Biosynthesis of silver nanoparticles using a non pathogenic *Alternaria solani* isolate: characterization and antifungal activity

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INTRODUCTION

Nanobiotechnology is one of the most fruitful outcomes in material research, particularly in synthesis of metallic nanoparticles [1]. Silver nanoparticles (AgNPs) seem to have attracted major interests in terms of their potential antimicrobial applications [2]. Chemical and physical methods have been reported to produce well-defined silver nanoparticles successfully, however these processes are usually costly and involve using toxic chemicals, which can cause adverse effects and limits their biological applications [3]. Nowadays, green synthesis approaches using fungal extracts appear to offer better and cost effective alternatives in silver nanomaterials synthesis [4].

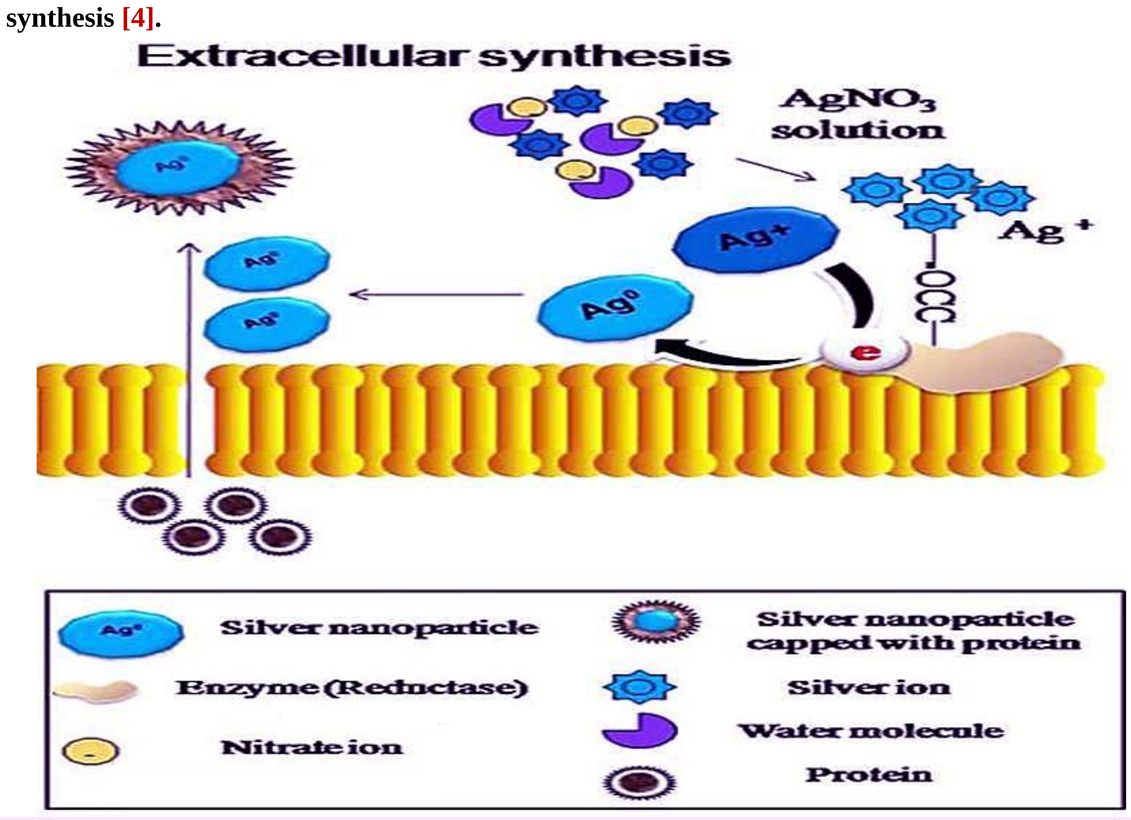


FIGURE 1: Schematic representation showing the mechanism of extracellular synthesis of silver nanoparticles using fungal extract.

The current investigation aims to:

- **Biosynthesize and characterize silver nanoparticles extracellularly using a non pathogenic *Alternaria solani* isolate.**
- **Evaluate the antifungal activity of the well characterized silver nanoparticles against pathogenic strains of *Alternaria solani* the causal agent of tomato early blight disease.**

MATERIALS & METHODS

- The genomic DNA of the isolated fungi was purified
- The internal transcribed spacer (ITS1-5.8S-ITS2) was amplified by PCR with the primers:
  ITS1 forward (5'CTTGGTCATTTAGAGGAAGTAA-3') and
  ITS4 reverse (5'- TCCTCCGCTTATTGATATGC-3').

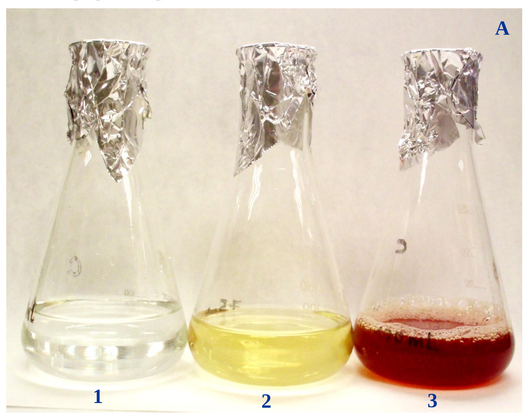
- The synthesized AgNPs were characterized by different techniques, including UV-Visible spectroscopy, Transmission electron microscopy (TEM), X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX), and Fourier transform infrared measurement (FT-IR).

- The antifungal activity of the biosynthesized-AgNPs was evaluated against pathogenic isolates of *Alternaria solani* using agar dilution method. The inhibition rate (%) was calculated by using the following formula:

$$\text{Inhibition rate (\%)} = \frac{R - r}{R} \times 100$$

Where **R** is Radial growth of fungi in control plate, and **r** is the radial growth of fungi in treated plates.

RESULTS



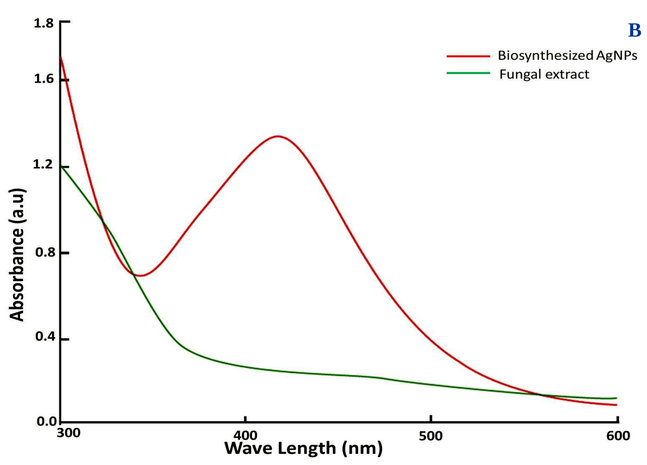


FIGURE 2.

(A) Biosynthesis of silver nanoparticles (1) $AgNO_3$ solution, (2) *Alternaria solani* filtrate and (3) AgNPs formation after 24 hrs incubation.

(B) UV spectra of the extracellularly synthesized silver nanoparticles.

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Characterization of silver nanoparticles

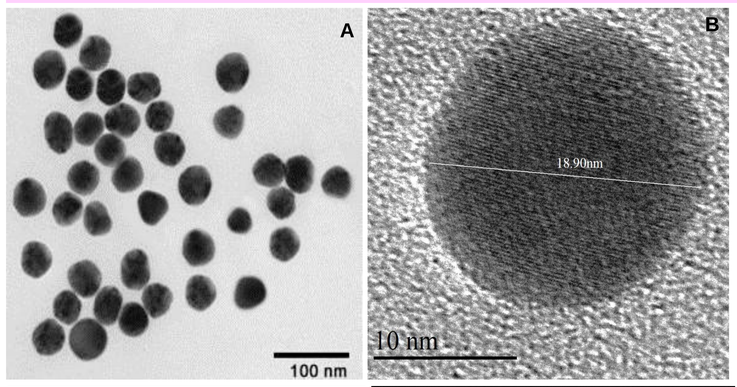


FIGURE 3 (A, B, C):
TEM analysis showing spherical and stable biosynthesized SNPs, with ~15-20 nm in size.

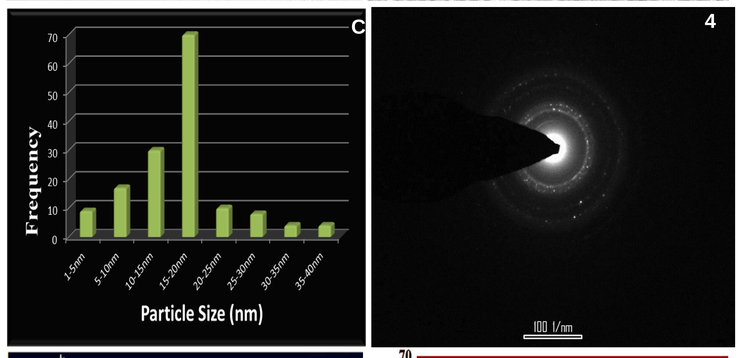


FIGURE 4:
SAED pattern indicates crystalline nature of the synthesized silver nanoparticles.

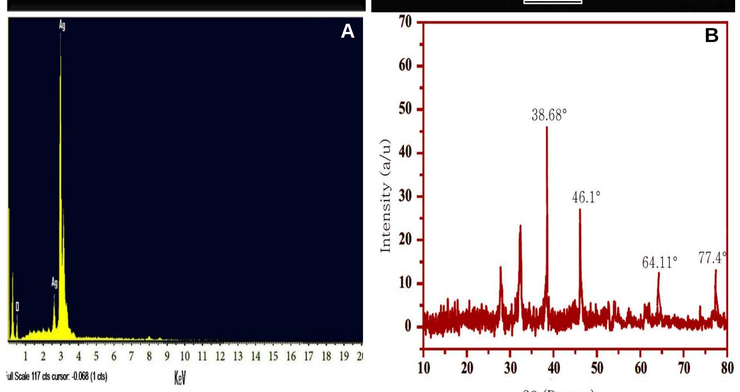


FIGURE 5 (A, B):
XRD pattern and EDAX analyses showing high crystallinity and purity of the synthesized SNPs.

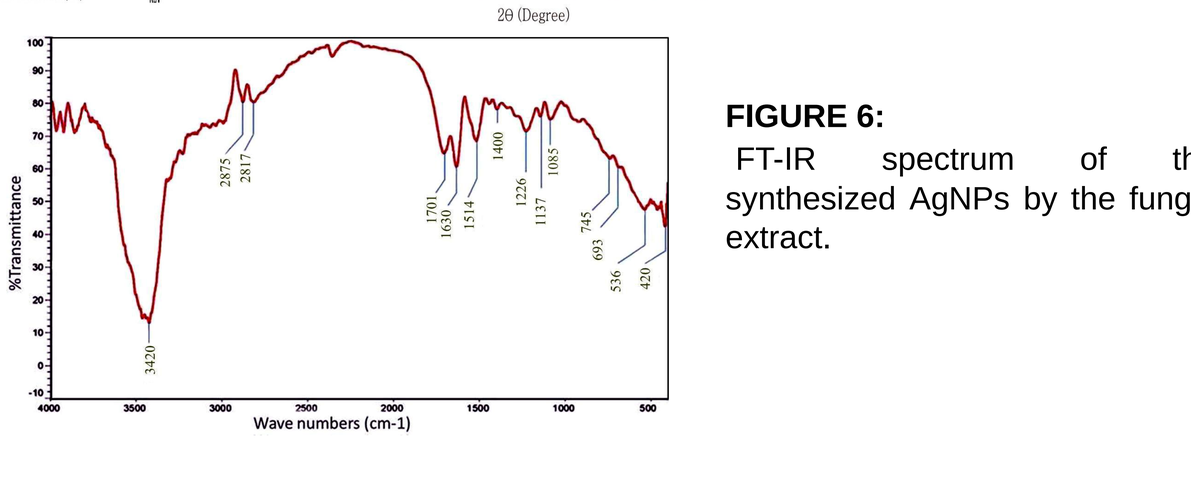
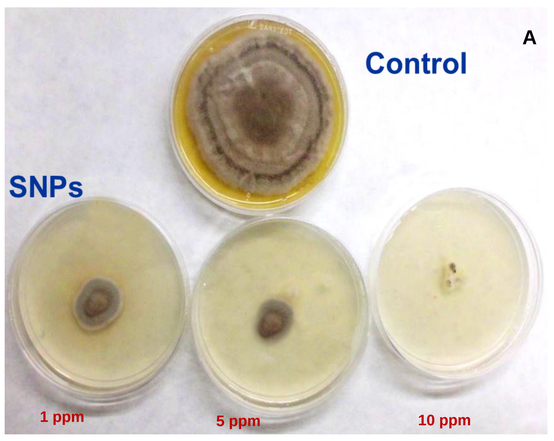


FIGURE 6:
FT-IR spectrum of the synthesized AgNPs by the fungal extract.

ANTI-FUNGAL ACTIVITY



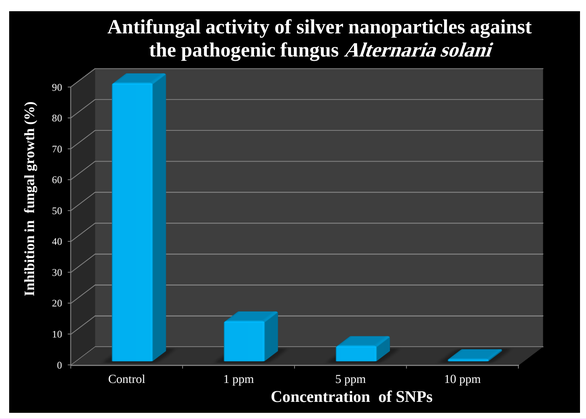


Fig. 7: Antifungal activity of silver nanoparticles against a pathogenic isolate of *Alternaria solani.*

CONCLUSIONS

- A simple, safe and ecofriendly rapid method was used to synthesize small size and stable AgNPs at room temperature without addition any toxic chemical reducing/capping agents.
- FT-IR spectrum revealed that protein molecules of fungal extract can acting as reducing and stabilizing agent as well by binding to AgNPs through free amino groups or through electrostatic attraction of negatively charged carboxylate groups in extracellular enzyme filtrate from fungal mycelia.
- The remarkably strong antifungal activity of the formed AgNPs against pathogenic isolates of the same species *(Alternaria solani)* suggests that green synthesized AgNPs can be used as better antimicrobial alternatives in controlling spore-producing fungal plant pathogens.
- Those findings will have beneficial applications for nanobiotechnology in crop improvement and plant disease management.

Acknowledgments

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