ABSTRACT

During the present research work, the histology of *in vivo* leaves and stem segments of *Tinospora cordifolia*, *Adhatoda vasica* and *Murraya koenigii* was done using the microtome technique. However, histological localization of endophytic fungi reside inside the healthy *in vitro* and *in vivo* leaves of *Tinospora cordifolia* was also done and compared. The anatomy of *in vitro* leaves has proved the occurrence of dense colonies of endophytic fungi as compared to *in vivo* leaves. Presence of endophytic fungi in intercellular spaces of ground and dermal tissues, sprinkled colonies in vascular bundles of leaf and stem explants were observed in case of all the selected plant species. Peculiarity of these structures is important because modifications are not observed in mycelia isolated from PDA plates. Through the histological study, it is possible to develop a complete inventory of fungal endophytes and their hosts, as well as enhance the level of understanding about the interaction of endophytes within their host.

Subsequently, Potato Dextrose Agar medium was found to be optimum for the emergence of endophytic fungi out of all the media tested in case of selected medicinal plants. Preservation of the isolated fungi was done in paraffin wax and stored at 4°C temperature in refrigerator, sterile glass vials containing 8.0 ml of sterile water, 0.5 ml dimethyl sulfoxide (DMSO) and 1.0 ml glycerol act as cryoprotectant.

The identification of isolated endophytic fungi was done on the basis of their morphological, biochemical and molecular approach.

The unique specific morphology was observed during identification of endophytic fungi in the pure culture of fungus (FGCC/MUJ-24) *Myceliophthora thermophila* isolated from *Tinospora cordifolia* leaves showed different morphological features as cottony-pink, but rapidly turn cinnamon-brown and granular in texture. On microscopic studies, this fungal mycelium was found to be obovate, hyaline, smooth and the conidia were thick-walled. *Mazzantia napellii* (FGCC/MUJ-1) isolated from *Adhatoda vasica* showed yellowish, septate hyphae which later on produced hyaline,
one celled, elongate conidia, filiform to phialidic conidiogenous cells in pycnidial locule. Further, *Aspergillus terreus* (FGCC/MUJ-3) which was isolated from *Murraya koenigii*, the hyphae was septate, hyaline and conidial heads were compact, biseriate and densely columnar.

*Myceliophthora thermophila* (FGCC/MUJ-24), *Mazzantia napelli* (FGCC/MUJ-1) and *Aspergillus terreus* (FGCC/MUJ-3) fungal strains were found to show maximum extracellular enzymatic activities like amylase, laccase, protease, lipase, cellulase etc. These enzymes have industrial applications such as in food and starch based industries. The market and the demand of amylases would always be high, laccases have also shown to be useful for bioremediation and biodegradation, food processing and paper & pulp industries. Besides, cellulases have shown potential applications in various industries including textile, laundry, biofuel production, food and feed industry, brewing, agriculture etc. Further, lipases serve important roles in human practices as ancient as yogurt and cheese fermentation, proteases are part of many laundry detergents, controlling blood clotting, pectin is used in confectionery jellies to give a good gel structure, used against constipation and diarrhoea etc.

Metabolic profiling of these fungi identified through 18 S rRNA technique using Gas Chromatography Mass Spectroscopy (GCMS) was carried out on a triple quadrupole mass spectrometer. During, the research work, GCMS analysis of *M. thermophila* extract showed the availability of important natural compounds like Speciofoline, Phenanthrene, Ezlopitant, Anthraquinone etc. Besides, the fungus *M. napellii* has revealed important secondary metabolites viz.; 1, 2, 4-Triazines, Furosemide, Desflurane, Ethyl oxamate etc. Furthermore, through GCMS analysis fungus *A. terreus* showed noteworthy secondary metabolites viz. Chloranthalactone A, Bismuthine, Diosgenin, Azomycin etc. These metabolites are of immense medicinal potential.

Using 18 S rRNA gene sequencing technology, three important fungi viz. *Myceliophthora thermophila* (FGCC/MUJ-24), *Mazzantia napelli* (FGCC/MUJ-1) and *Aspergillus terreus* (FGCC/MUJ-3) have been identified from *T. cordifolia* (Central Arid Zone Research Institute (CAZRI), Jodhpur), *A. vasica* (Amer region, Jaipur) and *M. koenigii* (Manipal University Jaipur), respectively. Further, 18 S rRNA gene sequences of these identified fungi were also retrieved from NCBI and matched
with morphological features. These identified fungi belonged to ascomycota phylum and these fungi have also been found to show unique morphological, extracellular enzymatic and antioxidant activities. *M. thermophila* efficiently degrades cellulose and is efficient in the production of biofuels and expressed laccase activity in the present research, that can act as clean substitutes for harmful chemical reagents used in the paper and pulp industry and textile dyes. *M. napelli* was found to be useful for production of maximum enzymatic activity viz. amylase, laccase, lipase, protease and cellulase etc. Besides, *A. terreus* was found to produce protease, cellulase and lipase enzymes and this fungus was the source for the drug mevinolin (lovastatin), a drug for lowering serum cholesterol and also helps in cardiovascular diseases. These fungi have the potential against *Staphylococcus aureus* and *Pseudomonas aeruginosa* etc. bacteria.

During the present research work, *Mazzantia napelli* had shown maximum free radical scavenging activity at 125 µg/ml concentration followed by *Aspergillus terreus* and *Myceliophthora thermophila*. These results show that these fungi are anti-oxidative in nature and may be used as alternative of antioxidants.

During the research endeavour, the fungal cell filtrate was used to synthesize silver nanoparticles. However, 10 ml of fungal cell filtrate of *M. thermophila, M. napelli* and *A. terreus* separately reduced the AgNO₃ solution (90 ml) after 48 h. Besides, 5 mM concentration of AgNO₃ solution at 7 pH and 30°C temperature found to be optimum for the production of AgNPs in all the fungal mediated synthesis of silver nanoparticles. Thereby exhibited the mycosynthesis of AgNPs of spherical shape & variable sizes viz. 20-59 nm as confirmed through SEM analysis. The synthesized AgNPs were characterized by fourier transforms infrared spectrometry (FTIR), scanning electron microscope (SEM), transmission electron micrographs (TEM), Selected area (electron) diffraction patterns (SAED), energy-dispersive X-ray spectroscopy (EDX) and X-ray powder diffraction (XRD) techniques.

The UV–vis spectrophotometer analysis of the fungal filtrates viz., *Myceliophthora thermophila* (FGCC/MUJ-24), *Mazzantia napelli* (FGCC/MUJ-1) and *Aspergillus terreus* (FGCC/MUJ-3) mixed separately with AgNO₃ showed a characteristic surface plasmon absorption band at 420, 430 and 440 nm, respectively.  

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SEM analysis of *Myceliophthora thermophila* mediated AgNPs showed nanoparticle size range of 25-30 nm, *Mazzantia napelli* revealed size of AgNPs in the range of 18-30 nm and *Aspergillus terreus* produced 35-59 nm silver nanoparticle.

TEM micrographs of AgNps synthesized using these fungal isolates also confirmed the formation of spherical nanoparticles with different sizes (5- 20 nm).

Selected area (electron) diffraction patterns recorded for single particle in the cumulative of all the nanoparticles tested samples match up to a characteristic polycrystalline ring pattern for a face-centred-cubic structure. During the examination of energy dispersive X-ray of the nanoparticles showed peaks in silver region at approximately 3 keV, which confirmed the presence of elemental silver in the AgNPs samples synthesized using fungi isolated during the study (*M. thermophila*, *M. napelli* and *A. terreus*).

XRD pattern showed peaks responsible for planes of face cubic crystal. Diffraction peaks were showing in the range of 2 Theta corresponding to (110), (202), (211) plane confirmed faced centered cubic structure of AgNPs synthesised by *Myceliophthora thermophila* isolated from *Tinospora cordifolia*. *Mazzantia napelli* mediated AgNPs characterized by XRD also showed in the range of 2 Theta corresponding to (111), (200), (220) plane confirmed faced centered cubic structure. AgNPs synthesised by *Aspergillus terreus* isolated from *Murraya koenigii* characterized by XRD and diffraction peaks were exhibiting in the range of 2 Theta corresponding to (111), (200), (311) plane authenticated faced centered cubic structure of AgNPs. Further, XRD results also confirmed that synthesized SNPs were crystalline in nature.
AgNp’s synthesized using crude fungal extracts of *Myceliophthora thermophila*, *Mazzantia napelli* and *Aspergillus terreus* have shown maximum zone of inhibition (12.2±0.4mm, 11.2±0.3mm, 12.4±0.3mm) against *E.coli* at 100 ppm, While against *S. aureus* at 100 ppm AgNPs concentration, least zone of inhibition 9.8±0.4mm, 9.3±0.4mm, 9.6±0.4mm respectively was recorded. Besides, AgNPs at 100 ppm concentration inhibited the growth of *P. aeruginosa* and has shown different zone of inhibition such as 10±0.3mm, 10.6±0.3mm, 11.6±0.3mm, respectively. At 100 ppm, concentration of AgNP’s, highest zone of inhibition 12.8±0.3mm, 12.2±0.3mm and 11.2±0.2mm against *F. oxysporum* were recorded.

In total, 2150 plant segments (leaves & stem) from *Tinospora cordifolia*, *Adhatoda vasica* and *Murraya koenigii* had cultured. Out of which, 263 segments were found to accommodate fungal endophytes (colonization rate 12.23%). In total, 20 Taxa comprising 3 Ascomycetes (*Aspergillus terreus*, *Mazzantia napelli* and *Myceliophthora thermophila*); 5 Coelomycetes (genera: *Colletotrichum* sp., *Pestalotiopsis* sp., *Phoma* sp., *Phomopsis* sp. and *Phyllosticta* sp.); 09 Hyphomycetes (genera: *Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Myrothecium* sp., *Penicillium* sp. and *Trichoderma* sp.) one Zygomycetes (genus: *Mucor* sp.) and 2-3 species were found to be Mycelia sterilia. During the present study, it was concluded that seasonal distribution of endophytic fungi in different tissues of *T. cordifolia*, *A. vasica* and *M. koenigii* showed variable colonization frequency, which was highest during monsoon followed by winter and minimum during summer.

It was concluded that leaves of *Tinospora cordifolia* and *Adhatoda vasica* harbours more number of endophytic fungi than their stem parts. However, stem of *Murraya koenigii* revealed comparatively high colonization rate than in leaves.

To the best of our knowledge, this is the first report which shows unique endophytic fungi *Myceliophthora thermophila*, *Mazzantia napelli* and *Aspergillus terreus* isolated from *Tinospora cordifolia*, *Adhatoda vasica* and *Murraya koenigii*. These fungi were also found to be exhibited diverse enzymatic, antioxidant and antimicrobial potential for pharmaceutical industry and combat dreadful diseases.