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Evaluation of Antimicrobial and Bacteriostatic-Bactericidal Activity of *Nigrospora*-Mediated Silver Nanoparticles Against MDR Strains.

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ABSTRACT

In the present study, *Nigrospora* sp. were isolated from infected *Malus domestica* fruit and identified on the basis of morphological and microscopic characteristics. Morphological, cultural and microscopic features of fungus were found to be significant markers for the preliminary identification. In current study, we synthesized silver nanoparticles (AgNPs) using *Nigrospora* spp. and evaluated their antimicrobial activity against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli*. We also determined the minimum bactericidal concentration (MBC) to minimum inhibitory concentration (MIC) ratio for isolates. AgNPs synthesized from *Nigrospora* spp. demonstrated remarkable antimicrobial activity against clinical isolates of methicillin resistant *S. aureus* (MRSA), *E. coli*, *K. pneumoniae*, *P. aeruginosa and Acinetobacter* sp. Highest value of MBC/MIC ratio is not ≤4. It reflects the both bacteriostatic as well as bactericidal activity with small quantity difference on different MDR isolates.
Keywords: *Nigrospora* sp., Silver nanoparticles, Multi drug resistant bacteria, MRSA

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INTRODUCTION

Nanotechnology has transformed multiple scientific disciplines, particularly in biomedical applications, due to its ability to manipulate materials at the molecular level. Among various nanoparticles, silver nanoparticles (AgNPs) have gained substantial attention for their potent antimicrobial properties, making them effective against multidrug-resistant (MDR) pathogens [1,2]. These pathogens, which exhibit resistance to multiple classes of antibiotics, pose a significant challenge to global healthcare systems, demanding the exploration of alternative therapeutic strategies [3].

Biological synthesis of AgNPs offers a environmentally friendly, sustainable and cost-effective method compared to conventional physical and chemical approaches [4]. Fungi-mediated synthesis is particularly advantageous due to the diverse biochemical capabilities of fungal species, including their ability to secrete extracellular enzymes and bioactive compounds that facilitate the reduction and stabilization of nanoparticles [5]. This study focuses on the synthesis of AgNPs using *Nigrospora* spp., an endophytic fungus isolated from infected Malus domestica fruit [6]. *Nigrospora* spp. is known for its active secretion of biomolecules, which may enhance nanoparticle synthesis and contribute to its stability and functional properties [7].

A crucial aspect of AgNPs lies in their antimicrobial efficacy against MDR bacteria. Traditional antibiotics target specific bacterial mechanisms; however, the advent of resistant strains has portrayed many of these treatments ineffective [8]. In contrast, AgNPs exert antimicrobial activity through multiple mechanisms, including the generation of reactive oxygen species, disruption of bacterial membranes, and interference with intracellular components [3]. Understanding the structural and functional characteristics of these nanoparticles is essential for evaluating their therapeutic potential and optimizing their biomedical applications [9].

By providing a comprehensive investigation into the synthesis and antimicrobial properties of mycogenic AgNPs, this study contributes to the ongoing efforts to develop novel and effective antimicrobial strategies [9]. The findings may hold potential applications in medical device coatings, wound dressings, and pharmaceutical formulations aimed at combating MDR bacterial infections [3].

MATERIALS AND METHODS

Isolation and identification of fungus

The isolated fungi were grown on PDA for 7-8 days and identified on the basis of morphological and cultural characteristics. Different morphological and cultural characteristics such as, growth pattern, hyphae, color of colony and medium, dorsal and ventral color and growth rate of colony, texture of mycelium etc. were observed. The microscopic characters such as fruiting bodies, e.g., pycnidia, conidia, septation, size of macroconidia and microconidia were considered for identification. Type of spore attachment, diameter of spores, attachment of cilia, setae, conidia with sheath, etc were observed and using different keys like Rai's key, Seifert's key [10] and Phoma identification manual by Boerema (2004) [11] used for identification of Phoma species as well as micro fungi on miscellaneous substrate (An identification Handbook) by Ellis (1988) [12] and Illustrated genera of imperfect fungi by Barnett (1972) [13], Barnett and Hunter (1956) [14] were used for identification of other fungi.

Synthesis of AgNPs

Fungal biomass for biosynthesis of AgNPs was grown aerobically in a potato dextrose broth (PDB). The flasks containing 100 ml of PDB were inoculated with different fungi and incubated at 26 ± 2 ^{0}C in incubator. The biomass was harvested after 5-7 days of growth by filtering through muslin cloth and then a Whatman filter paper No. 42. The harvested biomass was rinsed three to four times with distilled water to remove medium component from it. The obtained biomass was suspended and incubated in 100ml of sterilized distilled water for 24 hours at room temperature in Erlenmeyer flasks and agitated at 120 rpm. Again, cell filtrate was isolated by filtration and centrifuged to get rid of the traces of mycelium. Thereafter, the filtrates were treated with 1 mM $AgNO_3$ and kept in sunlight for 1 minute to complete reduction. Only fungal cell filtrate (without treatment with 1 mM AgNO3) as positive control and 1 mM AgNO3 as negative control were also maintained. All the experiments were performed in triplicate.

Characterization of AgNPs

UV-Visible spectroscopy

The initial detection of biosynthesized silver nanoparticles (AgNPs) was indicated by a visible color change in the fungal cell-free filtrate upon treatment with 1 mM silver nitrate. The development of a dark brown color suggested the formation of AgNPs, attributed to surface plasmon resonance. This color change was observed in the medium of the isolated fungal strain. Further confirmation was obtained using a UV-Visible double beam spectrophotometer (Shimadzu UV-1700, Japan), operated at a resolution of 1 nm. The absorbance spectra of the colloidal suspension were recorded in the range of 200–800 nm.

Characterization of AgNPs by Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy, which operates in the mid-infrared region (2.5–25 µm; 4000–400 cm^{-1}), is based on molecular vibrations associated with changes in dipole moments. In this study, FTIR analysis was employed to identify the functional groups of biomolecules potentially involved in the reduction of silver ions to AgNPs and in stabilizing the nanoparticles in colloidal form. FTIR spectra were recorded for both the mycosynthesized AgNPs (experimental) and the fungal cell-free filtrate (control) using a Perkin-Elmer FTIR-1600 spectrometer (USA). Each sample was analyzed in transmission mode at a spectral resolution of 4 cm^{-1}. Dried AgNP powders were scanned across the spectral range of 400–4000 cm^{-1} to detect characteristic absorption bands indicative of biomolecular capping agents.

Characterization of AgNPs by X-Ray Diffraction analysis (XRD)

X-ray diffraction (XRD) is a highly surface-specific analytical technique that provides detailed information on the crystallographic structure, surface properties, and elemental composition of crystalline and polycrystalline materials, based on Bragg's law. For sample preparation, colloidal silver nanoparticles (AgNPs) were centrifuged at 20,000 rpm for 15 minutes, and the supernatant was discarded. This washing step was repeated multiple times to collect a sufficient amount of purified AgNP powder. Thick films were prepared by depositing the AgNPs on a clean glass slide, followed by drying in an oven at 60 °C. The dried films were then subjected to XRD analysis. The diffraction pattern was recorded over a 2θ range of 10° to 80°, using a scanning speed of 10°/min and a chart speed of 20 mm/min, enabling accurate determination of the lattice parameters and crystalline nature of the mycosynthesized AgNPs.

Measurement of Zeta potential of mycogenic AgNPs

Zeta potential reflects the magnitude of electrostatic repulsion between particles in suspension. A higher absolute value of zeta potential generally indicates greater stability of the colloidal system. In this study, the zeta potential of the synthesized AgNPs was measured using a Malvern Zetasizer 3000HS equipped with a zeta dip cell. For sample preparation, the colloidal silver suspension was diluted with 1 mM KCl in a 1:10 ratio to ensure adequate ionic strength. A total volume of 1000 µL of the prepared sample was transferred into a clear disposable zeta cell for the measurement.

Characterization of AgNPs by Nanoparticles Tracking and Analysis (NTA).

Nanoparticle Tracking Analysis (NTA) is a light-scattering technique capable of measuring particles in the size range of approximately 30–1000 nm. In this study, particle size distribution of mycofabricated AgNPs was assessed using the NanoSight LM20 instrument (NanoSight Ltd., UK), as described by Malloy and Carr (2006). The technique involves tracking the Brownian motion of individual nanoparticles suspended in liquid and illuminated by a laser light source. The movement of the particles is captured on video and analyzed to determine their size on a particle-by-particle basis. Size calculations are derived using the Stokes-Einstein equation. The results are typically presented in terms of mean particle size (average size of all particles measured) and mode (most frequently occurring particle size), providing a detailed profile of the nanoparticle population in suspension.

Characterization of AgNPs by Transmission Electron Transmission (TEM).

The crystalline sample interacts with the electron beam mostly by diffraction rather than by absorption. The progress made in TEM has enabled the direct 2 D imaging of particle size, shape and surfaces. Morphology of AgNPs was evaluated using transmission electron microscopy. Samples of the mycosynthesized AgNPs by different fungi were prepared first by sonicating the sample in sonicator (Vibronics VS 80) for 15 minutes. Subsequently, a drop of the silver nanoparticle colloidal solution was placed on carbon coated copper grids, excess of colloidal solution was blotted and later it was allowed to dry under Infrared light for 30 minutes. TEM micrographs were captured by analyzing the prepared grids on Philips CM 200 super twin's TEM instrument operating at 200 kV (0.23 nm resolution). The crystalline nature of metallic AgNPs was confirmed by the particular area electron diffraction pattern.

Bacterial strains

The multidrug-resistant bacterial strains including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter* sp. were obtained from the S. S. Institute of Medical Sciences and Research Centre, Davangere, Karnataka. Bacteria were cultured on nutrient agar and maintained at 4 ^{0}C. The experiments were executed at the Biosafety Laboratory Level 2 (BSL-2) of the S. S. Institute of Medical Sciences and Research Centre, Davangere, Karnataka.

Disc diffusion assay for evaluation of activity against bacteria

All the clinical isolates were screened for sensitivity to antibiotics. It was found that bacteria showed resistance to antibiotics viz, Fluroquinolones, Aminoglycosides, Cephalosporin, Microlides, Cephalosporin+ beta lactamase inhibitor, Beta lactum penicillin, Lincomycin and Glycopeptides. The efficacy of antibiotics was evaluated by disc diffusion assay on Muller Hinton (MH) agar. The AgNPs powder was suspended in water to prepare the homogenized colloidal solution of known concentration. The colloidal solution thus prepared was used for further study.

The antimicrobial activity of AgNPs was assessed by disc diffusion method as per CLSI guideline on MH agar plates for bacteria and MH agar with 2 % extra glucose and 0.5 μg/ml methylene blue (Hi-media, Mumbai) for *Candida* spp. The bacterial cultures were incubated at 37 ^{0}C for 4-6 hrs. using direct colony suspension method until got an optical density equivalent to 0.5 McFarland turbidity standards, which results in yield a suspension having approximately 1 to 2 x 10^{8} CFU/ml. The lawn of culture was prepared on MH agar by using a sterilized cotton swab ensuring even distribution of bacterial cell. Sterile discs (Hi-media, Mumbai) were kept on the lawn and impregnated with 20 μg of AgNPs. The disc diffusion assay was performed against only one representative isolate of each organism viz. *E. coli*, methicillin-resistant (MRSA) *S. aureus, P. aeruginosa*, *K. pneumoniae* and *Acinetobacter* sp. [15]. The uniform lawn of culture was established on MH agar by using sterilized cotton swabs [16]. The zones of inhibition were measured using a scale specifically designed for this purpose (Hi-media, Mumbai). All experiments were performed in triplicate and average zones of inhibition were calculated.

Determination of MIC and MBC

The 'Minimum Inhibitory Concentration' (MIC) and Minimum Bactericidal Concentration (MBC) for multidrug-resistant bacteria were carried out by a microdilution method, using MH broth according to CLSI guidelines. Different concentrations of AgNPs such as 5, 10, 15, 20, 25, 30, 35, 40, 50...100 μg/ml were tested against clinical isolates of multidrug-resistant bacteria with final inoculum of 5×10^{5} CFU/ml. The effect on cell viability was determined after a 16-18 hrs. period of incubation [17]. The MIC value corresponded to the concentration that inhibited 99% of bacterial growth, while the MBC value signifies inhibitory effect of AgNPs with 100 % bacterial growth inhibition. The MBC was further evaluated through colony-forming capacity assay conducted on MH agar [18].

The microdilution plates were incubated at 35 ^{0}C and visible bacterial growth were monitored. Each microdilution well was assessed using a reading mirror, the observed growth in each well has been compared with the control (drug-free) well, following CLSI guidelines for standardized evaluation [19].

RESULTS

Nigrospora sp. was isolated from Malus domestica fruit. *Nigrospora* showed initially grey to dense grayish with wooly mycelia on dorsal side while black on ventral side on PDA plates. The growth rate was found to be 5.6 cm after 7-8 days of incubation at 250±20C. The conidiophores were found small, with dark cells [Fig. 1a]. The conidia were observed single cell on basal cell of conidiophores [Fig. 1b]. The conidia were observed flattened spherical with black in color [Fig. 1a]. The size of conidia ranges from 4.2±0.52 x 3.9±0.48 µm. On the basis of cultural and morphological characteristics the isolated fungus was confirmed as *Nigrospora* spp.

Nigrospora sp. were used for the synthesis of AgNPs. Pale- yellow color of the cell filtrate of *Nigrospora* sp. changes to dark-brown after treatment with silver nitrate which is a sign of the formation of AgNPs (Fig. 2 I A and B). The color change is due to excitation of surface plasmon after the synthesis of AgNPs. Synthesis of AgNPs reveals strong absorption in the visible range of light due to the local surface plasmon resonance. AgNPs synthesized by fungus were characterized by UV-Vis spectra (Fig. 2 II a and b). The synthesis of AgNPs recorded an absorbance peak around 420 nm (plasmon band), which is peculiar characteristic of AgNPs. The detection of a single absorbance peak indicates that there was a synthesis of spherical nanoparticles. For *Nigrospora* sp. distinctive absorbance peak was appeared at 440 nm.

Mycosynthesized AgNPs were subjected to FTIR analysis for further characterization. FTIR scanning of colloidal solution samples were conducted to study the possible interactions between silver nanoparticles and bioactive molecules. These biomolecules likely responsible for the synthesis and stabilization of AgNPs, functioning as a capping agent. In the present spectrum of *Nigrospora* spp., peaks at 1632, 1523 and 1038 [Fig. 3A, spectra (A)] are stretched to 1642,1542 and 1033 respectively [Fig. 3A, spectra (B)]. This specifies the involvement of different functional groups of protein, primarily amino acids, in the synthesis of AgNPs, contributing to their explicit spectral signature in electromagnetic spectrum.

XRD analysis was carried out for the mycosynthesized nanoparticles. The X ray diffraction of the *Nigrospora* spp. sample presented peaks at positions 38^0, $44^{0,}$ and 66^0 of 2θ, matching the (111), (200) and (220) planes of face centered cubic structure of AgNPs. These values align with reference standards mentioned by JCPD (Joint Committee on Powder Diffraction, standard file no. 04-0783). An analogous pattern of FCC structure was found in the nanoparticles synthesized from *Nigrospora* sp. Fig. 3E shows the powder X-ray diffractometry pattern described for AgNPs synthesized from *Nigrospora* sp.

Zeta potential analysis was conducted to study the stability and surface charges acquired by silver nanoparticles. The results indicated that the zeta potential for the AgNPs was found to be negative. It is most likely because of protein capping on AgNPs. This finding is supported by FTIR spectral analysis which revealed the stretching of the bond functional group of amino acids. The greater the zeta potential greater the stability of AgNPs. Zeta potential was found -24.1 mV for *Nigrospora* sp. (Fig. 3B).

Nanoparticle tracking and analysis (NTA) was made by using Nano Sight LM-20. It is used to measure the particle size distribution. The NTA of AgNPs was conducted extensively due to its capability to measure particle size on an individual basis which ensure accuracy and reliability. The NTA images show *Nigrospora* sp. synthesized AgNPs of having an average size diameter of 53 nm, which was calculated based on Brownian motion (Fig. 3C and D).

Further, TEM characterization gives an idea about actual shape and size of AgNPs. TEM revealed that synthesized AgNPs were spherical in shape and size ranging from 7-25 nm (Figure 3F).

Organisms that are resistant to two or more classes of antibiotics are called as multidrug-resistant (MDR) bacteria. Clinical isolates exhibited resistance against Beta lactum penicillin, Lincomycin, Aminoglycosides, Glycopeptides, Fluoroquinolones and Cephalosporin group of antibiotics etc. These MDR bacteria were tested for the antibacterial activity of mycosynthesized AgNPs. In disc diffusion assay antibiotic disc, antibiotic disc impregnated with AgNPs, and only sterile disc impregnated with AgNPs were kept on MH agar plate lawn with MDR. AgNPs synthesized by fungus exhibited antibacterial activity. Clinical isolates including MRSA were sensitive to AgNPs and showed zone of inhibition. *E. coli* showed resistance to antibiotics, while antibiotic discs impregnated with AgNPs demonstrated a zone of inhibition equal to AgNPs disc (Figure 4). AgNPs synthesized from *Nigrospora* sp. developed zone of inhibition ranging from 10-12 mm against all MDR isolates.

MIC was assessed by visual observation of microtiter plates while MBC was evaluated by colony forming capacity of bacteria on MH agar. AgNPs synthesized by *Nigrospora* sp. fungus revealed significant MIC and MBC values ranging from 10 and 50 µg/ml (Table 1). All MDR isolates were susceptible to AgNPs as the MBC/MIC ratio has not been exceeded than 2.

DISCUSSION

Mycosynthesis of AgNPs was found to be rapid and reliable method among biological sources. The pale-yellow color of filtrate changes to brown within 10 min. in sunlight when treated with 1 mM silver nitrate. In reaction mixture Ag^+ of silver nitrate converted to Ag^0. Color change on synthesis of AgNPs was due to the excitation of surface plasmon resonance in silver nanoparticles. In metal nanoparticles electrons can move freely from lower energy state to higher one after coming in contact with visible light. In visible light range, the oscillation of electron takes place, resulting into surface plasmon resonance, and ultimately brown color of solution appears [20]. It was found that both the fungi have potential of AgNPs synthesis. Our findings support the previous study carried out on mycosynthesis of AgNPs [21, 22].

Primary detection of synthesis of AgNPs was made by UV-Vis spectroscopy, which gives single peak of absorbance around 420 nm. It develops by the conversion of bulk material to nanomaterials which is characteristic peak for synthesis of AgNPs [23, 24]. It is well known that there is a very close relationship between size, shape and blue-shift or red-shift of UV-Vis spectrum [25]. AgNPs synthesized by *Nigrospora* sp. gives an absorbance peak at 440 nm, which showed red shift in spectra.

Since the synthesis of AgNPs takes place by fungal filtrate, which contains the biomolecule secreted by fungi provides stability to AgNPs in colloidal solution. The resulting FTIR spectra for both the AgNPs reveal the stretching of bonds in the infra-red region of the electromagnetic spectrum which produces the signature spectra for biomolecules, particularly proteins. In present study we obtained the stretching of bond–C=C–, C-N stretching vibration of aromatic amino acids, amide II and stretching vibration of –C-O-C- bond. It is reported that proteins can bind to nanoparticles through free amino acids, cystein residues or the negatively charged carboxylate group of enzymes present in cell wall of fungi [26, 27, 28, 29]. FTIR spectra revealed the vital role of proteins and amino acids in reduction and capping of silver ions to AgNPs.

XRD revealed the crystalline structure of metallic AgNPs. The nature of the metal particles is found to be "FCC". A similar diffractometry pattern was obtained or AgNPs synthesized from both fungi.

In our study, we observed the negative zeta potential for both the AgNPs. Surface zeta potential provides the characteristic features of stability to AgNPs in colloidal solution. Surface zeta potential is generally measured either in +ve or –ve which prevents particles from aggregation. The greater the zeta potential, the greater the stability of nanoparticles in the solution. If particles in solution possess a higher surface zeta potential particle repels each other and there will be no possibility of aggregation [30]. As the zeta potential lower the particles tend to aggregate and settle down and lose their property of nanomaterials. The AgNPs synthesized from -24.1 mV, which explains the stability of AgNPs in colloidal solution.

In the present study, the size of AgNPs was determined by NTA and TEM. NTA can focus the individual particles in a colloidal solution because particle size measurement depends upon the Brownian motion of the particles, which ultimately depends upon the size of nanoparticles. The NTA results in a large size due to the hydrodynamic radius of particles; on the other hand, TEM scrutinizes only the metallic core of nanoparticles and has a high-resolution capacity [31]. In other words, the nanoparticle size increases due to protein capping on them which is always larger than TEM. Similar to NTA, scanning electron microscopy also visualizes the larger particles due to lower resolution power. NTA results in to average size of particles dispersed in a colloidal solution while TEM gives an idea about exact size and shape of AgNPs. TEM shows a size ranging from 7-25 nm which is much smaller than NTA result.

Silver compounds have been used in medicine as the silver ions or silver salts possess the potent antimicrobial activity. Still, silver salts are used as a therapeutic agent in thermal injury to prevent the wound infection. Multidrug- resistant organisms are easily transferred among patients by hand-to-hand contact with the workers, medical utensils, catheters, and beds. In the present study, we intended to study antimicrobial activity of silver in nano form through different methods against the multi-drug resistance bacteria recovered from clinical isolates, which are life threatening manifestations. We evaluated antimicrobial potential of AgNPs against one *Acinetobacter* spp., *E. coli*, *P. aeruginosa*, *K. pneumoniae* and MRSA.

Firstly, for routine antibiotic sensitivity tests i.e. in disc diffusion assay, sterile discs impregnated with 20 µg/ disc of AgNPs and the antibiotic disc also impregnated with 20 µg AgNPs, synthesized from both the fungi, form approximately similar zone of inhibition (10-12 mm). It clearly reveals that mycosynthesized AgNPs possess potent antimicrobial activity. Our findings are corroborated with Durairaj et al. [32], who reported the zone of inhibition of 11 mm against the MDR *P. aeruginosa* with 10 µg/disc concentration. While no significant difference was found in the zone of inhibition among Gram- positive and Gram-negative bacterial strains (disc diffusion assay), which indicates that AgNPs are a broad-spectrum novel antimicrobial agent. It is who reported that AgNPs exert similar effects on both Gram positive and Gram-negative strains of bacteria [33]. As the AgNPs showed broad spectrum antimicrobial activity, all MDR strains were subjected to MIC and MBC assay. MBC/MIC ratio greater than one signifies that a larger amount of drug is needed to kill 100 % bacteria. If the value is 1 it is a sign of bactericidal property of compound. In our study, highest value of MBC/MIC ratio is not ≤2. It reveals both bacteriostatic as well as bactericidal activity with small quantity difference on different MDR isolates. In the present study, MBC value for *S. aureus* is much higher, ranging from 30 -100 µg/ml as compared to other bacteria. It provides evidence that for inhibition of Gram-positive bacteria, more quantities of AgNPs are required. In this context, many researchers reported that Gram-positive bacteria need higher concentrations of AgNPs as compared to Gram-negative bacteria for growth inhibition. The difference is probably due to the thick cell wall of peptidoglycan and having structural complexity, which is the main barrier to penetration of AgNPs into the cell [34,35]. TEM images revealed that AgNPs get easy entry through thin peptidoglycan into the interior of *E. coli.* Inside cell core AgNPs are surrounded by biological materials and develop a large zone of electron translucent cytoplasm that results in to the separation of cell membrane from cell wall. On the other hand, AgNPs were attached to cellular wall of *S. aureus* and surrounded by lighter biological material. Resistant to antibiotics and susceptibility to AgNPs clearly revealed that the mode of action and targets of AgNPs are definitely different than that of all groups of antibiotics. Inhibitory concentration of AgNPs, synthesized from *Nigrospora* sp., for drug susceptible *S. aureus, E. coli* and one of MRSA and drug-resistant *E. coli* was different. It can be said that multidrug resistance property honored by number of proteins do not affect the inhibitory efficiency of AgNPs. The reports by Ayala-Núñez et al. also supports to our study that AgNPs do not bind to any specific structure or protein of cell of drug sensitive and resistant bacteria but have a broad-spectrum target like cytoplasmic protein, genomic DNA or plasmid [36]. The mechanistic aspect of bactericidal effect of AgNPs is yet not clearly understood. The mode of action for AgNPs is independent of contact to the bacteria and the bactericidal activity is due to the gradual release of silver ions from zeolite membrane [37]. Silver ions also form complex with electron donor groups like sulfur, nitrogen, oxygen, which are normally present in and nucleic acid structure [38]. Silver ions also bind to the sulfhydral group which results to the protein denaturation by reducing disulphide bond. The main target site for AgNPs is bacterial membrane leading to a dissipation of the proton motive force which increases permeability of membrane thus finally killing the bacteria [39]. AgNPs act as vehicle to carry the silver ions up to the bacterial membrane ultimately whose protein motive force lowering pH up to 3 and enhance the release of silver ions [40]. AgNPs interact with phosphorous and sulfur containing component like proteins of membrane, disrupt cell membrane, attack respiratory chain, binding and dimerizing DNA and RNA leading to death of bacteria [41]. Cell membrane is the site of respiratory chain, active transportation, anchoring point of DNA and cell division. Dense AgNPs were observed around cell membrane with significant morphological changes and completely broken cell membrane [42]. TEM images of *E. coli* showed the AgNPs present at membrane and interior of cell. It clears that AgNPs get entry inside the cell by damaging cell membrane and interfere with the cytoplasmic content [43]. It is found that AgNPs located in membrane AgNPs on *E.coli*, *P. aeruginosa*, *Salmonella typhi*, *Vibrio cholerae* and [44]. The authors proposed the mechanism on size dependent activity of silver nanoparticles. According to them, binding strength of nanoparticles depend on the surface area to volume ratio. The small particles possess electronic effect, which changes the local electronic surface due to size, which enhances the reactivity of surface. Similarly, The rate of silver ions release is faster from smaller AgNPs than bigger one in AgNPs coated zeolite membrane [37].

CONCLUSION

This study highlights the successful mycosynthesis of silver nanoparticles (AgNPs) using *Nigrospora* spp., establishing a green, cost-effective, and environmentally sustainable approach with minimal downstream processing. To the best of our knowledge, this is the first report detailing the biosynthesis of AgNPs from *Nigrospora* spp. and their antimicrobial activity against multidrug-resistant (MDR) pathogens. The biosynthesized AgNPs demonstrated broad-spectrum antimicrobial efficacy, underscoring their potential as alternative therapeutic agents.

MDR in pathogens typically arises through genetic mutations, horizontal gene transfer, and repeated exposure to antibiotics. These organisms employ diverse resistance strategies, with biofilm-forming bacteria exhibiting particularly high resilience. Notably, the antimicrobial mechanism of AgNPs differs from that of conventional antibiotics, potentially circumventing common resistance pathways.

Our findings support the application of mycogenic AgNPs in the development of antimicrobial coatings for medical devices such as catheters, surgical tools, and hospital textiles, thereby aiding in infection control. Moreover, their unique mode of action positions AgNPs as promising candidates for future exploration as drug delivery systems.

Figure: 1. Morphological identification of Nigrospora sp. a) Conidia embedded in mycelia, b) Conidia attached to the basal cell of conidiophore.

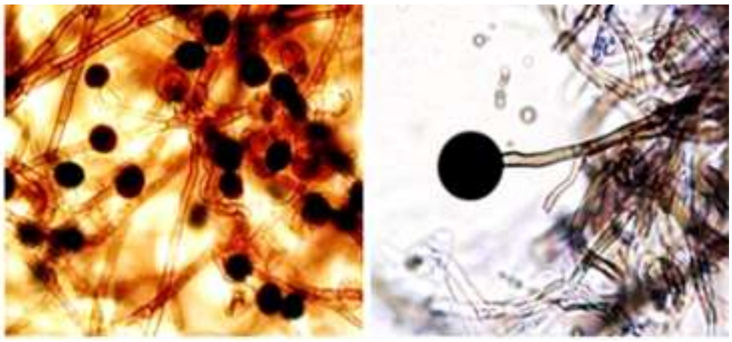


Figure: 2. Figure 3.6: Synthesis of AgNPs by using Nigrospora sp. (I): (A) Fungal filtrate (Control); (B) colour change of fungal filtrate after treatment with 1 mM AgNO3 (Experimental) (II): UV-Visible spectra recorded for AgNPs (A) Control (fungal filtrate); (B) Experimental (fungal filtrate + AgNO3).

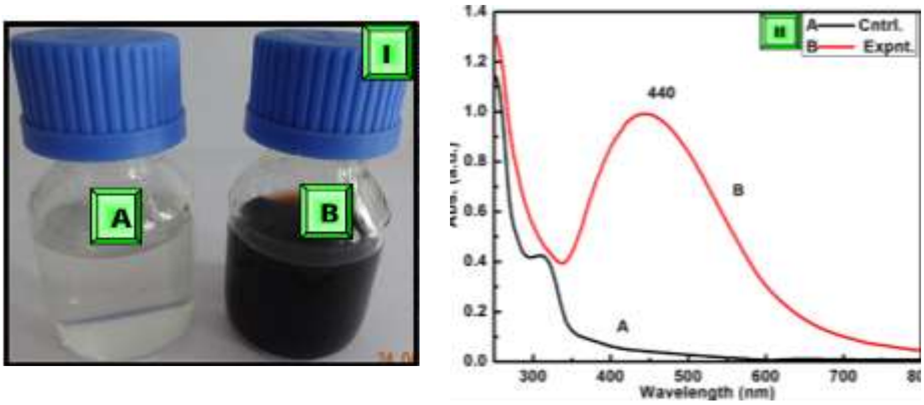
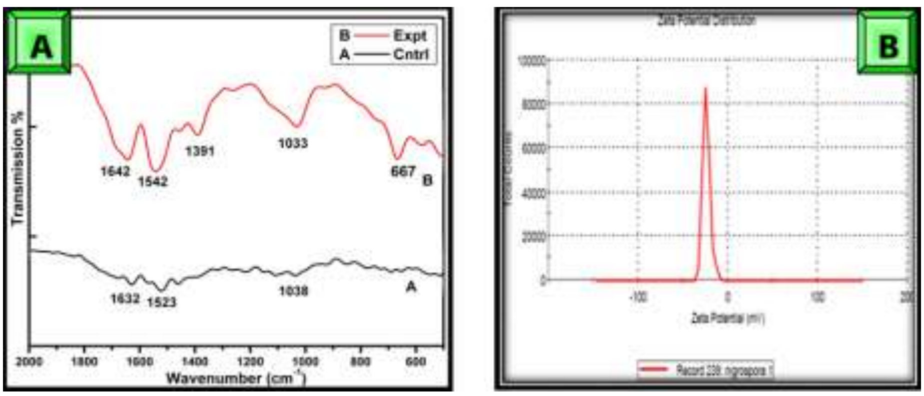


Figure: 3. Characterization of mycosynthesized AgNPs from Nigrospora sp. where, (A) FTIR spectrum, (B) Zeta potential, (C) NTA Particle size distribution, (D) NTA 3D plot of Particle size/ relative intensity, (E) XRD pattern, (F) TEM micrograph (scale bar 20 nm).



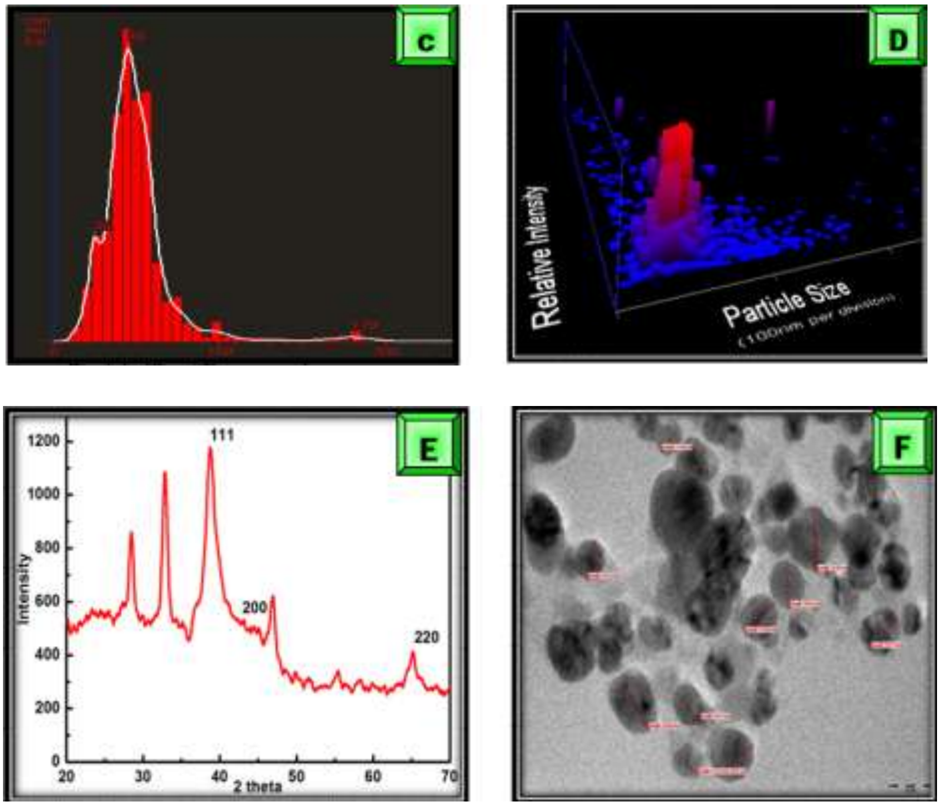


Figure 4: Antibacterial activity of AgNPs synthesized from, *Nigrospora* sp., against MDR *E. coli* Where, a- AgNO3 – 1 mM silver nitrate c- GEN + AgNPs e – Antibiotic (GEN) b- AgNPs – Silver nanoparticles d- CXM + AgNPs f- Antibiotic (CXM)

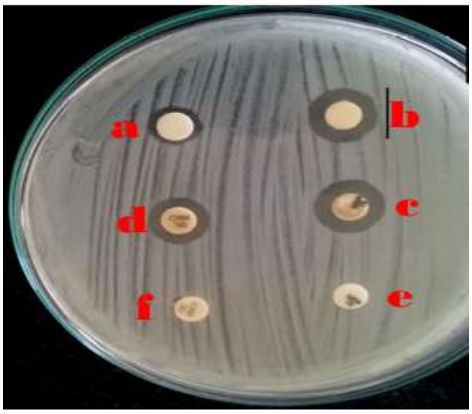


Figure: 5. Antibiogram of silver nanoparticles against multidrug resistant bacteria. Silver nanoparticles synthesized by *Nigrospora* sp.

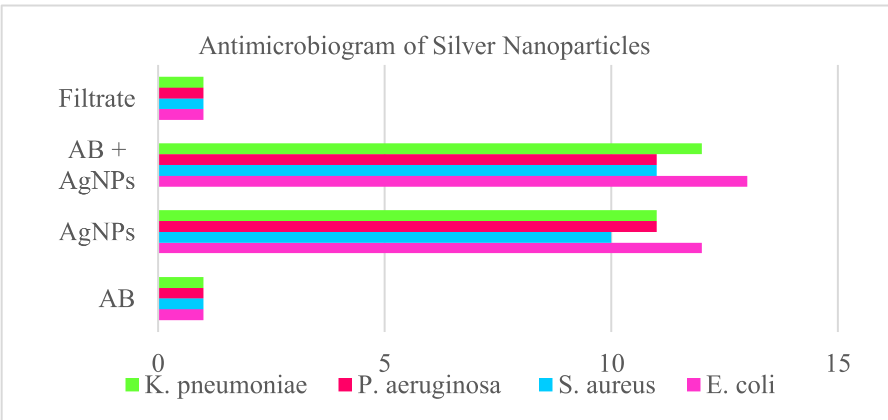


Table 1: MIC and MBC (µg/ml) of Mycosynthesized Silver nanoparticles tested against multi drug resistant bacteria (clinical isolates)

Sr. No.	MDR Bacteria	AgNPs synthesized by *Nigrospora* spp.		
		MIC	MBC	MBC/MIC ratio
1	*E. coli*	20	21	1
2	*P. aeruginosa*	10	20	2
3	*K. pneumoniae*	10	21	2.1
4	*S. aureus*	21	50	2.3
5	*Acinetobacter* spp.	20	21	1.08

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