyelitis following trauma and inoculation are the known spectrum of its manifestations in immunocompromised patients (Marr *et al.,* 2002). *Aspergillus* species have emerged as an important cause of life-threatening infections especially immuno-compromised patients (Stevans *et al.,* 2000). Jerome *et al.* (2020) diagnosed lower limb of an adult and isolated species of *Aspergillus flavus*. Some mucorales are also disease causing fungi. Such as mucormycosis is a rare fungal infection (Abdelmonem *et al.,* 2022) caused by *Rhizopus*, *Absidia* and *Mucor* (Tang and Wang, 1998). Mucormycosis is a rare disease typified by rapid clinical deterioration and progression to necrosis (Roden *et al.,* 2005), the cutaneous type has a better prognosis than other invasive types which have high mortality rate 44–80% (Spellberg *et al.,* 2012). *Absidia corymbifera* is also a saprophytic organism with worldwide distribution that is isolated from soil as well as decaying vegetation and grass. It is uncommon pathogen representing only 2% to 3% of all Zygomycetes infection in humans. Invasive fungal infection with *Absidia corymbifera* is rare opportunistic infection encountered in patient with burn injury (Noon and Jithendran, 2018). Invasive skin infection with *Absidia* is very rare entity with only few cases reported (Kaushik, 2012). *Mucor* spp. are the species which are most commonly associated with infection in burn patients, but other species including *Rhizopus* spp., *Absidia/Lichthiemia* spp. and *Rhizomucor* spp. may be present as single or co-infective agents (Tang and Wang, 1998). Abdelmonem *et al.* (2022) reported an adult male patient with 50% body burns who servived despite rapid deterioration following cutaneous and probable systemic mucormycosis. They applied a combination of antifungal treatment with extensive debridement and amputation likely resulted in the positive outcome in this case and prevent the mucormycosis. Wang *et al.* (2018) reported a 37-year-old person presented with a skin lesion on the left side of the chest wall, which had been present for 17 years. The patient was given antifungal agents without debridement and the antifungal treatment options consist of amphotericin B as the first-line therapy and posaconazole as salvage therapy. External application was given to his wound with silver sulfadiazine after cleansing with hydrogen peroxide and normal saline. After 5 months of treatment, the patient recovered satisfactorily with fresh granuloma and scarring of the skin lesion. Quino *et al.* (2004) reported that infections caused by *Cunninghamella bertholletiae* are rare but severe and this opportunistic mould in the order Mucorales infects immuno-compromized patients suffering from haematological malignancies or diabetes mellitus, as well as solid organ transplant patients. They also mentioned that the lung is the organ most often involved and infection is most often acquired by inhalation of airborne spores, particularly in pulmonary and rhinocerebral infections. Transmission *via* the gastrointestinal tract is also suggested by the prominent involvement of these organs in some cases. *C. bertholletiae* has been found in a wide variety of food materials, particularly seeds, nuts and vegetables.

Currently, silver nanoparticles occupy a prominent place as potential antifungal agents for clinical use due to their broad spectrum of antimicrobial activity and their enormous number of applications in the health sciences, ranging from topical formulations to catheters impregnated with AgNPs (Rai *et al.,* 2009; Ahamed *et al.,* 2010; Burdusel *et al.,* 2018; Mosleh-Shirazi *et al.,* 2021). The use of silver nanoparticles as antifungal agents has become more widespread, as scientific progress make their production more cost-effective. Thus, present work was done on biosynthesis of silver nanoparticles through green routes and their use as antifungal activities against some potential human pathogenic fungal strains.

MATERIALS AND METHODS

PRODUCTION OF FUNGAL BIOMASS

Aspergillus niger Link was taken from laboratory and grown in 500 ml Erlenmeyer flasks each containing 100 ml MGYP medium composed of malt extract (0.3%), glucose (1.0%), yeast extract (0.3%), and peptone (0.5%) at 25–28 ^{0}C

under shaking condition (200 rpm) for 96 hrs. After 96 hrs of fermentation, mycelia were separated from the culture broth by centrifugation (5000 rpm) at 10^0C for 20 min and the settled mycelia were washed thrice with sterile distilled water to remove any medium component from the fungus biomass pellet.

BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES

2mM aqueous solution of silver nitrate ($AgNO_3$) was prepared for the synthesis of silver nanoparticles. 10 gm of fungal biomass was taken and suspended separately in 100ml of the 2mM aqueous $AgNO_3$ solution in 250 ml Erlenmeyer flasks (at pH 5.5-6.0) for reduction of silver nitrate into Ag^+ ions. Whole mixture was placed on a shaker at 28 ^{0}C (at 200 rpm) and the reaction carried out for a period of 120 hours. The bio-transformation was routinely monitored visually and ultraviolet spectra after different time periods (0 hr, 4 hr, 12 hr, 24 hr, 48 hr, 72 hr, 96 hr and 120 hr) and through chemical test. The method of Mozhgan (2008) was followed with slight modifications for aggregation and colour test of silver nanoparticle solutions produced by tested mycomass. For this, 5 ml of silver nanoparticles solution was mixed with 30 ml 0.002M $NaBH_4$ solution in an Erlenmeyer flask and placed the flask in an ice bath on a magnetic stirrer plate for 20 min. A small portion of this solution was taken in a test tube and few drops of 1.5 M NaCl solution were added. After the formation of cloudy grey colour, solid polyvinyl alcohol (PVP) was slowly added to give a 4% solution and stirred again to mix it properly. Stirring was done untill a characterstic yellow paint like solution was formed. The spectra obtained from *Uv-vis* spectrophotometer was analyzed by Gaussian function according to the given formula: $f(x) = ae^{-\frac{(x-b)2}{2c2}}$, where, f(x) is Gaussian function, $e \approx 2.71828$ (mathematical constant), a = height of the peak in a curve, b = position of the centre of the peak, c = width of the bell shaped curve.

FUNGI ISOLATION AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES

The antimicrobial assay was done against six human pathogenic fungi (*Absidia* sp., *Aspergillus flavus, Aspergillus niger*, *Cunninghamella* sp., *Mucor* sp. and *Rhizopus* sp.) isolated from non-occlusive clothes of immunocompitent patients. The clothe was imprinted directly on sabouraud dextrose agar plates. After subculturing and morphological identification of the isolated fungal culture, antimicrobial activity of silver nanoparticles were tested against isolated fungal strains. Standard disc diffusion method was used and the diameters of the colonies were recorded and the percentage inhibition (I) was calculated using the formula (Wonglom *et al.*, 2019): $I = \frac{C - T}{C} \times 100$, where, I= mycelial growth inhibition percentage; C = radial growth to the fungus in control Petri plates; T= radial growth of the fungus in the Petri plates with medium containing the silver nanoparticles.

RESULTS AND DISCUSSION

AgNPs were synthesized using a reduction of aqueous Ag^+ with the culture supernatants of *Aspergillus niger* at room temperature. It was generally recognized that AgNPs produced brown solution in water, due to the surface plasmon resonances (SPR) effect of reduced $AgNO_3$ (Huang *et al.*, 2007). After the addition of $AgNO_3$ solution, the cell filtrate of *A. niger* changed from light yellow to brown in a few hours, while no color change was observed in the culture supernatant without $AgNO_3$. Thus, color change of the solution clearly indicated the formation of AgNPs. The color intensity of the cell filtrate with $AgNO_3$ was sustained even after 24 hour incubation, which indicated that the particles were well dispersed in the solution, and there was no obvious aggregation. All these reactions were monitored by ultraviolet-visible spectroscopy of the colloidal AgNPs solutions. The ultraviolet-visible spectra of the cell filtrate with $AgNO_3$ showed a strong broad peak at 440 nm which is surface Plasmon resonances (SPR band), which indicated the presence of AgNPs (Fig. 1). The intensity of the SPR band steadily increased from 6 hr to 24 hr as a function of time of reaction. It was also observed that the AgNPs formed were quite stable in the supernatant of *A. niger*. It is generally accepted that *UV-Vis* spectroscopy could be used to examine size and shape of nanoparticles in aqueous solutions (Hussain *et al.*, 2016). The *Uv-Vis* absorbance peak was found to fit into a Gaussian curve with the test concentration of fungal biomass. Peak broadening was noticeable with increasing in absorbance intensity. The peak broadening is attributed to an increase in polydispersity as a result of increased biomass extract solution employed during synthesis. A decrease in FWHM suggests an increase in silver nanoparticles core-diameter (Mie *et al.*, 2014).
The addition of sodium borohydride prevented the aggregation of colloidal silver nanoparticles and by providing a particle surface charge through adsorption, it stabilized the growing silver nanoparticles (Mozhgan, 2008). The addition of sodium chloride speed up the aggregation of colloidal silver nanoparticles by shielding the charges and allowing the particles to clump together to form aggregates (Hyning *et al.*, 1998, 2001), which turns the darker yellow solution into grayish. Further, addition of polyvinylpyrrolidone (PVP) prevented the aggrigation and stabilized the colloidal nanoparticles solution (Malynych *et al.*, 2001; Xiong *et al.*, 2006). These yellow colloidal silver remains stable for as long as several weeks or months (Mozhgan, 2008).

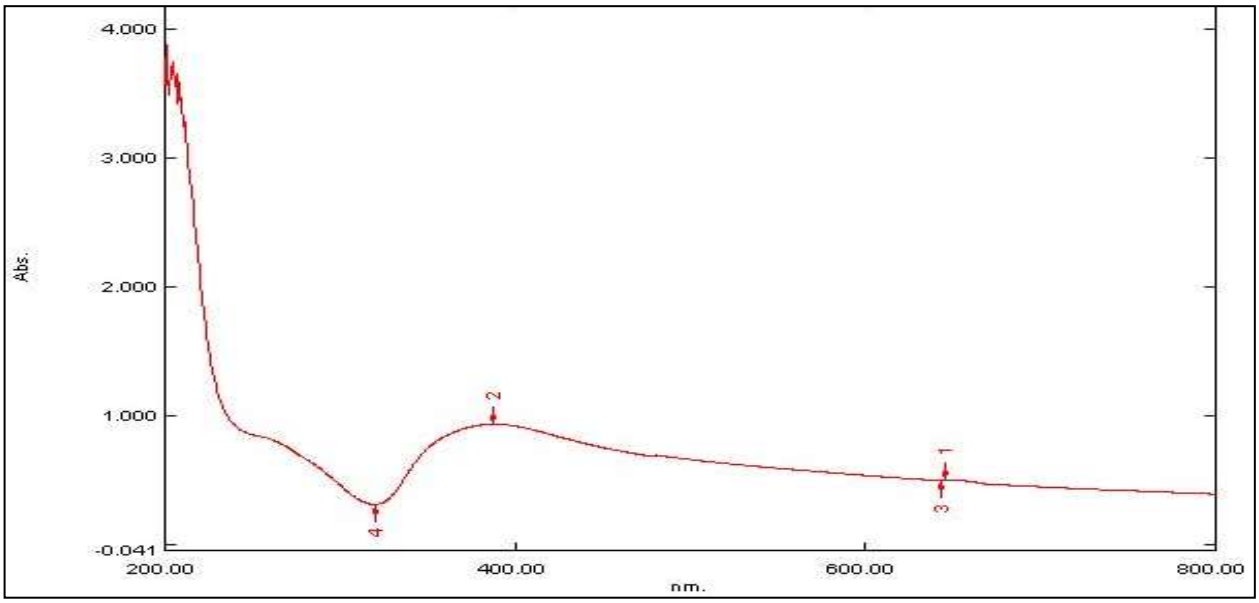

Fig. 1 Ultraviolet-visible spectra of synthesized silver nanoparticles

Silver nanoparticles prepared with a variety of methods have effective antimicrobial activity (Devi and Joshi, 2015). Table 1 and figure 2 provides the percentage reduction in growth of different fungal strains when exposed to silver nanoparticles. The percentage reduction represents the extent to which the growth of each fungal strain was inhibited by the silver nanoparticles. For *Absidia* sp., the growth was reduced by 50.98% when exposed to silver nanoparticles. This indicates that the silver nanoparticles had a significant inhibitory effect on the growth of *Absidia* sp., suppressing approximately half of its growth. *Aspergillus flavus* exhibited a reduction in growth of 27.90% when exposed to silver nanoparticles. This suggests that the silver nanoparticles had a moderate inhibitory effect on the growth of *Aspergillus flavus*, reducing it by nearly one-fourth compared to the control group. The growth of *Aspergillus niger* was significantly reduced by 59.61% in the presence of silver nanoparticles. This indicates that the silver nanoparticles had a substantial inhibitory effect on the growth of *Aspergillus niger*, suppressing approximately 60% of its growth. *Cunninghamella* sp. showed the highest reduction in growth among the tested strains, with a percentage reduction of 67.44% when exposed to silver nanoparticles. This demonstrates that the silver nanoparticles were highly effective in inhibiting the growth of *Cunninghamella* sp., suppressing more than two-thirds of its growth. *Mucor* sp. exhibited a reduction in growth of 46.42% when exposed to silver nanoparticles. This suggests that the silver nanoparticles had a moderate inhibitory effect on the growth of *Mucor* sp., reducing it by nearly half compared to the control group. *Rhizopus* sp. displayed a reduction in growth by 26.41% when exposed to silver nanoparticles. This indicates that the silver nanoparticles had a relatively modest inhibitory effect on the growth of *Rhizopus* sp., suppressing approximately one-fourth of its growth. Overall, the results indicate that silver nanoparticles had varying degrees of effectiveness in inhibiting the growth of different fungal strains, with *Cunninghamella* sp. being the most susceptible and *Aspergillus flavus* and *Rhizopus* sp. showing relatively lower susceptibility to the silver nanoparticles. The tested concentration of crude silver nanoparticles solution was found toxic to tested potential human pathogenic fungal strains. (Pulit *et al.*, 2013) was found that nanosilver suspension at the concentration of 50 ppm inhibited the growth of *Cladosporium cladosporoides* and *Aspergillus niger* by 90% and 70%, respectively. Rout *et al.* (2011) the nanoparticles synthesized by green route (using *Ocimum sanctum*) were found to be highly toxic against clinically isolated fungal species. At a concentration of 50 µl silver nanoparticles revealed a higher antifungal activity against *C. albicans, C. kefyr, A. niger* whereas intermediated activity were showed against *C. tropicalis, C. krusei, A. flavus, A. fumigatus.* Rajeshkumar *et al.* (2014) carried out a study on the antifungal activity of *Sargassum longifolium* mediated synthesized silver nanoparticles against harmful pathogenic fungi *viz. Fusarium* sp., *Candida albicans* and *A. fumigatus*.

Table 1 Antifungal activity of aqueous solution of silver nanoparticles

Test Fungal Strain	Control (cm)	Growth Diameter (cm)	% Reduction
Absidia sp.	5.10 ± 0.30	2.50 ± 0.02	50.98
Aspergillus flavus	4.30 ± 0.12	3.10 ± 0.08	27.90
Aspergillus niger	5.20 ± 0.14	2.10 ± 0.15	59.61
Cunninghamella sp.	4.30 ± 0.10	1.40 ± 0.03	67.44
Mucor sp.	2.80 ± 0.13	1.50 ± 0.04	46.42
Rhizopus sp.	5.30 ± 0.07	3.90 ± 0.04	26.41

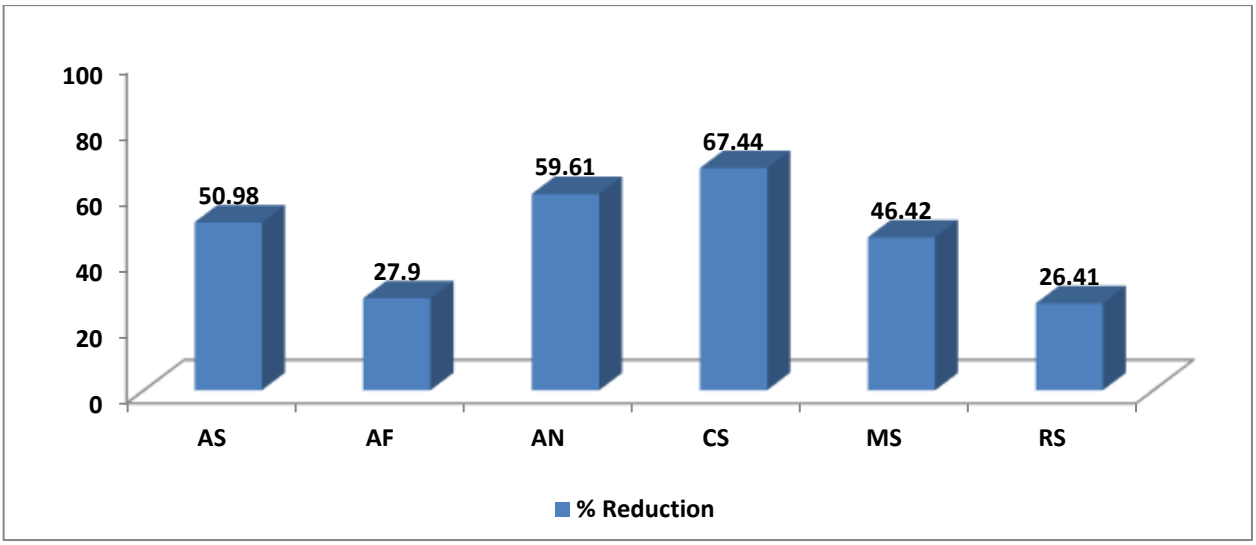

Fig. 2 Percent reduction in growth of *Absidia* sp., *Aspergillus flavus, Aspergillus niger, Cunninghamella* sp., *Mucor* sp. and *Rhizopus* sp. after treatment with silver nanoparticles solution

The precise mechanism underlying the antifungal activity of colloidal silver nanoparticles is not yet fully understood. However, it is known that the antifungal efficacy of these nanoparticles is influenced to a significant extent by their size and shape. Smaller nanoparticles have a larger surface area, which enables them to effectively inhibit the growth of microbes. Additionally, silver nanoparticles with a spherical shape and reduced size ions have an increased contact area, allowing them to eliminate bacterial growth. The activity of silver nanoparticles produces effects similar to those of silver ions, as demonstrated by Pal *et al.* (2007). Positively charged silver ions can attach to the negatively charged cell membranes of microbes through electrostatic attraction, as observed by Dibrov *et al.* (2002). Silver nanoparticles can also create pits in the cell wall, leading to damage in cell permeability, as reported by Raffi *et al.* (2008). Furthermore, silver nanoparticles induce proton leakage caused by reactive oxygen species (ROS) in the membrane, as noted by both Dibrov *et al.* (2002) and Dehkordi *et al.* (2011), ultimately resulting in cell death (Sondi and Salopek-Sondi, 2004). Noon and Jithendran (2018) have concluded that cutaneous zygomycosis is often under-diagnosed in humans, despite its frequent occurrence. Zygomycosis is caused by fungi of the Mucorales order, including genera such as Mucor, Rhizopus, Absidia, Cunnighmella, Rhizomucor, and Apophysomyces. Rhino-cerebral and pulmonary zygomycosis are the most common manifestations, while invasive skin infections are less common. Boyce *et al.* (1981) and Mostaza *et al.* (1989) have reviewed two cases of primary cutaneous infection following soft tissue injuries. Only two cases of primary cutaneous infection with *C. bertholletiae* have been reported to date: one in an AIDS patient, who died, and one in a diabetic patient, who survived after undergoing leg amputation. According to John and Shadomy (1987), cutaneous aspergillosis is primarily caused by *A. flavus* and *A. fumigatus*, and rarely by *A. niger*. Clinically, the lesion is characterized by macules, papules, plaques, or hemorrhagic bullae, which may progress into necrotic ulcers covered with a heavy black eschar. Stevans *et al.* (2000) reported that cutaneous aspergillosis typically occurs in immuno-compromised patients. Zayet *et al.* (2021) concluded that mucormycosis and aspergillosis are two opportunistic and invasive fungal infections that have clinical and para-clinical similarities. The diagnosis of these fungal strains is challenging, often misleading practitioners (Maiorano *et al.*, 2005; Davoudi *et al.*, 2014; Rit *et al.*, 2014; Boras *et al.*, 2019). These severe infections could be disseminated involving multiple organs and are frequently fatal in immuno-compromised patients (Rit *et al.*, 2014; Boras *et al.*, 2019; Safai *et al.*, 2019; Boroujeni *et al.*, 2020).

CONCLUSIONS

Based on the findings of this study, it can be concluded that the fungal biomass used in the experiment was effective for producing silver nanoparticles. The silver nanoparticles exhibited distinct characteristics in the ultraviolet range. The antimicrobial properties of the silver nanoparticles were enhanced as their concentration increased. Consequently, there was a direct correlation between the concentration of silver nanoparticles and the formation of inhibition zones around the well. These results highlighted the significant potential of silver nanoparticles in controlling spore-producing fungi and their role as effective antimicrobial agents against fungal diseases caused by the tested fungal strains. The chances of unhygenic non-occlusive clothing of immuno-compromised individuals might be reduced by the treatment of non-occlusive cloting with silver nanoparticles solutions and this, in turn, also minimized the chances of invasive fungal infections due to spores inhalation, unhygenic occlusive and semi occlusive clothing/ dressings.

REFERENCES

1. Abdelmonem, R., Backx, M., Vale, L., Healy, B. and Williams, D. (2022). Successful treatment of *Mucor circinelloides* in a burn patient. *Burns Open* **6**: 77–81.
2. Arun, P., Shanmugaraju, V., Ramanujam, J.R., Prabhu, S.S. and Kumaran, E. (2013). Biosynthesis of silver nanoparticles from *Corynebacterium* sp. and its antimicrobial activity. *Int. J. Curr. Microbiol. App. Sci.* **2**(3): 57–64.
3. Banerjee, P. Satapathy, M., Mukhopahayay, A. and Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresour. Bioproc.* **1**(3): 1–10.
4. Boras, V.V., Jurlina, M., Brailo, V., Vukovic, K.D., Roncevic, P., Kinda, S.B., Juras, D.V. and Gabric, D. (2019). Oral mucormycosis and aspergillosis in the patient with acute leukemia. *Acta Stomatol. Croat.* **53**(3): 274.
5. Boroujeni, Z.B., Shamsaei, S., Yarahmadi, M., Getso, M.I., Salimi-Khorashad, A. and Haghighi, L. (2021). Distribution of invasive fungal infections: molecular epidemiology, etiology, clinical conditions, diagnosis and risk factors: a 3-year experience with 490 patients under intensive care. *Microb. Pathog.* **2021**: 104616.
6. Boyce, J.M., Lawson, L.A., Lockwood, W.R. and Hughes, J.L. (1981). *Cunninghamella bertholletiae* wound infection of probable nosocomial origin. *South Med. J.* **74**: 1132–1135.
7. Davoudi, S., Anderlini, P., Fuller, G.N. and Kontoyiannis, D.P. (2014). A long-term survivor of disseminated *Aspergillus* and *Mucorales* infection: an instructive case. *Mycopathol.* **178**(5–6): 465–70.
8. Dehkordi, S.H., Hosseinpour, F. and Kahrizangi, A.E. (2011). An *in vitro* evaluation of antibacterial effect of silver nanoparticles on *Staphylococcus aureus* isolated from bovine subclinical mastitis. *Afri. J. Biotechnol.* **10**(52): 10795–10797.
9. Denning, D.W., Perlin, D.S., Muldoon, E.G., Colombo, A.L., Chakrabarti, A., Richardson, M.D. and Sorrell, T.C. (2017). Delivering on antimicrobial resistance agenda not possible without improving fungal diagnostic capabilities. *Emerg. Infect. Dis.* **23**(2): 177–183.
10. Devi, L.S. and Joshi, S.R. (2015). Ultrastructures of silver nanoparticles biosynthesized using endophytic fungi. *J. Micros. Ultrastr.* **3**: 29–37.
11. Dias, M., Antony, B., Pinto, H. and Rekha, B. (2003). Primary cutaneous aspergillosis due to *Aspergillus niger* in an immunocompetent patient. *Ind. J. Med. Microbiol.* **27**(4): 367–370.
12. Dibrov, P., Dzioba, J., Gosink, K.K. and Hase, C.C. (2002). Chemiosmotic mechanism of antimicrobial activity of Ag^+ in *Vibrio cholera*. *Antimicrob. Agents Chem.* **46**(8): 2668–2670.
13. Enoch, D.A., Yang, H., Aliyu, S.H. and Micallef, C. (2017). The changing epidemiology of invasive fungal infections. *Methods Mol. Biol.* **1508**: 17–65.
14. Fadel, Q.J., Al-Mashhedy, L.A.M. (2017). Biosynthesis of silver nanoparticles using peel extract of *Raphanus sativus* L. *Biotechnol. Ind. J.* **13**(1): 120(1–10).
15. Friedman, D.Z.P. and Schwartz, I.S. (2019). Emerging fungal infections: new patients, new patterns, and new pathogens. *J. Fungi* **5**(3): E67.
16. Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H, Wang, Y., Shao, W., He, N., Hong, J. and Chen, C. (2007). Biosynthesis of silver and gold nanoparticles by novel sun dried *Cinnamomum camphora* leaf. *Nanotechnol.* **18:** 105104 (1–11).
17. Hussain, I., Singh, N.B., Singh, A., Singh, H. and Singh, S.C. (2016). Green synthesis of nanoparticles and its potential application. *Biotechnol. Lett.* **38**: 545–560.
18. Hyning, D.L.V. and Zukoski, C.F. (1998). Formation mechanisms and aggregation behavior of borohydride reduced silver particles. *Lang.* **14**: 7034–7046.
19. Hyning, D.R., Klemperer, W.G. and Zukowski, C.F. (2001). Characterization of colloidal stability during precipitation reactions. *Lang.* **17**: 3120–3127.
20. Jerome, T.J., Kandasamy, L, Terrence, T.K., Bhalaj, S. and Shanmugasundaram, B. (2020). *Aspergillus flavus* infection of lower limb in an immune-competent patient. *Cureus* **12**(7): e9342.
21. John, P.U., Shadomy, H.J. (1987). Deep fungal infections. In: *Dermatology in General Medicine* 3rd ed. (eds., Fitzpatrick, T.B., Eisen, A.Z., Wolff, K., Freedberg, I.M. and Austen, K.F.), New York: McGraw-Hill; pp: 2266–2268.
22. Kainz, K., Bauer, M.A., Madeo, F. and Carmona-Gutierrez, D. (2020). Fungal infections in humans: the silent crisis. *Micro. Cell.* **7**(6): 143–145.
23. Kaushik, R. (2012). Primary cutaneous zygomycosis in India. *Ind. J. Surg.* **74**: 468–475.
24. Kaviya, S., Santhanalakshmi, J., Viswanathan, B., Muthumary, J. and Srinivasan, K. (2011). Biosynthesis of silver nanoparticles using citrus sinensis peel extract and its antibacterial activity. *Spectrochimica Acta Part A: Mole. Biomole Spect.* **79**(3): 594–598.

25. Leon, E.R., Palomares, R.I., Navarro, R.E., Urbina, R.H., Tanori, J., Palomares, C.I. and Maldonado, A. (2013). Synthesis of silver nanoparticles using reducing agents obtained from natural sources (*Rumex hymenosepalus* extracts). *Nanoscale Res. Lett.* **8**(1): 318(1-9).
26. Lockhart, S.R. and Guarner, J. (2019). Emerging and reemerging fungal infections. *Semin. Diagn. Pathol*. **36**(3): 177–181.
27. Maiorano, E., Favia, G., Capodiferro, S., Montagna, M.T. and Muzio, L.L. (2005). Combined mucormycosis and aspergillosis of the oro-sinonasal region in a patient affected by Castleman disease. *Virchows Arch. Int. J. Pathol.* **446**(1): 28–33.
28. Malynych, S., Luzinov, I. and Chumanov, G. (2001). Poly(vinyl pyridine) as universal surface modifier for immobilization of nanoparticles. *J. Phy. Chem. B* **106**(6): 1280–1285.
29. Marr, K.A., Patterson, T. and Denning, D. (2002). Aspergillosis: pathogenesis, clinical manifestations, and therapy. *Infect. Dis. Clin. North Am.* **16**: 875–894.
30. Mie, R., Samsudin, M.W., Din, L.B., Ahmad, A., Ibrahim, N. and Adnan, S.N.A. (2014). Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema praesorediosum. Int. J. Nanomed*. **9**: 121–127.
31. Moon, P. and Jithendran, N. (2018). Invasive fungal infection with *Absidia Corymbifera* in immunocompetent patient with electrical scalp burn. *World J. Plast. Surg.* **7**(2): 249–252.
32. Mostaza, J.M., Barbado, F.J., Fernandez-Martin, J., Pena-Yanez, J., Vasquez-Rodriguez, J.J. (1989). Cutaneoarticular mucormycosis due to *Cunninghamella bertholletiae* in a patient with AIDS. *Rev. Infect. Dis*. **11**: 316–318.
33. Mozhgan, B. (2008). *Synthesis of Noble Metal Nanoparticles*. Ph.D. Thesis, Drexel University, Philadelphia, Pennsylvania.
34. Pacioni, N.L., Borsarelli, C.D., Rey, V. and Veglia, A.V. (2015). Synthetic routes for the preparation of silver nanoparticles: a mechanistic perspective. In: *Silver Nanoparticle Applications, Engineering Materials* (Alarcon, E.I. *et al.* (eds.), Springer International Publishing Switzerland, pp: 13–46.
35. Pal, S., Tak, Y.K and Song, J.M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. *App. Env. Microbiol.* **73**(6): 1712–1720.
36. Person, A.K., Chudgar, S.M., Norton, B.L., Tong, B.C. and Stout, J.E. (2010). *Aspergillus niger*: an unusual cause of invasive pulmonary aspergillosis. *J. Med. Microbiol.* **59**: 834–838.
37. Pierce, C.G., Srinivasan, A., Ramasubramanian, A.K. and Lopez-Ribot, J.L. (2015). From biology to drug development: new approaches to combat the threat of fungal biofilms. *Microbiol Spectr* **3**(3): 1–18.
38. Pulit, J., Banach, M., Szczygłowska, R. and Bryk, M. (2013). Nanosilver against fungi.Silver nanoparticles as an effective biocidal factor. *Acta Biochim. Pol.* **60**(4): 795–798.
39. Quinio, D., Karam, A., Leroy, J.P., Moal, M.C., Bourbigot, B., Masure, O., Sassolas, B. and Le Flohic, A.M. (2004). Zygomycosis caused by *Cunninghamella bertholletiae* in a kidney transplant recipient. *Medical Mycology*, **42**: 177–180.
40. Raffi, M., Hussain, F., Bhatti, T., Akhter, J., Hameed, A. and Hasan, M. (2008). Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. *J. Mater. Sci. Technol.* **24**: 192–196.
41. Rajeshkumar, S., Malarkodi, C., Paulkumar, K., Vanaja, M., Gnanajobitha, G. and Annadurai, G. (2014). Algae mediated green fabrication of silver nanoparticles and examination of its antifungal activity against clinical pathogens. *Inter. J. Metals* **2014**: 1–8.
42. Rit, K., Saha, R., Dey, R. and Barik, G (2014). Rhino-oculo-cerebral *Aspergillus* and *Mucor* co-infections in an immunocompromised patient with type 2 diabetes mellitus. *Med. J. Dr. Patil. Univ*. **7**(4): 486.
43. Roden, M.M., Zaoutis, T.E., Buchanan, W.L., Knudsen, T.A., Sarkisova, T.A., Schaufele, R.L. (2005). Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin. Infect. Dis*. **41**(5): 634–653.
44. Rout, Y., Behera, S., Ojha, A.K. and P.L. Nayak (2011). Green synthesis of silver nanoparticles using *Ocimum sanctum* (Tulashi) and study of their antibacterial and antifungal activities. *J. Microbiol. Antimicrob*. **4**(6): 103–109.
45. Safai, N.S., Dehghan, M.S., Jahanbin, B., Khodaveisi, S., Giasvand, F., Seifi, A. and Salehi, M. (2019). Invasive fungal consecutive infections in a patient with acute myeloid leukaemia. *Niger J. Clin. Pract.* **22**(4): 582.
46. Sondi, I. and Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J. Coll. Inte. Sci.* **275**: 177–182.
47. Spellberg, B., Kontoyiannis, D.P., Fredricks, D., Morris, M.I., Perfect, J.R., Chin-Hong, P.V., Ibrahim, A.S. and Brass, E.P. (2012). Risk factors for mortality in patients with mucormycosis. *Med. Mycol.* **50**(6): 611–618.
48. Stevans, D.A., Kan, V.L., Judson, M.A., Morrison, V.A., Dummer, S., Denning, D.W., Bennett, J.E., Walsh, T.J., Patterson, T.F. and Pankey, G.A. (2000). Practice guidelines for diseases caused by *Aspergillus*. *Clin. Infect. Dis.* **30**(4): 696–709.

49. Tang, D. and Wang, W. (1998). Successful cure of an extensive burn injury complicated with mucor wound sepsis. *Burns* **24**(1): 72–73.
50. Uppuluri, P., Pierce, C.G. and Lopez-Ribot, J.L. (2009). *Candida albicans* biofilm formation and its clinical consequences. *Future Microbiol.* **4**(10): 1235–1237.
51. Walsh, T.J., Anaissie, E.J., Denning, D.W., Herbrecht, R., Kontoyiannis, D.P., Marr, K.A., Morrison, V.A., Segal, B.H., Steinbach, W.J., Stevens, D.A., Burik, J.A.V., Wingard, J.R. and Patterson, T.F. (2008). Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis.* **46**(3): 327–360.
52. Wang, Y., Zhu, M., Bao, Y., Li, L., Zhu, L., Li, F., Xu, J. and Liang, J. (2018). Cutaneous mucormycosis caused by *Rhizopus microsporus* in an immunocompetent patient: a case report and review of literature. *Medicine* (2018) **97**: 25.
53. Xiong, Y.J., Washio, I., Chen, J., Cai, H., Li, Z. and Xia, Y. (2006). Poly(vinyl pyrrolidone): A dual functional reductant and stabilizer for the facile synthesis of noble metal nanoplates in aqueous solutions. *Lang*. **22**(20): 8563–8570.
54. Zayet, S., Zaghdoudi, A., Ammari, L., Kilani, B. and Benaissa, H.T. (2021). Cerebro-rhino-orbital mucormycosis and aspergillosis coinfection in a patient with diabetes mellitus: A case report. *IDCases* **23**: e01022
55. Zhu, S., Du, C.L. and Fu, Y. (2009). Fabrication and characterization of rhombic silver nanoparticles for biosensing. *Opt. Mat.* **31**: 769–774.