

Original Article

“MYCO SYNTHESIS OF SILVER NANOPARTICLES USING *GLIOCLADIUM ROSEUM* (*CLONOSTACHYS ROSEA* (LINK) SCHROERS, SAMUELS) AND ITS ANTIMICROBIAL EFFICACY AGAINST SELECTED PATHOGENS”

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ABSTRACT

Objective: Antimicrobial efficacy of silver nanoparticles from *Gliocladium roseum*, culture filtrate (C. F.) and mycelial mat extract (M. E.) against selected pathogens.

Methods: Culture filtrate (C. F.) and Mycelial mat extract (M. E.) of *Gliocladium roseum* were subjected to 10 Mm silver nitrate solution for the synthesis of silver nanoparticles. Formed silver nanoparticles were evaluated via UV-vis spectroscopy and the structural elucidation was done by FT-IR and TEM. Antimicrobial efficacy was tested against bacterial (*Salmonella typhi* and *Klebsiella pneumonia*) and fungal (*Cladosporium cladosporioides* and *Alternaria alternata*) pathogens. Different nanoparticle concentrations-50, 100, 150 and 200 µl were checked via disc diffusion method.

Results: *Gliocladium roseum* (C. F. and M. E.) on interaction with silver nitrate solution effectively reduced metallic silver exhibiting a colour change from yellow to dark brown within 24 h due to the formation of silver nanoparticles. The UV-vis spectrum of C. F. and M. E. showed maximum absorption peaks at 350-400 nm and 400-450 nm respectively and FT-IR and TEM showed strong N-H bonding and spherical shaped silver nanoparticles with the size of 11-19 nm (C. F.) and 25-38 nm (M. E.). Antimicrobial analysis resulted in efficient inhibitory activity against *Salmonella typhi*, *Klebsiella pneumonia* and also showed moderate inhibitory activity against *Alternaria alternata* and *Cladosporium cladosporioides*.

Conclusion: The synthesis of silver nanoparticles from fungus *Gliocladium roseum* is simple, cheap, safe and eco-friendly thus emphasising on large scale scientific application.

Keywords: *Gliocladium roseum*, Silver nanoparticles, Culture filtrate (C. F.), Mycelial mat extract (M. E.), Antimicrobial activity

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INTRODUCTION

Microorganisms have a salient role in the present world. They are used for various purposes in different industries like chemical industry, food industry, medicinal fields, environmental benefits etc. Despite of tremendous progress, they are deleterious to animals, human beings and plants. Human beings and plants are often infected by microorganisms such as bacteria, moulds, yeasts and viruses present in their living environments. Innovation of multiple antibiotic-resistant microorganisms as well as the appearance of undesirable side effects of certain antibiotics forced to develop new effective antimicrobial agents that can overcome the resistances of these microorganisms and are also cost-effective. Nowadays, development of nanotechnology-based medicines has opened a new territory to confront multidrug resistance in microorganisms and its alleviated obstacles [1-4].

Nanoparticles are of enormous scientific interest as they link the gap between bulk materials and atomic or molecular structures [5]. Among various nanoparticles, metal nanoparticles are the most promising ones and amid silver nanoparticles form prime product in the field of nanotechnology. Silver metal comprises strong inhibitory effect of bacterial cell as well as broad spectrum of antibacterial, antiviral, antifungal, in addition to anti-inflammatory activities [6]. Furthermore, nanosilver is comparatively less reactive than silver ions, so it is used for clinical and therapeutic applications [7, 8].

Silver nanoparticles have the ability to anchor the bacterial cell wall and actively penetrate it thereby causing structural changes in the cell membrane and it lead to the cell death. The formation of free radicals by the silver nanoparticles is scrutinized as another mechanism of cell death. Electron spin resonance spectroscopy studies revealed that formation of free radicals had the ability to damage the cell membrane and make it porous which can ultimately lead to cell death [9].

Fungi produce a large amount of nanoparticles because they can secrete large amounts of protein which is directly in cooperated with the higher productivity of nanoparticles [10]. Fungi include organisms like molds, yeasts and mushrooms; some of them may cause disease in humans and inflict losses on crops and others provide essential nutrients for the growth of the plants. Fungi are used in the production of chemicals and also in the drug manufacturing industries. The mechanism of silver nanoparticles production by fungi is trapping of Ag⁺ ions at the surface of the fungal cell and subsequent reduction of silver ions by enzymes like naphthoquinones and anthraquinones present in the fungal system [11].

Gliocladium roseum is an unusual hyphomycete that produce one-celled conidia on two distinct types of conidiophores, one penicillately branched and the other verticillately branched [12]. *Gliocladium roseum* is used as a biocontrol agent against various pathogens in different crops and is reported to have remarkable efficiency, dependability, cost-effectiveness, and safety in plant disease management [13-15]. The present study aimed to analyse the antimicrobial ability of bio-synthesized silver nanoparticles from *Gliocladium roseum* and against selected bacterial and fungal organisms.

MATERIALS AND METHODS

Isolation of fungus *Gliocladium roseum* and production of mycelia free media

Gliocladium roseum was provided from Kerala Forest Research Institute, Peechi, Thrissur, Kerala. To prepare biomass, fungus was grown aerobically in antibiotic amended PDA medium at 25±2 °C. From the actively growing regions 10 mycelial discs (7 mm) were cut and inoculated into an Erlenmeyer flask of 250 ml capacity and incubated at 25±2 °C for 15 d. After 15 d of incubation, mycelia were

separated from the culture broth by filtration through Whatman filter paper No.1. 15 ml of broth culture were filtered and the filtered broth was denoted as culture filtrate (C. F.). Separated mycelial mat was then kept for air drying. Dried mycelial mat was grinded in sterile distilled water and centrifuged at 10000rpm for 10 min. The supernatant was separated out and denoted as mycelial mat extract (M. E.).

Preparation of silver nitrate solution (10 Mm)

0.1698 gm of Silver Nitrate was added to 100 ml of sterile distilled water. Solution was stirred continuously until the silver nitrate dissolved completely. Being light-sensitive, the solution was kept in darkness and stored at 4 °C till further use.

Synthesis of silver nanoparticles

15 ml Silver nitrate solution was aseptically transferred into *Gliocladium roseum* mycelial mat and culture filtrate extracts. The extracts were then kept in darkness at 28 °C for seven days.

Characterization of synthesized silver nanoparticles (UV-vis spectroscopic studies)

The samples were subjected to UV visible spectra analysis on a Perkin-Elmer (lambda-25) spectrophotometer and the absorption maxima were analyzed at wavelength 300–600 nm. The de-ionized water was kept as blank.

FTIR analysis

FTIR analysis was performed on synthesized fungal (C. F. and M. E.) silver nanoparticles to identify the functional groups of the chemical components of these samples. Fourier transform infrared spectroscopy (FTIR) studies were carried out using a Bruker Vertex 70 spectrophotometer.

Transmission electron microscopy (TEM)

The size and shape of synthesized silver nanoparticles were visualized through the 200 kV Ultra High-Resolution Transmission Electron Microscope. TEM grids were prepared by placing a drop of the particles solution on a carbon-coated copper grid and drying under lamp.

Evaluation of antimicrobial activity

Test bacteria

Klebsiella pneumonia and *Salmonella typhi* were selected to assess the activity of silver nanoparticles because these organisms are frequently involved in hospital-acquired infections [16].

Test fungus

Cladosporium cladosporioides and *Alternaria alternata* were selected to assess the activity of silver nanoparticles because these organisms frequently cause plant foliar diseases [17].

Antibacterial assay

Evaluation of the antimicrobial activity of synthesized fungal silver nanoparticles (C. F. and M. E.), were carried out by disc diffusion method against two human pathogenic bacteria, namely-*Klebsiella pneumonia* and *Salmonella typhi* and two plant foliar fungal pathogens *Cladosporium cladosporioides* and *Alternaria alternata*. The discs were loaded with 50, 100, 150 and 200 µl of silver nanoparticle solution. Fungal extracts were taken as positive control and silver nitrate solution as the negative control. The Nutrient agar and Potato dextrose agar plates were swabbed with bacterial and fungal cultures, respectively. Prepared discs were placed on agar plates. Bacterial plates were incubated at 37 °C for 24-48 h and fungal plates were incubated at 25±2 °C for 7-14 d and observations were recorded.

RESULTS

After mixing of aqueous AgNO₃, the C. F. and M. E. of *Gliocladium roseum* showed a colour change at room temperature from yellowish to dark brown within 24 h and later colour is due to Surface Plasmon resonance (SPR) characteristics of silver nanoparticles and control set not showed any colour change within the same environment. The presence of AgNO₃ was confirmed by an Extinction spectroscopy of ultraviolet (UV) and visible (Vis) light (UV-Vis spectrum). The UV-vis spectrum of silver nanoparticles synthesised from mat extract and culture filtrate of *Gliocladium roseum* showed maximum absorption peaks at 350-400 nm and 400-450 nm, respectively (fig. 1 and 2).

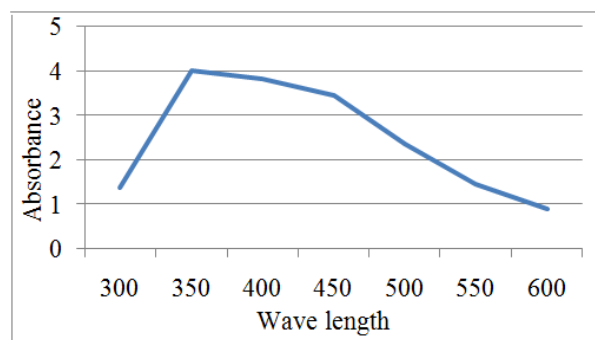


Fig. 1: UV-vis spectra analysis of silver nanoparticles of *Gliocladium roseum* mycelial mat extract

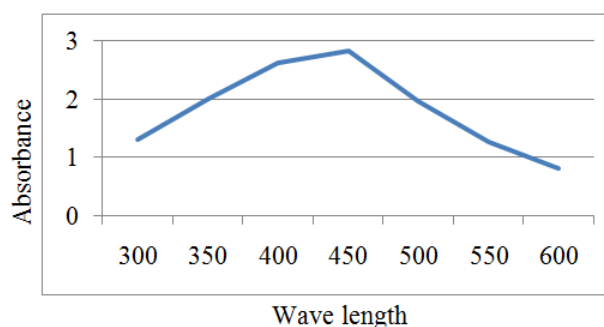


Fig. 2: UV-vis spectra analysis of silver nanoparticles of *Gliocladium roseum* culture filtrate

The results of UV-vis spectra confirmed by using transmission electron microscope. TEM was used to view the morphology and size of silver nanoparticles. C. F. of *Gliocladium roseum* produced

spherical-shaped nanoparticles of size 11-19 nm whereas M. E. formed nanoparticles were of 25-38 nm in size and of spherical to subspherical in shape (fig. 3 and 4).

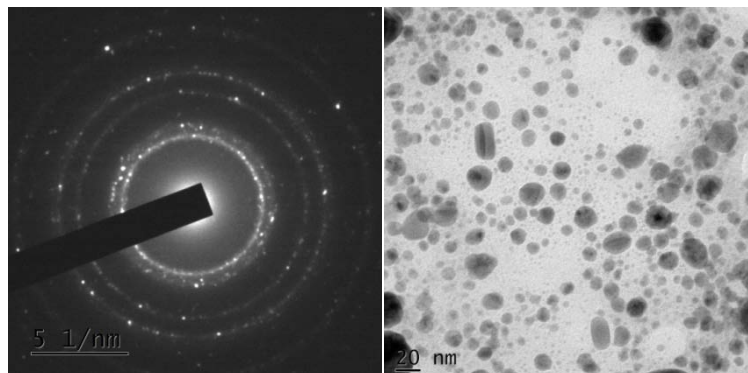


Fig. 3: TEM analysis of silver nanoparticles of *Gliocladium roseum* culture filtrate

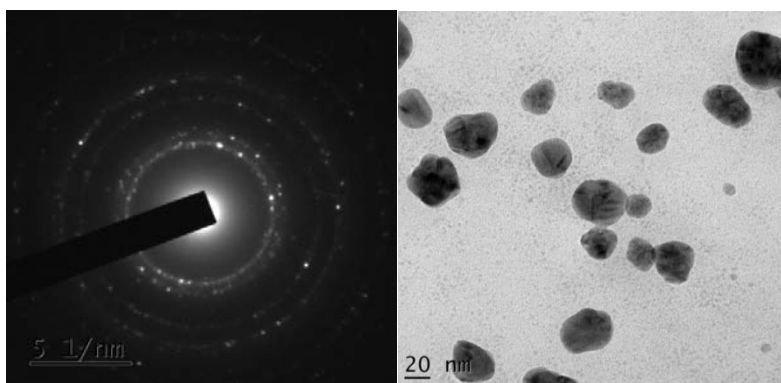


Fig. 4: TEM analysis of silver nanoparticles of *Gliocladium roseum* mycelial mat extract

The FTIR spectra obtained shows the spectral absorption peaks for different extracts in the region 500–4000/cm. The analysis for *G. roseum* C. F. revealed strong N-H group assigned to primary amines for 3448.47/cm, a weak C=C terminal alkynes at

2075.88/cm, a medium C-N bond at 1636.74/cm, a weak N-O bond assigned to methyl group at 1385.70/cm and a medium to strong chloroalkanes or bromoalkanes group at 563.54/cm (fig. 5).

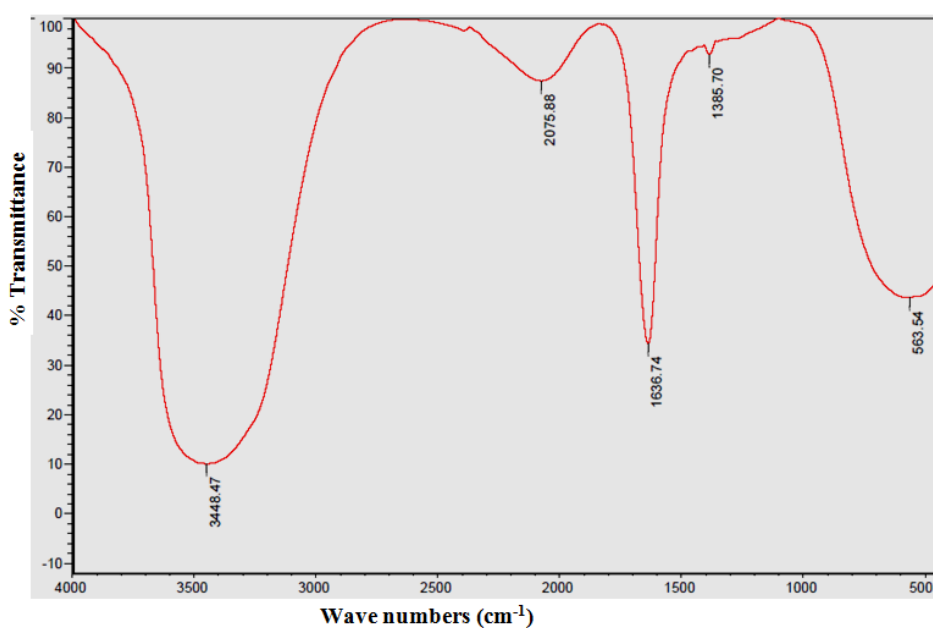


Fig. 5: FT-IR analysis of silver nanoparticles of *Gliocladium roseum* culture filtrate

The analysis for *G. roseum* M. E. revealed strong N-H group assigned to primary amines for 3448.47/cm, a weak C≡C terminal alkynes at 2075.88/cm, a medium C-N bond at 1636.74/cm, a weak N-O bond assigned to methyl group at

1384.85/cm, a medium P-C bond assigned to aromatic organophosphorus compounds at 1460.81/cm and a medium to strong chloroalkanes or bromoalkanes group at 572.13/cm (fig. 6).

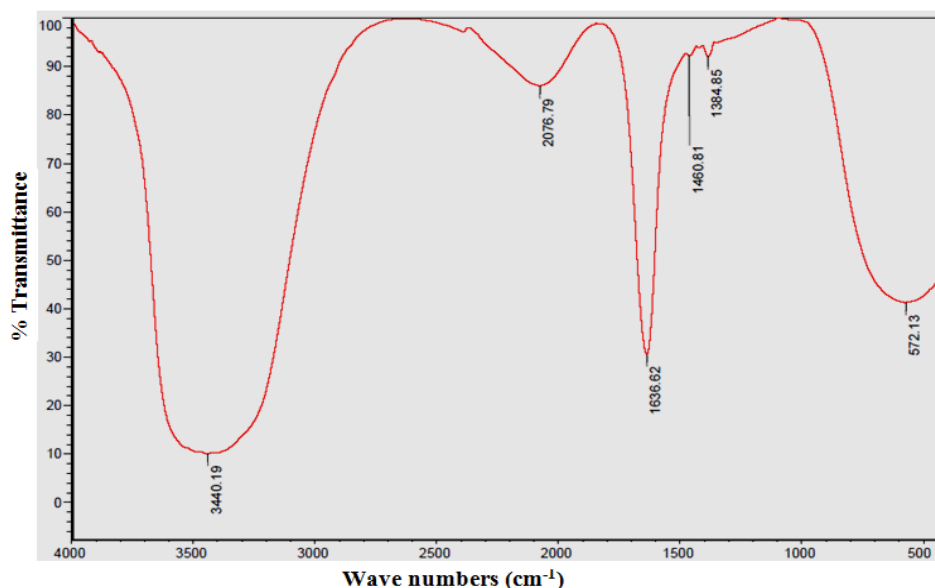


Fig. 6: FT-IR analysis of silver nanoparticles of *Gliocladium roseum* mycelial mat extract

Antimicrobial assay

The synthesised silver nanoparticles, fungal extracts (C. F. and M. E.) and silver nitrate solution were tested for their antimicrobial activity against two human pathogenic bacteria, *Salmonella typhi* and *Klebsiella pneumoniae* and two plant foliar fungal pathogens *Cladosporium cladosporioides* and *Alternaria alternata* by disc diffusion method using different concentrations-50, 100, 150 and 200 μ l.

Antibacterial activity

Silver nanoparticles (Snp. C. F. and Snp. M. E.) of *Gliocladium roseum* showed considerable inhibition zone against *Salmonella typhi* and *Klebsiella pneumoniae*. Culture filtrate (Snp. C. F.) of *Gliocladium roseum* showed inhibition of 7 mm against *S. typhi* at 200 μ l but against *K. pneumoniae* 6 mm inhibition zone was noticed. Similarly Mat extracts (Snp. M. E.) *G. roseum* exhibited 9 mm zone of inhibition against both the bacterial species respectively (table 1 and fig. 7-10).

Table 1: Antibacterial activity of silver nanoparticles of *Gliocladium roseum* against selected human pathogenic bacteria

S. No.	Antibacterial activity of silver nanoparticles of <i>Gliocladium roseum</i> (mm)				
	Different concentration	<i>Salmonella typhi</i>		<i>Klebsiella pneumoniae</i>	
		C. F.	M. E.	C. F.	M. E.
1	50	3	6	5	6
2	100	5	7	5	7
3	150	7	7	6	8
4	200	7	9	6	9
5	AgNO ₃	5	5	5	5
6	Control	Nil	Nil	4	4

Antibacterial activity of silver nanoparticles

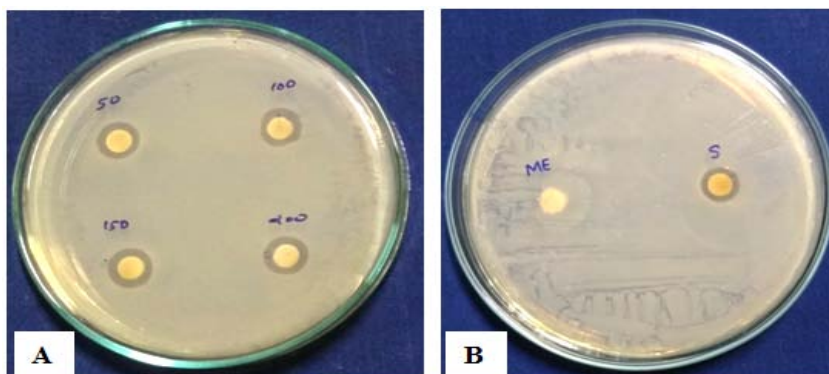


Fig. 7: (A) *Gliocladium roseum* mycelial mat extract silver nanoparticles against *Salmonella typhi* (B) Control

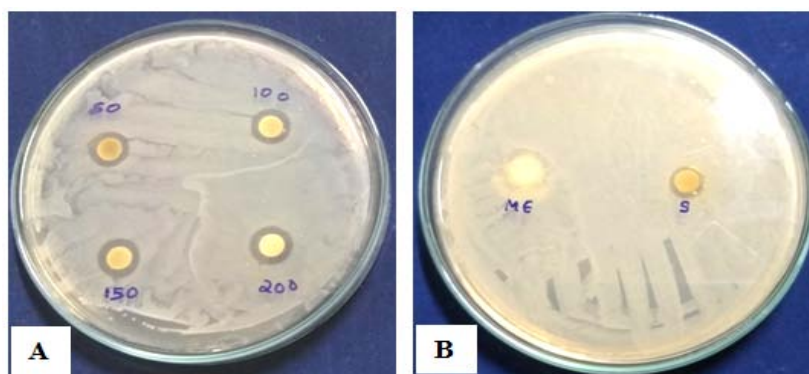


Fig. 8: (A) *Gliocladium roseum* mycelial mat extract silver nanoparticles against *Klebsiella pneumoniae* (B) Control

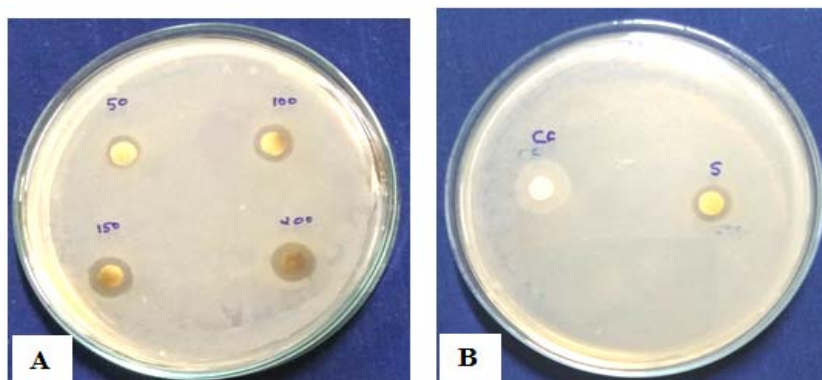


Fig. 9: (A) *Gliocladium roseum* culture filtrate silver nanoparticles against *Salmonella typhi* (B) Control

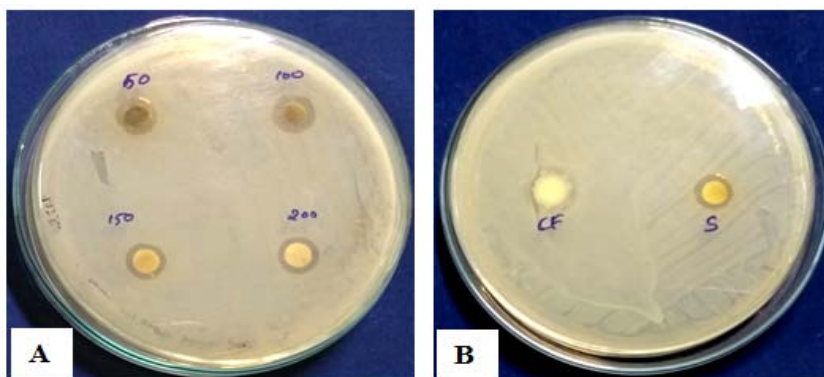


Fig. 10: (A) *Gliocladium roseum* culture filtrate silver nanoparticles against *Klebsiella pneumoniae* (B) Control

Table 2: Antifungal activity of silver nanoparticles of *Gliocladium roseum* against selected plant foliar fungal pathogens

S. No.	Antifungal activity of silver nanoparticles of <i>Gliocladium roseum</i> (mm)					
	Different Concentrations	<i>Cladosporium cladosporioides</i>		<i>Alternaria alternata</i>		
		C. F.	M. E.	C. F.	M. E.	
1	50	4 mm	4 mm	Nil	4 mm	
2	100	4 mm	5 mm	3 mm	4 mm	
3	150	5 mm	5 mm	3 mm	5 mm	
4	200	5 mm	6 mm	4 mm	5 mm	
5	AgNO ₃	5 mm	5 mm	4 mm	5 mm	
6	Control	Nil	Nil	Nil	Nil	

Antifungal activity

Silver nanoparticles (Snp. C. F. and Snp. M. E.) of *Gliocladium roseum* were also tested for their antifungal activity against plant foliar

pathogens-*Alternaria alternata* and *Cladosporium cladosporioides*. Nanoparticles of both fungal extracts showed a moderate inhibitory activity against studied pathogens (table 2 and fig. 11-14).

Antifungal activity of silver nanoparticles

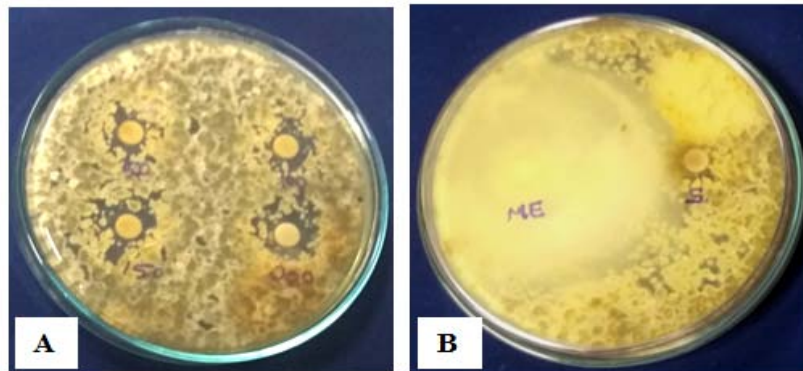


Fig. 11: (A) *Gliocladium roseum* mycelial mat extract silver nanoparticles against *Cladosporium cladosporioides* (B) Control

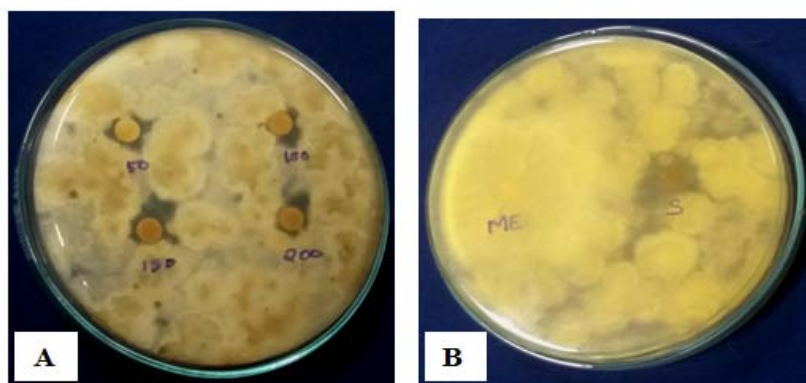


Fig. 12: (A) *Gliocladium roseum* mycelial mat extract silver nanoparticles against *Alternaria alternata* (B) Control

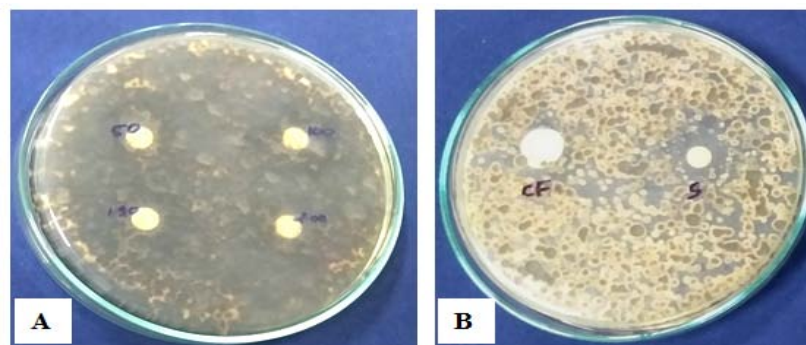


Fig. 13: (A) *Gliocladium roseum* culture filtrate silver nanoparticles against *Cladosporium cladosporioides* (B) Control

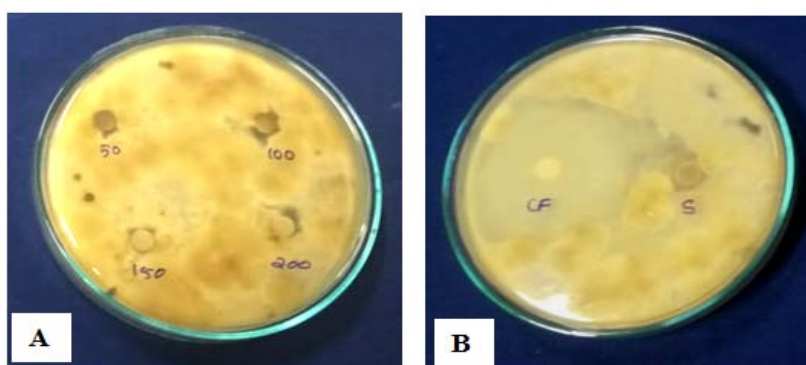


Fig. 14: (A) *Gliocladium roseum* culture filtrate silver nanoparticles against *Alternaria alternata* (B) Control

DISCUSSION

Gliocladium roseum popularly, a bio-control agent, has been found associated with other peculiar abilities as well. *G. roseum*, an endophytic fungus, possessing different kinds of compounds that could act as mycodiesel [18] and also the ability to synthesise different compounds on different growing media exhibiting versatility of the fungus in various scientific applications [19]. Current approach evaluated silver nanoparticle production by the fungus and its antimicrobial potential. Synthesis of nanoparticle using microorganisms such as bacteria, fungi, and plants, or the byproducts of their metabolism has been described by [20]. Advantages of fungal cultures over bacteria and plants were described by [21, 22] and also they produce large quantities of proteins and enzymes, some of which can be used for the fast and sustainable synthesis of nanoparticles [23, 24]. The method of synthesis of silver nanoparticles using fungi were reported by various researchers [25-28].

Fungal culture filtrate and mat extract exhibited colour change reducing silver ions at room temperature from yellow to dark brown within 24 h and later colour is due to Surface Plasmon resonance (SPR) characteristics of silver nanoparticles [29, 30] which is correlated with the results obtained by Mukherjee et al. 2001. Silver nanoparticles production was again confirmed via UV-Vis spectrophotometry exhibiting a peak range between 350-450 characteristic to silver nanoparticle [31-33].

FTIR analysis revealed the presence of biomolecules and its different functional groups which reduce the silver nitrate and bind onto the NPs and helps in its stabilization [34, 35]. The broad band at the range of 3400 cm^{-1} was attributed to the -NH stretching of amines and the transmittance in this region was due to bonds with nitrogen atoms, which are the binding sites for silver [36]. The weak bands at range 2000 cm^{-1} and 525 cm^{-1} were attributed to the presence of CN and the C-O stretching of aliphatic esters [37].

On the evaluation of the formed nanoparticles via disc diffusion method over microbes exhibited varied activity with significant inhibition over bacterial agents but a moderate to low inhibition over fungal organisms. Similarly, Mat extract was found to exhibit higher inhibitory activity compared with culture filtrate more over the formed silver nanoparticle size also higher format extract compared with that of culture filtrate. *Aspergillus terreus*, fungus-mediated green synthesis of silver nanoparticles and its antimicrobial activity by disc diffusion assay resulted in efficient activity against various microbes namely *P. aeruginosa*, *S. aureus*, *E. coli*, *C. albicans*, *C. Krusei*, *C. tropicalis*, *A. fumigates* and *A. flavus* [38]. The antibacterial activity of silver nanoparticles against various bacterial species such as *P. aeruginosa*, *K. pneumoniae* and *B. Cereus* etc have been reported by various authors [39, 40]. The variation in the diameter of the zone of inhibition against bacterial species may be due to the difference in their cell wall composition [41]. *In vitro* analysis of *Trichoderma harzianum* silver nanoparticles efficiently inhibited bacteria *Staphylococcus aureus* and *Klebsiella pneumonia* [41]. Efficacy of silver nanoparticles using the fungus *Guignardia mangiferae* was evaluated against gram-negative bacteria [42]. *Aspergillus flavus* nanoparticles were found to be effective in controlling the bacteria *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, and *Staphylococcus aureus*, with *B. subtilis* and *E. coli* being most sensitive and against the fungi *Aspergillus niger* and *Trichoderma harzianum* [43]. *Aspergillus versicolor* nanoparticles were found to be effective against *Sclerotinia sclerotiorum* and *Botrytis cinerea* in strawberry plants [44]. Antifungal activity of silver nanoparticles was observed against a wide range of fungi including *Gloeophyllum abietinum*, *G. trabeum*, *Chaetomium globosum*, *Phanerochaete sordid*, *Fusarium oxysporum*, *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea*, *Curvularia lunata*, *Aspergillus niger*, *A. flavus* and *A. fumigatus* [45-49].

Nanotechnology or Nano-science is an important branch of modern science technology with applications in different fields of science. Different types of metallic nanoparticles can be used; among them, silver nanoparticles gave emphasis to modern world because of their broad-spectrum antimicrobial potential [50-53]. Current scenario

also emphasised the potentiality of *Gliocladium roseum* ability to synthesise silver nanoparticles and its efficacy against microbial agents emphasizing large scale industrial application but peculiarly speculating over the synthesis substrate for enhanced activity.

CONCLUSION

Characterisation of silver nanoparticles synthesised by fungus *Gliocladium roseum* done by various analytical methods such as UV-vis spectrophotometry, FT-IR and TEM resulted in the synthesised nanoparticles from both fungal extracts, mat extract (M. E.) and culture filtrate (C. F.) showed maximum absorption peak between the range of 350-450 nm possessing strong N-H bonding and spherical shape. We demonstrated that the antimicrobial activity of silver nanoparticle showed efficient inhibitory activity against two human pathogenic bacteria-*Salmonella typhi*, *Klebsiella pneumonia* and also showed moderate inhibitory activity against two plant foliar pathogenic fungi-*Alternaria alternata* and *Cladosporium cladosporioides* that may suggest their future use in pharmaceutical formulation (antibacterial agents or drugs) and also emphasized in the green synthesis of silver nanoparticle with the application of fungi and can be strengthened in future studies. Further analysis for the actual mechanism of silver nanoparticles against microbes needs to be done.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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