

***Botrytis Cinerea*, One of the Most Destructive Plant Pathogens, as a Potent to Produce Silver Nanoparticles**

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Abstract

*Nanoparticles are synthesized using different physical and chemical methods. However, the development of an eco-friendly approach for the synthesis of nanoparticles is of critical importance to nanotechnology. Types of fungi which secrete a high amount of proteins are ideal candidates for the eco-friendly synthesis of nanoparticles. In this research, the extracellular biosynthesis of silver nanoparticles was implemented, using *Botrytis cinerea*. UV-vis spectroscopy illustrated a sharp peak at 420 nm, demonstrating the presence of silver nanoparticles in the fungal cell filtrate. Further analysis was accomplished through TEM and FTIR. Silver nanoparticles were spherical and 5.1-13.95 nm in diameter with an average size of 8.55 nm. NPs were stable three months after their formation, which is, quite likely, due to their capping with proteins which were secreted by the fungus.*

Keywords: *Biosynthesis, UV-vis spectroscopy, TEM, FTIR.*

1. INTRODUCTION

Owing to the outbreak of various infectious diseases, as well as the development of new, resistant strains of bacteria, pharmaceutical companies and researchers face the challenge of designing and screening newer and more efficient drugs [1, 2]. Despite the availability of a plethora of antimicrobial agents, researchers have been adamant in using silver, especially in the form of nanomaterial, for decades. Thanks to its broad-spectrum biocidal activity toward bacteria, fungi, and viruses, silver is a potential alternative in the development of a new antibacterial agent [3]. It is quite unlikely for microbes to develop resistance against silver, as they do against conventional and narrow-target antibiotics. Metal attacks a broad range of targets in microorganisms, making it difficult for them to generate resistance which requires multiple mutations simultaneously [4, 5]. These criteria make silver

nanoparticles appropriate for wound dressings and as coatings for medical devices [3]. Silver nanoparticles with antimicrobial properties have also been used in clothing, respirators, household water filters, contraceptives, antimicrobial sprays, and cosmetics, detergents, dietary supplements, cutting boards, socks, shoes, cell phones, laptop keyboards, and children's toys [6, 7].

Although there are several physical and chemical methods for the synthesis of metallic nanoparticles, researchers in the field of nanotechnology have turned to biological systems to develop a simpler and more eco-friendly approach [8, 9]. Amongst biological systems, filamentous fungi have certain advantages over other microorganisms that make them good candidates for these eco-friendly processes. Secretion of large amounts of protein makes fungi capable of the

production and stabilization of more nanoparticles. Furthermore, they are easy to cultivate, and their downstream processes are less complex than those of the alternatives [10–13].

Botrytis cinerea is one of the most destructive plant pathogens, and it attacks more than 235 plant species [14]. Such a wide host range shows the wide range of capabilities that this fungus has to secrete different kinds of metabolites [15]. In producing such high volumes of metabolites, *B. cinerea* is an ideal candidate for the green synthesis of silver nanoparticles. In the present work, we investigate the potentials that different strains of *B. cinerea* have in the biosynthesis of silver nanoparticles.

2. MATERIALS AND METHODS

2.1. Fungal Isolates and Silver Nanoparticles Biosynthesis

Eighteen *B. cinerea* isolates were received from the department of Plant Pathology at the University of Kurdistan (Iran). In an effort to obtain biomass for biosynthesis studies, the researchers inoculated the fungus in flasks containing 100 ml of potato dextrose broth (PDB). These flasks were incubated at 26°C on a rotary shaker (150 rpm) for four days. The biomass was harvested by filtration through filter paper, followed by extensive washing with sterile distilled water to remove any medium component.

Afterwards, 5 g of biomass was transferred to 100 ml of sterilized double-distilled water and incubated for 72h under the aforementioned condition. The biomass was then filtered through Whatman filter paper No.1 and cell-free filtrate was treated with AgNO₃ solution to yield the final concentration of 1 mM. The flasks were then incubated at 26°C on a shaker (150 rpm) in the dark. Simultaneously, cell-free filtrate without AgNO₃ and de-ionized water containing AgNO₃ were incubated under the same condition as controls. This experiment was conducted

according to Bhainsa and D'Souza [16] with some modifications.

2.2. Characterization of Silver Nanoparticles

The formation of silver nanoparticles (AgNPs) was primarily monitored through changes in color in the solution, which ranged from colorless to dark brown. To measure the time-dependent production of AgNPs, periodically, aliquots of solution were withdrawn and subjected to UV-vis spectrophotometry (Varian Caryconc 100) from 200 to 800 nm at the resolution of 1 nm.

The color-changed solution was kept in the oven at 40°C to prepare the necessary powder for TEM and FTIR analysis. The morphology and size of nanoparticles were detected by Transmission Electron Microscopy (TEM) (CM 120, Philips) using a conventional carbon-coated copper TEM grid. FTIR was also carried out to study the interaction of nanoparticles with proteins. The spectrum was recorded on a PerkinElmer Spectrum, CM 120; Philips, in the range of 550-4000 cm⁻¹ at a resolution of 4cm⁻¹.

3. RESULTS AND DISCUSSION

Eighteen *B. cinerea* isolates were investigated for their potential of NPs biosynthesis. Color change, which is the first indicator of NPs [17], was observed in most isolates. All isolates were subjected to UV-vis spectrophotometry (data not shown) and of the eighteen, three isolates were selected for further investigation. The mentioned experiments were done on these three isolates several times, and finally, isolate B9 was selected for further studies. Having added AgNO₃, the researchers observed that the colorless crude cell filtrate of *B. cinerea* changed its color first to light brown and then to dark brown. To confirm the formation and stability of AgNPs, aliquots of the cell filtrate were removed and subjected to UV-vis spectroscopy at different time intervals. Cell filtrate, which had been treated with

AgNO₃, showed a strong, yet broad, peak at around 420 nm (Figure 1), which illustrates the presence of silver nanoparticles.

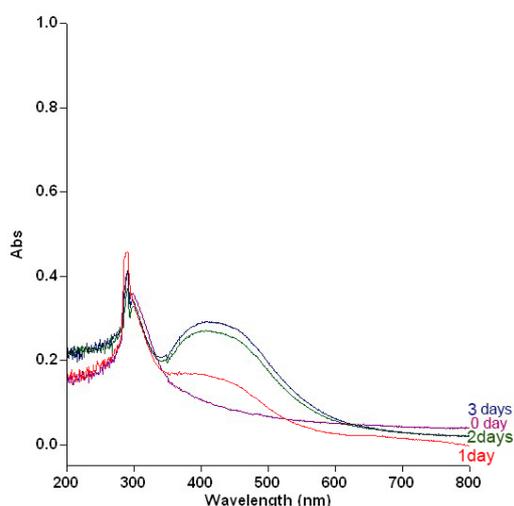


Figure 1. UV-vis spectra of silver nanoparticles synthesized by *Botrytis cinerea*.

Broad plasmon resonance is proposed to be the result of the small aggregation of NPs [4, 18]. The absorption increased as a function of time, which was consistent with the results of Li, et al. [19] and Bhainsa and D'Souza [16].

To investigate whether NPs are produced intra- or extracellularly, AgNO₃ (at the concentration of 1 mM) was added to 100 ml of distilled water containing 5 g of biomass. Mycelia showed no color change after 72 hours, thus indicating the extracellular production of NPs. Examining mycelia through Scanning Electron Microscopy (SEM) is necessary for further confirmation.

The shape and size of silver nanoparticles are important criteria which is considered in their production [20]. Transmission Electron Microscopy was used to visualize the size and shape of AgNPs. Silver nanoparticles were spherical in nature, with an average size of 8.55 nm (Figure 2). They were in the range of 5.1-13.95 nm with the majority of them (94.15%) being 6-12 nm (Figure 3).

Fungi are believed to change Ag ions to AgNPs enzymatically [17]. Therefore, it seems that more protein secretion means more NPs production. In order to determine whether the amount of secreted proteins influences the AgNPs production, the researchers immersed biomass in distilled water for 8 days rather than 72 h. After 8 days of shaking, the biomass was filtered through Whatman filter paper No.1 and the cell-free filtrate was treated with AgNO₃ at a concentration of 1 mM. Afterwards, flasks were incubated at 26°C on a rotary shaker in both dark and light conditions. Longer incubation resulted in more severe color change and more absorption, confirming that the reaction is enzymatic. The stability of NPs is one of the biggest challenges in NPs biosynthesis. Consequently, the solution was examined three months later. No sedimentation was observed, and despite the decrease in absorbance, there was a sharp peak at 420 nm wavelength (Figure 4), indicating the stability of the NPs. Using UV-vis spectrophotometry Radhi et al. [21] studied the colloidal stability of silver nanoparticles biosynthesized by *B. cinerea*. They observed decreasing in the intensity of peak and it also broadened, owing to the reduction of nanoparticles and formation of aggregates respectively.

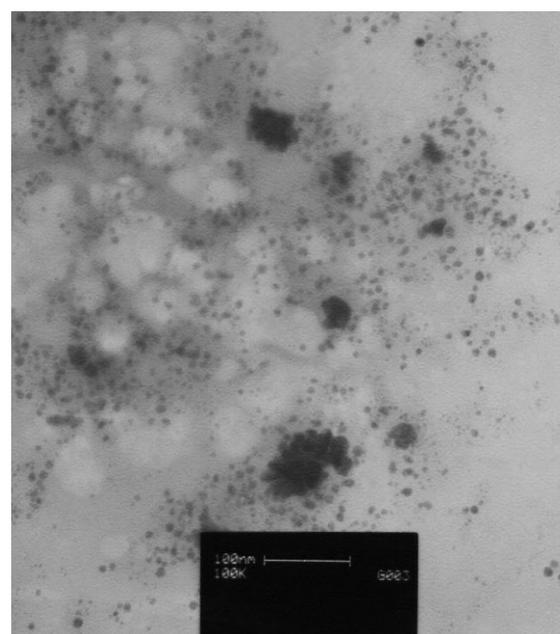


Figure 2. TEM image of the silver nanoparticles synthesized by *Botrytis cinerea*, showing their spherical nature.

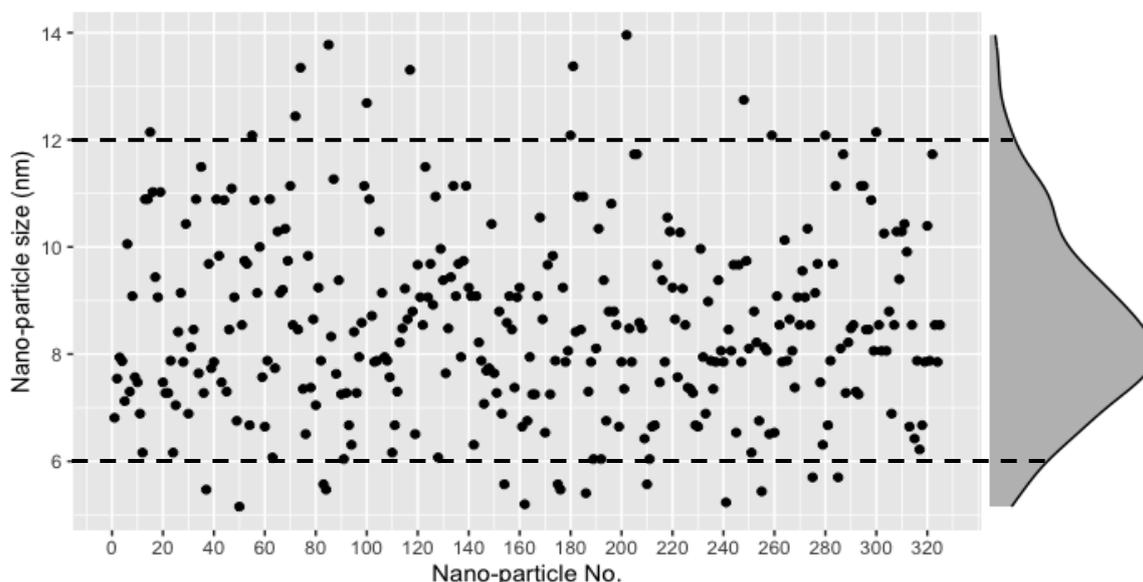


Figure 3. Distribution of silver nanoparticle size.

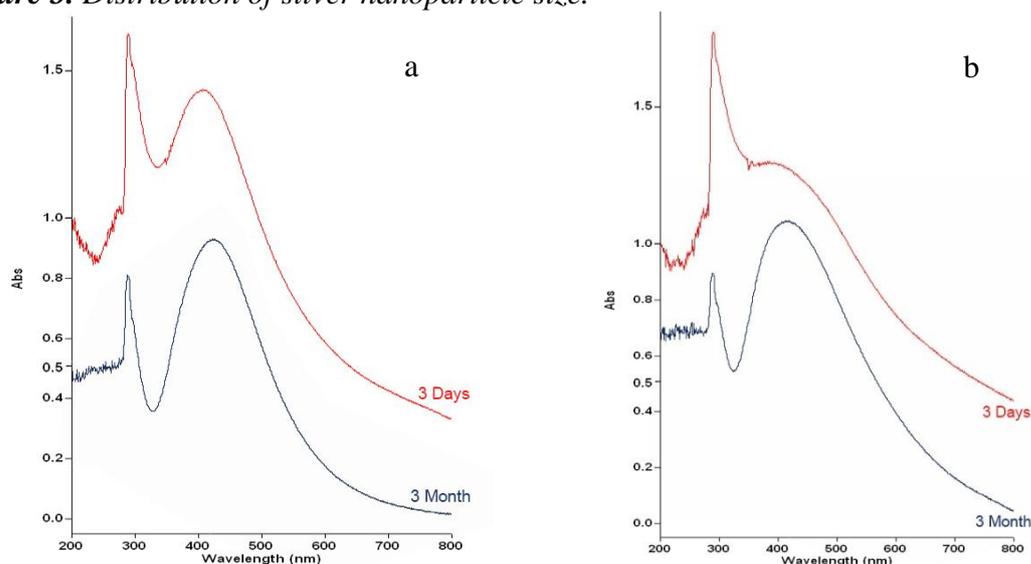


Figure 4. UV-vis spectra of silver nanoparticles synthesized by *Botrytis cinerea*, confirming the nanoparticles' stability three months after formation. a: in light and b: in a dark condition.

AgNPs have been reported to interact with enzymes [17], and it seems that their formation and stability rely on proteins. The FTIR spectrum of AgNPs (Figure 5) showed the presence of several bands at 3427.48, 2925, 2858, 1748, 1641, 1584, 1388, 1353, 1232, 1149, 1081 and 1037. Maximum absorption was at 3427.48, which is associated with the O-H stretch of the carboxylic acid groups [22]. Other

absorption bands are identified as the C-H stretch of the methylene groups of the proteins, amid I and amid II, N-H vibration of the amid III groups, O-H stretch of symmetric and antisymmetric modes of aliphatic and aromatic, C-O or C-O-C, and residual NO_3^- in the solution [22, 23]. Moreover, FTIR confirmed the presence of microbial proteins in the solution. These proteins can bind to AgNPs through their

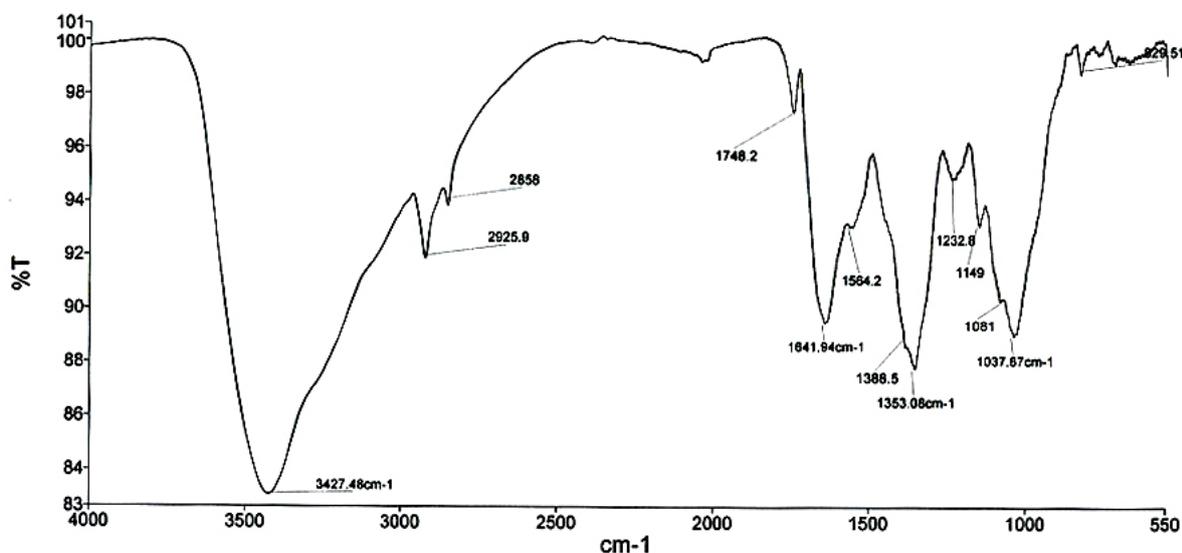


Figure 5. FTIR of synthesized silver nanoparticles by *Botrytis cinerea*.

carboxylic group, amine group, or residual cysteine. Furthermore, as capping agents, fungal proteins stabilize AgNPs. Physical and chemical methods are able to produce large amounts of nanoparticles in a relatively short time, but they usually require high pressure and temperature and consume a lot of energy. Moreover, due to the use of chemicals, these methods usually lead to the retention of some toxic reagents which limit their use in biological applications [11, 24]. The low-cost, nontoxic, highly-efficient, as well as eco-friendly nature of biological approaches draws attention to them.

4. CONCLUSION

Fungi which secrete high amount of proteins might result in the significant mass productivity of nanoparticles. Fungal proteins have the potential for hydrolyzing metal ions. It is noteworthy to point out that fungi are easy to isolate and cultivate, and the handling of fungal biomass is less complex compared to the synthetic methods [11]. In this research, *B. cinerea* was used for the extracellular biosynthesis of silver nanoparticles. AgNPs were

spherical, with an average size of 8.55 nm, and they were stable three months after their formation. All 18 *B. cinerea* isolates were able to produce silver nanoparticles, confirming the capacity of this fungus as a promising candidate to produce NPs. More importantly, the results showed that *B. cinerea* is reliable in overcoming the main challenges of NPs biosynthesis approach, producing small-sized NPs and their stability. Green synthesized NPs are supposed to be stable through protein capping agents which are secreted in enormous amounts by *B. cinerea*. This is an efficient and eco-friendly procedure, and it would be appropriate for the large-scale production of silver nanoparticles.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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