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Molecular identification and *in vitro* bioactivities of silver nanoparticles (AgNPs) biosynthesized by an endophytic fungus, *Plectosphaerella oligotrophica*, isolated from *Anaphalis contorta*

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In this study, a fungal endophyte was isolated from *Anaphalis contorta*, a medicinal plant and identified as *Plectosphaerella oligotrophica* using ITS-rDNA gene sequencing that shows 99.61% similarity. Production of hydrolytic enzymes by this fungus was determined. The isolate was allowed to biosynthesize silver nanoparticles(AgNPs)which was confirmed using UV-Vis spectrophotometer. The biological activities of *P. oligotrophica* and AgNPs were assessed *in vitro*. The antifungal activity of *P. oligotrophica* was evaluated using a modified dual culture method against ten phytopathogens and found to show highest activity against *A. flavus*, *U. virens*, *A. tenuisimma*, and no activity against *A. niger*. The AgNPs have shown antifungal activity against all the tested pathogens with the highest inhibition against *U. virens* (60%). Antibacterial activity was conducted using the agar well diffusion method and 96-well microtitre method against six pathogenic bacteria. The ethyl acetate extract of *P. oligotrophica* was found to possess weak to moderate antibacterial activity showing highest activity against *S. typhi* with the MIC of 62.50 µg/ml. The AgNPs have shown strong antibacterial activity with the greatest inhibition against *S. typhi* having MIC of 15.62 µg/ml. The antioxidant activity was assessed using DPPH assay. The *P. oligotrophica* extract and AgNPs have shown inhibitions of 49.85% and 59.56%, respectively.The biosynthesized AgNPs displayed potential biological activities and hence provided an important application for agricultural sciences, medical sciences, and pharmaceutical industries.

Keywords: *Anaphalis contorta*, cellulase, endophytic fungi, ethyl acetate, nanoparticles

INTRODUCTION

Endophytic fungi colonize the healthy tissues of the host plants for all or a portion of their life cycle without exhibiting any disease symptoms. Tropical medicinal plants offer great promise as hosts for endophytic fungi, which release bioactive secondary metabolites with antibacterial, antifungal, antiviral, anti-inflammatory, anti-tumor, and antioxidant properties. These compounds have potential uses in agriculture, medicine, and the food industry (Abo Nouh, 2019; Banerjee, 2011). *Anaphalis contorta* is a high-altitude medicinal plant that is used in traditional medicine by several ethnic groups.

Endophytic fungi are useful in modern agricultural applications for their capacity to promote plant growth, suppress pests, withstand environmental stress, and be ecologically friendly (Shah *et al.* 2021; Ripa *et al.* 2019). According to several research, fungal endophytes lessen insect and pathogenic fungus attacks on the host plant (Bamisile *et al.* 2018; Gange *et al.* 2012). The need for sustainable agriculture is the main target area for recent scientific research, which will help to protect and reduce the negative impacts on the environment in the future (Umesha *et al.* 2018). Nanoparticles' size ranges from 1–100 nm and because of their small size exhibit unique physical properties and increase catalytic activities. Endophytic fungi are more suitable for the production of nanoparticles because they can generate a large amount of biomass fast in an *in vitro* condition.

Plant diseases and postharvest crop loss caused by fungi are a huge threat to global food security accounting for more than 20% of yield loss (Kumar and Kalita,2017). The mycotoxins

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produced by this pathogenic fungi have harmful effects on humans after consumption (Moretti *et al.* 2017). In recent studies, silver nanoparticles of endophyte origin were used as effective and environment-friendly antifungal agents. The antibiotic-resistant microbes are increasing at a high rate (Frieri *et al.* 2017). Silver has been used as an effective antimicrobial agent since ancient times, and it is commonly used in the treatment of open wounds and chronic ulcers. Silver metal is less harmful to mammalian cells in comparison to other metals exhibiting higher toxicity to a broad spectrum of microorganisms (Bondarenko *et al.* 2013). The free radicals are produced in most of the cells as part of the metabolic process and cause a number of chronic diseases, especially neurological disorders and cancers (Phaniendra *et al.* 2015). Recent studies showed that endophytic fungi produces secondary metabolites having antioxidant activity (Ibrahim *et al.* 2021; Ujam *et al.* 2021). AgNPs have been reported to possess strong antioxidant properties (Nirmala and Sridevi, 2021; Govindappa *et al.* 2020). In the present study, an endophytic fungus *Plectosphaerella oligotrophica* was used to synthesize silver nanoparticles and evaluate the antifungal, antibacterial, and antioxidant activities of both *P. oligotrophica* extract and biosynthesized AgNPs.

MATERIALS AND METHODS

Plant sample collection and isolation of endophyte

The fungal endophyte was isolated from the medicinal plant *Anaphalis contorta* collected from the Phangrei region (latitude 25° 8' 41.464" N; longitude 94° 27' 38.289'' E; altitude 2062.14 m) of Ukhurl district, Manipur during December. The isolation of fungal endophyte was conducted following the protocol given by Hallmann *et al.* (2006). The collected plant was authenticated and deposited at the Manipur University Museum of Plants (MUMP), Manipur University.

Identification

The fungal endophyte was identified based on morphological characteristics like colony colour, growth rate, pigmentation, texture, spore, and spore-producing structures (Watanabe, 2002; Barnett and Hunter, 1972). Further, the fungal isolate was identified using partial ITS-rDNA gene sequencing. Five days old mycelial plug was inoculated into a 250 ml potato dextrose broth (PDB) medium and cultured in an incubator for 7 days at 28±1ºC and the mycelial mat was collected after filtration. Deoxyribonucleic acid (DNA) extraction, amplification, and gel electrophoresis was performed following the modified Cetyltrimethylammonium bromide (CTAB) method given by Guo *et al.* (Gou *et al.* 2000).

DNA extraction

The fungal mycelium (0.2 g) was transferred into a 1.5 mlmicrocentrifuge tube containing 700 µl of preheated (60°C) 2X CTAB extraction buffer of 2% (w/v) CTAB, 100 mMTris-HCl, 1.4 M NaCl, 20 mMethylenediaminetetraacetic acid (EDTA), pH 8.0, and 0.2 g sterilized quartz sand. The mycelia were ground using a glass pestle and incubated in a 60°C water bath for 30 min with constant swirling. Each tube was added with 500 µl of phenol:chloroform (1:1) and centrifuged at 12000 g for 20 min and the liquid phase was collected. The liquid phase containing DNA was re-extracted with chloroform:isoamyl alcohol (24:1) and was added with 50 µl of 5MKOAc and 400 µl of isopropanol and mixed. The genomic DNA was precipitated at 9200 g for 2 min in a microcentrifuge and the DNA pellet was washed with 70% ethanol and air dried for 30 min. The DNA pellet was then re-suspended in 100 µl TE buffer (10 mMTris-HCl, 1 mM EDTA). DNA samples were analyzed for the presence of endophyte DNA using polymerase chain reaction (PCR) and gel electrophoresis.

Amplification

The PCR amplification was performed using GeneAmp PCR System 9700 (Applied Biosystems, Massachusetts, USA). The DNA coding for internal transcribed spacers (ITSI & ITSII) and 5.8S rDNA region was amplified using the primers ITS4 (52 -TCCTCCGCTTATTGATATGC-32) and ITS5 (52 -GGAAG TAAAAGTCGTAACAAGG-32). The amplification was performed in a 50 µl reaction

volume containing 25 µl of PCR master mix (Thermo Scientific, USA), 2.5 µl of DMSO, 1 pmol l^{-1} of each primer, and 100 ng of template DNA. The thermal cycling program was as follows: 5 min initial denaturation at 94ºC, followed by 30 cycles of 1 min denaturation at 94ºC, 1 min primer annealing at 50ºC, 1 min extension at 72ºC, and a final 7 min extension at 72ºC. A negative control using water instead of template DNA was included in the amplification process.

Gel Electrophoresis

From each PCR reaction 5 µl of PCR products were examined by electrophoresis at 75 V for 2 h in a 0.8% (w/v) agarose gel in 1×TAE buffer (0.4 M Tris, 50 mMNaOAc, 10 mM EDTA, pH 7.8) and visualized under ultraviolet (UV) light after staining with ethidium bromide (0.5 µg ml^{-1}).

DNA Sequencing: The PCR products were purified using a GeneJET PCR Purification Kit (Thermo Scientific Massachusetts, USA), according to the manufacturer's protocol, and adjusted to 5 ìg/ìL concentrations before sequencing. The purified PCR products were sequenced using the ITS1 and ITS4 primers on ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit (Applied Biosystems, Massachusetts, USA) following the manufacturer's protocol. Analysis of the sequences was carried out by using SeqStudio Genetic Analyzer (Applied Biosystems, Massachusetts, USA) and manually adjusted for any inconsistencies. The nucleotide sequences were deposited in National Center for Biotechnology Information (NCBI) to obtain the GenBank accession number.

Phylogenetic analysis

The nucleotide sequences were analyzed in Basic Local Alignment Search Tool (BLAST) through the NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare with other nucleotide sequences available in the GenBank database and identify the maximum similarity sequence. The phylogenetic tree was constructed with the Maximum Likelihood method using the MEGA software, version 10, with 1000 bootstrap values (Tamura and Nei, 1993). Sequences in the GenBank database having more than 98% similarity with the sequence of the fungal endophytes were used to construct the phylogenetic tree.

Qualitative extracellular enzyme production

The isolate *P. oligotrophica* was tested for the production of protease, lipase, amylase, cellulase, and laccase by inoculating on modified culture media containing different substrates. For protease, amylase, cellulose, and laccase activity, glucose yeast peptone agar (GYPA) medium was used after adding skim milk, soluble starch, carboxymethylcellulose, and 1-naphthol, respectively. For lipase activity, peptone agar (PA) medium added with Tween 20 was used. The mycelial plug of *P. oligotrophica* was inoculated in the agar petriplates and incubated for 7 days at 28±1ºC (Rajput *et al.* 2016; Satish *et al.* 2012).

Biosynthesis of silver nanoparticles (AgNPs)

The fungal endophyte was grown on potato dextrose broth for 10 days at 27±1°C. After incubation, the biomass was harvested by filtering through Whatman filter paper No. 1. and washed two times with distilled water. The fungal mat was suspended in 100 ml of sterilized distilled water for 48 h at 27±1°C and filtered. The filtrates were treated with 1 mM silver nitrate solution and incubated for 24 h at room temperature in dark conditions for reduction and observed for colour change. The synthesized silver nanoparticles were analyzed using UV-Vis spectrophotometer. The absorbance of silver nanoparticle aliquots was measured between 200 and 800 nm range. The silver nitrate solution and the fungal filtrate were used as the positive and negative controls. By using the absorbance data collected from the spectrophotometer a graph was plotted between absorbance and wavelength. Further, the AgNPs solution was centrifuged at 10,000 rpm for 15 min and the AgNPs pellets obtained were over-dried and kept for further analysis (Devi *et al.* 2014).

Biological activities of Plectosphaerella oligotrophica and biosynthesized AgNPs

The antifungal, antibacterial, and antioxidant activities of the *P. oligotrophica* and AgNPs were evaluated to assess their bioactive properties.

Antifungal activity of Plectosphaerella oligotrophica

P. oligotrophica was screened for its antifungal property against ten fungal plant pathogens, *Curvularia lunata* (ITCC 7170), *Fusarium oxysporum* (ITCC 4998), *Aspergillus niger* (ITCC 5406), *Aspergillus flavus* (ITCC 6972), *Sclerotium oryzae* (ITCC 6972), *Rhizoctonia solani* (ITCC 4576), *Alternaria alternata* (ITCC 6778), *Colletotrichum capsici* (ITCC 6078),*Alternaria tenuissima* (ITCC 7736), and *Ustilaginoidea virens* (ITCC 7046), following a modified petriplate culture method (Pei *et al.* 2019). Five days old 9 mm mycelial plugs of both the pathogen and *P. oligotrophica* were placed on PDA petriplates in such a way that the pathogens were inoculated at the centre of each plate, and *P. oligotrophica* on both sides of the pathogen 2 cm apart and incubated at 28±1°C. Observations were recorded on 6th day and 11th day of incubation.

Antifungal activity of AgNPs

The antifungal activity of the synthesized AgNPs was evaluated following a modified petriplate culture method (Ghojavand *et al.* 2020). The pathogens were inoculated on 90 mm petriplates containing 25 ml of PDA medium mixed with 100 µl of 100 µg/ml and 200 µg/ml of AgNPs solution and incubated at 28±1°C. The diameter of the colonies formed were recorded after seven days after incubation. Pathogens cultured on AgNP-free PDA plates were used as controls. The inhibition percentage was calculated using the formula, growth inhibition percentage (I%) = (C – T)/ C × 100, where C is the diameter of the control colony and T is the diameter of the treatment colony.

Extraction of crude fungal metabolites

The endophytic fungal isolate *P. oligotrophica* was allowed to produce secondary metabolites. The mycelial plug of size 0.5 cm cube was cut off from a 7-day-old culture and inoculated on 500 ml potato dextrose broth (PDB) and incubated for 15 days with constant steering using an orbital shaker. The fungal hyphae mat was separated from the liquid part by using a muslin cloth and filtering through Whatman No. 1. The filtrate obtained was extracted using equal volumes of ethyl acetate thrice. Then the ethyl acetate (EtOAc) extract was evaporated at 40°C to remove the ethyl acetate from the mixture, and the dried crude extract was stored at 4°C for further analysis (Sharma *et al.* 2016).

Antibacterial activity of Plectosphaerella oligotrophica extract and AgNPs

The ethyl acetate extract of *P. oligotrophica* and AgNPs were evaluated for antibacterial activity against six pathogenic bacteria, *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 121), *Enterococcus faecalis* (MTCC 439), *Salmonella typhi* (MTCC 531), *Escherichia coli* (MTCC 443), and *Shigella flexneri* (MTCC 1457), by using the well diffusion method, and minimum inhibitory concentrations (MICs) were calculated using the 96-well microtitre plate method.

Agar-well diffusion method

All the test bacteria were grown overnight at 37±1°C and the bacterial suspension was adjusted to 1×10^7 CFU/ml using a spectrophotometer. By using sterile cotton swabs, bacteria were applied to Muller-Hinton agar (MHA) plates, and five holes were made with a 6 mm cork borer. The samples were dissolved in Dimethyl sulfoxide (DMSO) at 1 mg/ml concentration. Holes were filled with 100 µl of different concentrations (25 µg/ml, 50 µg/ml, and 75 µg/ml) of the samples; streptomycin sulphate (25 µg/ml) was used as a positive control and DMSO as a negative control. The plates were put at room temperature for 30 mins to allow diffusion and incubated for 24 h at 37±1°C. The appearance of a halo zone around the well was observed and recorded (Rani *et al.* 2017).

Minimum inhibitory concentration (MIC)

The MICs of the test bacteria were calculated as given by Perumal *et al.* (2012) with few modifications by using a 96-well microtitre plate. The first well of each row was left bank, and wells from column 2 to column 8 were filled with 100 µl of Muller-Hinton broth (MHB). The wells of columns 1 and 2 of each row were added with 100 ml of crude extract solution and followed by

a two-fold serial dilution from column 2 to column 8, giving a concentration range of 1000 µg/ml to 7.81 µg/ml. A 30 µl of streptomycin sulphate solution (1 mg/ml) was added to the wells of column 9 and served as a positive control. Lastly, all the wells from column 1 to column 10 were added with 100 µL of respective bacterial inoculum and incubated for 24 hrs at 37±1°C. The wells of column 10 serve as negative control. The MICs of the crude extract against the bacteria were determined by adding 20 µL of iodonitrotetrazolium chloride solution (1mg/ml) to all the wells and incubating for another 60 min at 37±1°C. The appearance of a pinkish-red colour indicates bacterial growth. The lowest concentration that does not show a pinkish colour was recorded as the MIC of the extract against the particular pathogen.

Antioxidant activity of P. oligotrophica extract and AgNPs

The free radical scavenging activity was evaluated following the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay given by Caicedo*et al.* (Caicedo *et al.* 2019). The crude extract and AgNPs samples were dissolved in methanol and absorbance of different concentrations (20, 40, 60, 80, and 100 µg/ml) was determined at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as a standard antioxidant. The negative control used was a mixture of methanol and DPPH solution. Antioxidant activity percentage was calculated using the formula, RSA%=(A control-A sample)/(A control) ×100, where A control is the absorbance of the DPPH radical with methanol, A sample is the absorbance of the DPPH radical with the sample. The IC_{50} values were calculated for the crude extract, AgNPs, and ascorbic acid using linear regression to determine the concentration of the sample required to inhibit 50% of the radicalantioxidant activity.

RESULTS

Isolation and identification of endophytic fungus

The fungal endophyte in the study was isolated from the stem of *Anaphalis contorta*. Morphological characteristics have identified the isolate as *Plectosphaerella oligotrophica* and confirmed by molecular characterization. The partial ITS-rDNA sequencing has revealed a nucleotide sequence of 515 base pair long. The BLAST alignment has shown maximum similarity of 99.61% with *Plectosphaerella oligotrophica* GFRS31 (MT447499), *Plectosphaerella oligotrophica* GFRS24 (MT447492), and *Plectosphaerella* sp. RFE5 (MT221576) (Table1, Fig. 1).

Qualitative enzyme production

P. oligotrophica could solubilize all the added substrates and was shown to produce protease, lipase, amylase, cellulose, and laccase. The appearance of a clear zone on solid agar media indicates the release of protease and lipase enzymes. The appearance of a clear white zone after the addition of iodine solution shows the production of amylase. The appearance of yellowish colour around the culture colony after the addition of congo red and destaining shows the release of cellulose enzyme. The colour change of culture media from white to purple colour was due to the oxidation of 1-naphthol by the produced laccase enzyme (Fig. 2).

Biosynthesis of AgNPs by P. oligotrophica

The endophytic fungus *P. oligotrophica* was used for the first time for the mycosynthesis of silver nanoparticles. After 24 h of incubation, colour changes from light yellow to dark brown indicating the formation of AgNPs by the reduction of silver nitrate solution. AgNPs formation was confirmed by the surface plasmon absorption peak at 430 nm (Fig. 3).

Antifungal activity of P. oligotrophica and AgNPs

After 10 days of incubation, *P. oligotrophica* has shown antifungal activity against all the tested pathogens except A. niger. Strong antifungal activity was exhibited against *A. flavus*, *U. virens*, and *A. tenuisimma*; moderate activity against *C. lunata*, *F. oxysporum*, *S. oryzae*, and *A. alternata*; and weak activity against *R. solani* and *C. capsici*. The biosynthesized AgNPs were effective against all ten pathogens. With the increase of AgNPs concentration from 100 µg/ml to 200 µg/ml, the

Table 1: Identification and accession details of the endophyte and host plant.

	Name	Family	Accession no.		Plant part used
Host plant					
	Anaphalis contorta (D.Don) Hook.f.	Asteraceae	MUMP 001372		Stem
	Name	Family	Accession no.		Identification remarks
Endophyte			NFCCI	GenBank	
	Plectosphaerellaoligotrophica	Plectosphaerellaceae	NFCCI 5222	OR357719	Slow growth rate; light pink colony; circular margin, no pigmentation; spores after 7 days

Table 2: Antifungal growth inhibition (%) of ten phytopathogens with different concentrations of biosynthesized AgNPs after 7 days of incubation.

Pathogens	Growth inhibition percentage (I%)	
	100 µg/ml	200 µg/ml
Curvularia lunata	22.63	41.03
Fusarium oxysporum	27.58	48.15
Aspergillus niger	34.90	56.15
Aspergillus flavus	37.26	57.60
Sclerotium oryzae	28.25	35.71
Rhizoctonia solani	28.55	31.88
Alternaria alternata	28.05	45.65
Colletotrichum capsici	21.61	49.60
Alternaria tenuissima	31.17	52.71
Ustilaginoidea virens	33.90	60.00

growth of the fungal colony decreases showing maximum inhibition against *U. virens* (60%) and least against *R. solani* (31.88%). The silver nanoparticles were observed to increase antifungal activity as compared to that of *P. oligotrophica* as the former could inhibit *A. niger* by upto 56% (Table 2, Fig. 4).

Antibacterial activity of P. oligotrophica extract and AgNPs

The ethyl acetate extract of *P. oligotrophica* has shown antibacterial activity against the six pathogenic bacteria with higher inhibition against *S. typhi* (15.33±0.23 mm) with MIC of 62.50 µg/ml. The biosynthesized AgNPs were found to increase the antibacterial activity against all the tested bacteria showing a maximum clear zone against *S. typhi* (19.83±0.29 mm) with MIC of 15.62 µg/ml (Table3).

Antioxidant properties of AgNPs

The free radical scavenging activity of *P. oligotrophica* crude extract and AgNPs have shown increased inhibition with increasing concentration. At the concentration of 100 µg/ml, the *P. oligotrophica* extract could inhibit 49.85% and that of AgNPs inhibits 59.56%. The IC_{50} values of *P. oligotrophica* extract and AgNPs were found to be 99.71 µg/ml and 84.25 µg/ml respectively. Lower IC_{50} indicates higher antioxidant activity. The AgNPs were observed to possess greater antioxidant potential than that of standard ascorbic acid with IC_{50} of 89.62 µg/ml (Fig. 5).

DISCUSSION

ITS-rDNA gene sequencing is the most common and important tool for fungal DNA analysis. The ITS is considered the universal barcode marker for fungi (Badotti *et al.* 2017). No previous studies

Table 3: Antibacterial activity of ethyl acetate extract of *P. oligotrophica* and AgNPs against six pathogenic bacteria by well diffusion method and MIC calculation by 96-well microtitre plate method.

Sample	Conc. (µg/ml)	Inhibition clear zone diameter (mm)					
		Gram positive bacteria			Gram negative bacteria		
		B. subtilis	*S. aureus*	*E. faecalis*	*S. typhi*	*E. coli*	*S. flexneri*
P. oligotrophica extract	25	0.00	0.00	0.00±0.00	12.15±0.40	0.00±0.00	0.00±0.00
	50	8.83±0.23	8.42±0.08	0.00	14.09±0.26	8.15±0.15	8.05±0.40
	75	10.25±0.01	10.63±0.29	8.13±0.46	15.52±0.08	10.08±0.29	10.19±0.32
	+ve control	12.51±0.08	13.19±0.23	12.09±0.30	12.51±0.19	12.50±0.19	14.53±0.09
	MIC	125.00	125.00	250.00	62.50	62.50	250.00
AgNPs	25	9.27±0.17	10.53±0.46	8.21±0.29	16.42±0.35	10.52±0.33	0.00±0.00
	50	11.45±0.21	11.58±0.61	10.69±0.33	18.63±0.41	13.84±0.13	9.38±0.26
	75	13.33±0.09	13.68±0.19	14.25±0.08	20.83±0.15	14.53±0.16	12.19±0.41
	+ve control	13.18±0.33	13.45±0.41	12.23±0.52	12.28±0.11	13.08±0.29	15.03±0.16
	MIC	62.50	62.0	31.25	15.62	31.25	125.00

Data are means of clear zone diameter (triplicate) ± standard deviation (SD); 25 µg/ml streptomycin sulphate was used as positive control

have been made regarding the isolation of *P. oligotrophica* as an endophyte or the evaluation of its bioactivities. Enzymes of microbial origin, especially endophytic fungi, are of interest due to their low time requirements, cost-effectiveness, and environment-friendly nature. Hydroplytic enzymes produced by endophytes aid in the absorption of nutrients from the host plant and, in return, protect the host plant from the invasion of pathogens (Afzal *et al.* 2019). In recent studies, endophytic fungi were found to be good producers of silver nanoparticles that show a wide range of biological activities. In our study, the biosynthesized AgNPs were shown to increase antifungal activity against plant pathogens as compared with the endophyte extract. This highlights their potential as novel antifungal agents, which are highly needed in organic agriculture. In a previous study, silver nanoparticles synthesized by the endophytic fungus, *Guignardia mangiferae* isolated from *Citrus* sp. showed strong antifungal activity against the plant pathogens *Colletotrichum* sp., *R. solani*, and *C. lunata* (Balakumaran *et al.* 2015). Fungal pathogens have infected most of the major crops for human consumption across the globe, and in several instances, they have caused epidemics. Fungi not only infect standing plants but are also the major cause of many post-harvest diseases in cereals, fruits, and vegetables (Lamenew *et al.* 2019). In another study, biosynthesized silver nanoparticles using the endophytic fungus, *Aspergillus versicolor* associated with *Centella asiatica* showed potential antifungal activity against the human pathogens, *Candida albicans* and *Candida nonalbicans* (Netala *et al.* 2016). Endophyte-origin silver nanoparticles might play an important role in developing antifungal agents applicable for both plant and animal treatment. It has been estimated that more than 1.6 billion people have died due to fungal infections (Almeida *et al.* 2019). The ethyl acetate extract of *P. oligotrophica* has shown weak antibacterial activity, and that of AgNPs has moderate to strong activity. The AgNPs showed higher inhibition than positive control in the cases

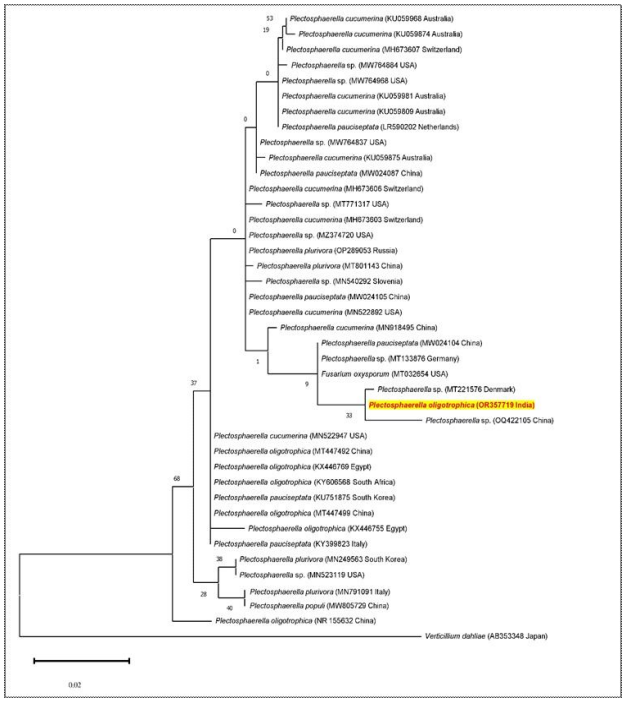

Fig.1: Phylogenetic tree depicted by Maximum Likelihood method between *Plectospaerella oligotrophica* (OR357719) and closely related 39 species based on ITS1-5.8S-ITS2 rDNA sequences. *Verticillium dahliae* (OM801883) was used as the out-group.

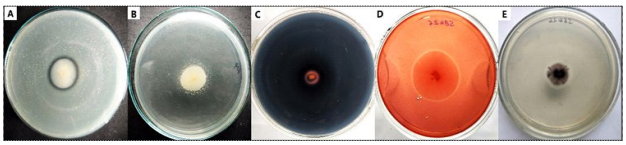

Fig.2: Qualitative extracellular enzyme production by *P. oligotrophica* on agar media (A) protease, (B) lipase, (C) amylase, (d) cellulase, and (e) laccase.

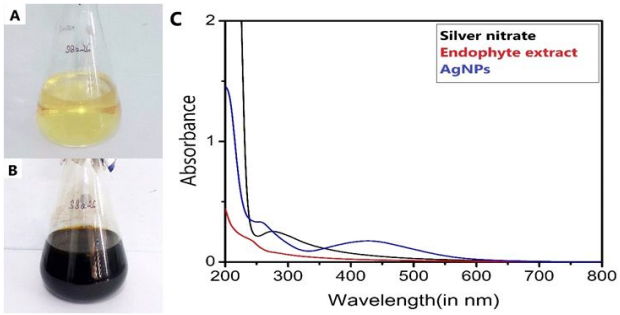

Fig. 3: Biosynthesis of AgNPs (A) *P. oligotrophica* extract before addition of silver nitrate, (B) *P. oligotrophica* extract after addition of silver nitrate, and (C) UV-vis spectroscopy of AgNPs, *P. oligotrophica* extract, and standard silver nitrate solution.

of *Enterococcus faecalis*, *Salmonella typhi*, and *Escherichia coli*. The AgNPs were effective against both gram-positive and gram-negative bacteria and could be used in the formulation of strong broad-spectrum antibiotics. In a recent study, the AgNPs synthesized by the endophytic

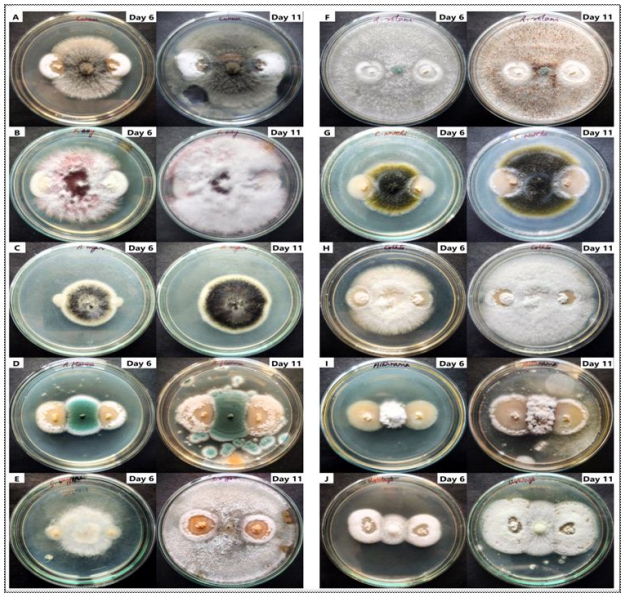

Fig. 4: Antifungal activity of *P. oligotrophica* against ten phyto-pathogens (A) *C. lunata*, (B) *F. oxysporum*, (C) *A. niger*, (D) *A. flavus*, (E) *S. oryzae*, (F) *R. solani*, (G) *A. alternata*, (H) *C. capsici,* (I) *A. tenuissima*, and (J) *U. virens*, after incubation for 5 days and 10 days on PDA media. Pathogens are inoculated at the centre of each petriplate

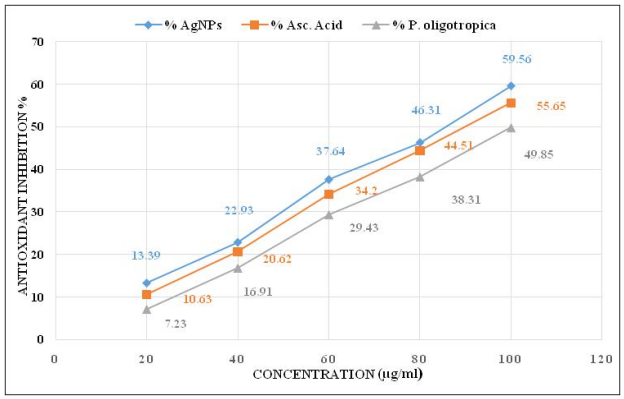

Fig. 5: Radical scavenging activity of *P. oligotrophica* extract and biosynthesized AgNPs at different concentrations by using DPPH antioxidant assay.

fungus, *Talaromyces purpureogenus* isolated from the leaves of *Pinus densiflora* have shown potent antibacterial activity against gram-positive and gram-negative bacteria, with higher inhibition zones against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Hu *et al.* 2019). Antibiotic-resistant bacteria have increased over the last few decades and have become a major health concern globally. Biosynthesized silver nanoparticles are a safe and effective alternative to conventional antibiotics, which have no or minimal side effects on mammalian cells. The incorporation of silver with the secondary metabolite of *P. oligotrophica*

enhances the antioxidant activity. According to Phongpaichit *et al.* (2007), the IC_{50} value of the AgNPs was found to be in the range of intermediate antioxidant activity. Govindappa *et al.* (2022) reported that AgNPs synthesized by an endophytic fungal isolate, *Alternaria alternata*, from *Dendrophthoe falcate* have shown strong antioxidant activity, exhibiting 90% 2,2-diphenylpicrylhydrazyl (DPPH) scavenging activity, 86.47% hydrogen peroxide (H_2O_2) scavenging activity, and 70% nitric oxide (NO) scavenging activity at 100 ìg/ml. Oxidative stress causes severe neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, and Huntington's disease, which are incurable (Ienco *et al.* 2011). Environmental pollution is an important factor in causing oxidative stress (Leni *et al.* 2020). The development of strong antioxidant agents is very much required, and endophytic fungi and their biosynthesized AgNPs are an important option.

CONCLUSION

The goal of this study was to use the endophytic fungus *P. oligotrophica* to biosynthesize AgNPs and evaluate the biological activities of the endophyte and the AgNPs. In a comparative observation, the AgNPs were found to show greater biological activity than *P. oligotrophica*. This enhancing effect may be due to the combined action of silver and the fungal metabolite. Based on the results, it can be said that adding nano-sized metal ions to the fungal secondary metabolite makes its bioactive properties much stronger. This could be very important in nanomedical science, the pharmaceutical industry, and agro-nanotechnology. Further studies are needed in the quantification of extracellular enzymes and the complete characterization of AgNPs, and in vivo experiments will aid the importance of this endophyte research.

DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

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