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SILVER NANOPARTICLES: MYCOGENESIS, CHARACTERIZATION AND ITS ANTI PLANT PATHOGENIC APPLICATIONS

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ABSTRACT

Nanotechnology is an emerging field in the area of plant disease management. Silver nanoparticles (AgNPs) have been heavily studied as antimicrobial materials. Silver nanoparticles synthesized by the means of physical, chemical and biological ways but bio synthesis is the most eco-friendly approach. In bio synthesis of silver nanoparticles, the use of fungi gives good mono dispersity and dimensions. The synthesized silver nanoperticles are characterized by different techniques such as UV-Vis spectroscopy for confirmation of AgNPs synthesis, Particle size Analyzer for Hydrodynamic diameter of AgNPs, Fourier Transmission Infrared Spectroscopy (FTIR) for identifying the molecules over AgNPs and Transmission Electron Microscopy (TEM) studies to know the surface morphology and size of AgNPs. Application of nano-sized silver particles as antimicrobial agents has become more common as technological advances make their production more economical. Since silver displays multiple modes of inhibitory action to microorganisms, it is used for controlling various plant pathogens in a relatively safer way compared to synthetic fungicides.

KEYWORDS: Review, Silver Nanoparticles, Mycogenesis, Plat Pathogen Control

INTRODUCTION

Nanotechnology is an emerging field in the area of interdisciplinary research especially in biology. The advancement of nanotechnology mainly requires the development of reliable and ecofriendly protocols for the synthesis of nanomaterial over a range of biological composition, sizes, shapes and high monodispersity. Nanoparticles possess exceptional physical and chemical properties which lead to rapid commercialization. Nanoparticles are considered as fundamental molecular building blocks for nanotechnology. They are pre-requisites for preparing many nanostructure materials and devices. Biosynthesis of nanoparticles is an attractive possibility of advancement of green nanotechnology which has potential to find numerous applications in biology, agriculture in particular. Bansal *et al.*, 2011 reported the biosynthesis of nanoparticles is a kind of bottom-up approach which involves reduction or oxidation process. Recently the utilization of biological systems provides a novel idea for the production of nanomaterials.

CONCEPT OF NANOTECHNOLOGY

The idea of Nanotechnology was first conceived by Noble laureate Richard Feynman, in his famous lecture at the California Institute of Technology, 29th December, 1959. In one of his articles published in 1960 titled, “There is plenty of

room at the bottom" discussed about the properties of nanomaterials. He pointed out that if a bit of information required only 100 atoms, then all the books ever written could be stored in a cube with sides 0.02 inch long. Norio Taniguchi first defined the term Nanotechnology, in 1970. (Taniguchi, 1974)

A nanoparticle is defined as a small object or particle that behaves as a whole unit in terms of its transport and properties. Nanoparticles are particles that have at least one dimension in the range of 1 to 100 nm. Nanotechnology takes advantage of the fact that when a solid material becomes very small, its specific surface area increases, which leads to an increase in the surface reactivity and quantum-related effects. Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macroscaled counterparts. (Sharma *et al*., 2009)

IMPORTANCE OF SILVER AND NANO SILVER

Silver has been valued throughout the history for many of its properties that are useful to humans. It is used as a precious commodity in currencies, ornaments, jewelry, electrical contacts and photography, among others. One of the most beneficial uses of silver has been as a potent antibacterial agent that is toxic to fungi, viruses and bacteria. Silver has long been used as a disinfectant; for example, the metal has been used in treating wounds and burns because of its broad-spectrum toxicity to bacteria as well as because of its reputation of limited toxicity to humans. Silver is incorporated in textiles to inhibit the growth of bacteria and to keep odor at minimum (Clement *et al*., 1994). In 1954, silver was registered in the US as a pesticide for use in disinfectants, sanitizers and fungicides.

Silver nanoparticles are fine particles of metallic silver that have at least one dimension less than 100 nm. Nanosilver is not a new discovery, it has been known for over 100 years Previously, nanosilver or suspensions of nanosilver were referred to as colloidal silver. To produce colloidal silver, a positive electrical current is applied through pure silver bars suspended in water resulting in colloidal silver particles with a size range of 15-500 nm (Lindemann, 1997). By converting bulk silver into nanosized silver, its effectiveness for controlling bacteria and viruses was increased multifold, primarily because of the nanomaterials extremely large surface area when compared to bulk silver, thus resulting in increased contact with bacteria and fungi. Nanosilver, when in contact with bacteria and fungus, adversely affects the cellular metabolism of the electron transfer systems, and the transport of substrate in the microbial cell membrane.

METHODS OF SYNTHESIS OF NANOSILVER

Generally, metal nanoparticles can be prepared and stabilized by chemical, physical and biological methods; in chemical approach, techniques such as chemical reduction, electrochemical techniques, photochemical reduction and pyrolysis are used.

Turkevich *et al*. (1951) reported a wet chemistry technique to synthesize nanosilver using silver nitrate as a silver ion source and sodium citrate as the reducing agent for the first time. Janardhanan *et al*. (2009) synthesized silver nanoparticles by an aqueous chemical method with an organic base and with no external capping agents. Silver nanoparticles of 40–80 nm size are formed in the process of oxidation of glucose to gluconic acid by amine in the presence of silver nitrate, and the gluconic acid caps the nanosilver. Tien *et al.* (2008) reported that chemical methods for metal nanoparticle fabrication usually involve toxic chemicals, which can be harmful to our environment. Although these methods may successfully produce pure silver nanoparticles, they require the use of stabilizers to protect the Ag

nanoparticles against agglomeration. Additionally, these methods are usually expensive and potentially harmful to the environment. In Physical Methods nanoparticles were produced from larger structures (top down) by use of ultrafine grinders, lasers, and vaporization followed by cooling.

Biological synthesis method has been developed to obtain biocompatible, inexpensive, ecofriendly, and size-controlled nanoparticles which were considered as main drawbacks in physical and chemical methods (Sadasivum *et al*., 2010). In biological synthesis, living organisms such as bacteria, fungi and plants were used for the production of metal nanoparticles.

MYCOGENESIS

Myconanotechnology is the study of nanoparticles synthesis using fungi and their applications. Fungi have gained much importance for synthesis of silver nanoparticles because they are easy to culture and they produce higher amount of proteins than bacteria (Sastry *et al*., 2003). Fungal-mediated synthesized nanoparticles have good monodispersity and good dimensions (Rai *et al*., 2009). The intracellular and extracellular methods were used for the synthesis of nanoparticle from fungi. The extracellular method is more advantageous than intracellular method because the intracellular method needs an additional step to obtain the purified nanoparticles (Kuber *et al*., 2006).

METHODOLOGY

Synthesis of silver nanoparticles involves the addition of ten ml of fungal culture filtrate to Fifty ml of aqueous solution of 1mM Silver nitrate (AgNO3) followed by incubation of the mixture at room temperature for 24 hrs. The color change of silver nitrate from colorless to brown color indicates the formation of silver nanoparticles through reduction of silver ionic forms (Ag^{+}) to Ag^{0}. (Figure.1)(Bhaskar *et al*., 2014)

Table 1: List of Fungi Used in Synthesis of Silver Nanoparticles

Fungi	Extra Cellular/Intra Cellular	Reference
Trichoderma asperallum	Extra cellular	Mukherjee *et al.* (2008)
Trichoderma viride	Extra cellular	Fayaz *et al.* (2010) and Chitra and Gurusamy (2013)
Trichoderma reesei	Extra cellular	Vahabi *et al*. (2011)
T. asperallum, T. harzianum, T. longibrachiatum, T. pseudokoningii and *T. virense*.	Extra cellular	Devi *et al.* (2013), Bhaskar *et al*, (2014)
Fusarium semitectum	Extra cellular	Basavaraja *et al*. (2008)
Aspergillus feotidus	Extra cellular	Roy *et al*. (2013)
Aspergillus terreus	Extra cellular	Abeer *et al*. (2013), Al-Othman *et al*. (2014)
A. niger	Extra cellular	Soni and Soam (2013)
Aspergillus fumigatus	Intra and Extra cellular	Bhainsa and Souza.(2006)
Penicillium citrinum	Extra cellular	Soheyla *et al.* (2011)
Rhizophus stolonifer	Extra cellular	Afreen and Vandana (2011)
Verticillium	Extra cellular	Mukherjee *et al.* (2001)
Pleurotus ostreatus	Extra cellular	Devika *et al*. (2012)
Agaricus bisporus	Extra cellular	Papaiah *et al*. (2014)
Fusarium oxysporum	Extra cellular	Vanmathi and Siva (2012)

CHARACTERIZATION OF SYNTHESIZED SILVER NANOPARTICLES

Characterization refers to the study of material's features such as composition, colour, size, structure and various properties like physical, chemical, and magnetic properties. Nanoparticles characterization is necessary to establish understanding and control of nanoparticle synthesis and applications. Characterization was done by using a variety of different techniques.

VISUAL OBSERVATION (COLOUR CHANGE)

In bio-synthesis of silver nanoparticles the silver ion solution changed from colorless to brownish color which indicates formation of silver nanoparticles. Silver nanoparticles exhibit brown color in water due to excitation of surface plasmon vibrations in metal nanoparticles (Singh and Raja, 2011).

UV-VIS SPECTROSCOPY ANALYSIS

UV-Vis spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample. The spectra recorded from AgNPs solution showed an absorption peak at 420 nm which was specific for the silver nanoparticles.

PARTICLE SIZE ANALYSIS

The hydrodynamic diameter of the silver nanoparticles in the solution is measured by using the principle of Dynamic Light Scattering (DLS) technique. DLS can be used to determine the size distribution profile of small particles in suspension. (Figure.2)

FOURIER TRANSMISSION INFRARED SPECTROSCOPY (FTIR)

FTIR is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum (Figure 3). The FTIR measurements are carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for the synthesis and stabilization of silver nanoparticles with the capping agent available in the culture filtrate (Roy *et al*. 2013). Vahabi *et al*. (2011) recorded the FTIR spectrum from a drop-coated film of an aqueous solution incubated with *Trichoderma reesei* and reacted with *Ag+* ions for 72 hours. The amide bands are identified at 1650 cm-1 and 1450 cm-1 which are due to *–C=O* and *N-H* stretch vibrations present in the amide linkages of the proteins, respectively. Afreen and Vandana (2011) studied the FTIR spectrum of SNPs produced by *R. stolonifer* This spectrum shows the presence of band at 1645(1), 1537(2) and 1454(3) cm-1, the bands at 1645 cm-1 corresponds to primary amine NH band 12 The band atca.1454 cm-1 due to methylene scissoring vibrations present in the proteins.

TRANSMISSION ELECTRON MICROSCOPY (TEM)

Transmission electron microscopy provides the details of surface morphology, size and shape of the silver nanoparticles (Figure 4). Mukherjee *et al.* (2001) reported the biological synthesis of silver nanoparticles using the fungus *Verticillium* and found the size of silver nanoparticles were in the range of 25 + 2 nm. Singh and Raja (2011) studied the size and shape of the silver nanoparticles synthesized from *T. harzianum* and found that they were in the range of 30-50nm in size and spherical in shape. Devika *et al*. (2012) studied the TEM analysis of silver nanoparticles synthesized from the

fungus *Pleurotus ostreatus* and it has been reported that the size of the silver nanoparticles were in the range of 8-50 nm. Vanmathi and Siva (2012) studied the shape and size of silver nanoparticles, which were isolated from *Fusarium oxysporum* through TEM analysis and found that they were in the range of 5-60nm in dimension and spherical in shape.

ANTI-PLANT PATHOGENIC ACTIVITY OF SILVER NANOPARTICLES

Roy *et al*. (2013) studied the anti fungal activity of silver nanoparticles synthesized from *Aspergillus feotidus* MTCC8876 against different fungal strains through agar well diffusion method. The zone of growth inhibition (cm) of AgNO3, AgNPs against *A. niger* (1.5, 2.0), *A. oryzae* (1.5, 1.8), *A. parasiticus* (1.4, 1.9), *A. foetidus* (1.1, 1.7), *A. flavus* (1.2, 1.5) and *Fusarium oxysporum* (1.3, 1.7). Papaiah *et al*. (2014) evaluated the silver nanoparticles synthesized from white button mushroom (*Agaricus bisporus*) against three plant pathogens *Sclerotium rolfsi, Aspergillus niger, Rhizoctinia solani* causing stem rot, collar rot, root rot in groundnut respectively. As the dosage of silver nanoparticles increased per well, the diameter of zone of inhibition also increased and the highest zone of inhibition (0.38, 0.38, 0.3cm rerspectively) was found at 150 µl for all three pathogens. Kaur *et al*. (2012) used silver nanoparicles, chitosan nanoparticles and silver-chitosan nanocomposite as an antifungal agent against seed borne pathogens. In this study, seed borne pathogens *Rhizoctonia solani, Aspergillus flavus, Alternaria alterneta* were isolated from chickpea seeds. The zone of inhibitions of Ag-Ch nanoformulations was much higher than silver or chitosan nanoparticles used independently. In agar well diffusion method Ag-Ch exhibited highest inhibition against *Aspergillus flavus* (19.66 ± 0.28) followed by *Alternaria alternate* (16.33 ± 0.29) and *Rhizoctonia solani* (12.66 ± 0.76). In mycelia growth inhibition method Ag-Ch showed inhibitions 94%, 67% and 78% against *Aspergillus* spp, *Rhizoctonia* spp, and *Alternaria* spp. respectively. Jolanta *et al*. (2013) synthesized the silver nanoparticles from an aqueous raspberry extract. Nanosilver suspensions were tested for their antifungal activity against *Aspergillus niger* and *Cladosporium cladosporioides.* The results showed that nanosilver suspension at the concentration of 50 ppm inhibited the growth of *Cladosporium cladosporoides* and *Aspergillus niger* by 90% and 70%, respectively. Al-Othman *et al*. (2014) evaluated the effect of silver nanoparticles which were biosynthesized from *Aspergillus terreus* (KC462061) on growth and aflatoxin production by Five isolates of *A. flavus* isolated from groundnut pods. All five *A. flavus* isolates were inhibited to various extents by different concentrations of silver nanoparticles but the best inhibition was recorded at 150 ppm and inhibition % of aflatoxin production at 50ppm ranged from 48.2 to 61.8%, at 100 ppm ranged from 46.1 to 82.2% whereas at 150ppm inhibition % reached to 100%. Rao and Savitramma (2011) studied the efficacy of biologically synthesized silver nanoparticles from *Svensonia hyderabadensis* leaf extract against different fungi (*Fusarium oxysporum, Curvularia lunata, Aspergillus niger, Rhizopus arrhizus*). The zone of inhibition was found as 8mm, 10mm, 11mm, 10mm for *Fusarium oxysporum, Curvularia lunata, Aspergillus niger and Rhizopus arrhizus* respectively. Kim *et al.* (2012) studied the effect of silver nanoparticles (AgNPs, WA-CV-WA13B (CV), WA-AT-WB13R (AT), and WA-PR-WB13R (PR)) provided by Bio plus co. (Pohang, Korea) against eighteen plant pathogenic fungi on different media (PDA, MEA and CMA). Higher inhibition of fungal growth was recorded at a concentration of 100 ppm. Most of the fungi showed growth inhibition with the increment of incubation time, and the inhibition showed similar patterns for each type of media. Absolute inhibition (100%) was observed on PDA medium against A-3, C-10, F-5, P-8, and P-9, and greater than 90% inhibition on PDA against C-1, D-1, G-1, M-1, and S-3. The lowest level of inhibition was observed against G-1 on PDA treated with a 10 ppm concentration of AgNPs. Kabir *et al.* (2011a) applied the silver nanoparticles (WA-PR-WB13R) for the control of *Colletotrichum* species *in vitro* and pepper anthracnose in field. Complete inhibition was observed on PDA treated with 100 ppm silver nanoparticles against

isolates C-3 and C-5. The C-7 and C-8 isolates also showed more than 90% inhibition on PDA treated with 100 ppm silver nanoparticles. The inhibition of fungi was significantly high when silver nanoparticles were applied before disease outbreak on the plants when compared with the application after the disese outbreak. The lowest disease incidence was observed on plants treated with 50 ppm silver nanoparticles before the disease outbreak (9.7%). Kabir *et al.* (2011b) evaluated the inhibition effect of silver nanoparticles (WA-CV-WA13B) against powdery mildews on cucumber and pumpkin. The disease incidence was observed as 57.8, 48.8, 40.2 and 20% in 10, 30, 50 and 100 ppm concentrations of silver nanoparticles treated after disease outbreak on plants. In similar way, the disease incidence was observed as 45, 40, 27 and 18% in 10, 30, 50 and 100 ppm concentrations of silver nanoparticles treated before the disease outbreaks on plants, respectively. Bhaskar *et al*. (2014) synthesized silver nanoparticles using *Trichoderma* sp. and evaluated against *Aspergillus niger* causing collar rot of Groundnut. At 100 ppm concentration, the per cent inhibitions were observed as 74.8% and 68.5% for AgNPS and AgNO3 solutions respectively. Gan *et al*. (2010) studied antibacterial activity of nanosilver against *Xanthomonas campestris* pv. campestris towards control of cabbage black rot in the pot.

CONCLUSIONS

The use of fungi for the synthesis of silver nanoparticles results good monodispersity and dimensions of AgNPs. Silver nanoparticles act as strong antimicorobial agents because of its size dependent properties. The main problem with usage of available fungicides for control of plant pathogens is residues in crop plants and improvement of resisitance in pathogens. Therefore, application of nanoformulations such as silver nanoparticles will be a better alternative to the synthetic fungicides.

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APPENDICES

Figure 1: Synthesis of Silver Nanoparticles Using Fungi

A: *Trichoderma* sp Culture filtrate

B: 1 mM AgNO3 solution before tratment with culture filtrate

C: AgNPs after 24 hrs incubation

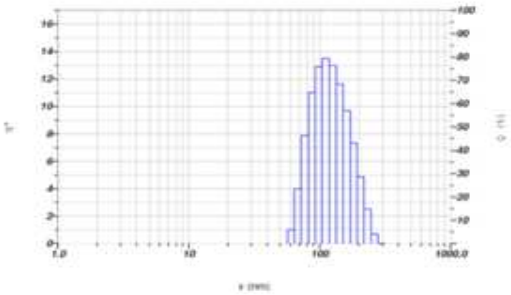

Figure 2: Dynamic Light Scattering (DLS) Study of Synthesized Silver Nanoparticles Showing the Size Distribution of Silver Nanoparticles

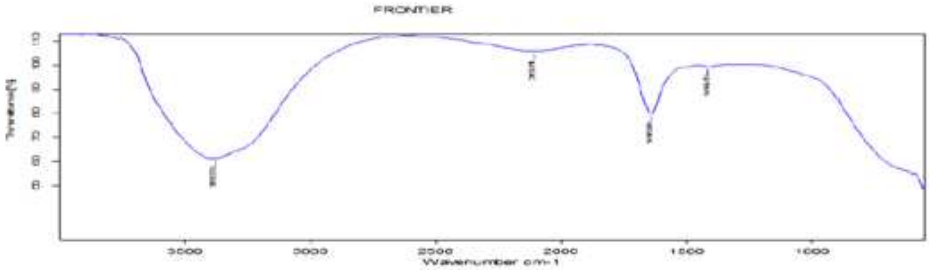

Figure 3: FTIR Spectrum Recorded From a Drop-Coated Film of an Aqueous AgNPs Solution Showing Characteristic Peaks at 1414.78, 1640, 2108.51, 3380 Cm^{-1}

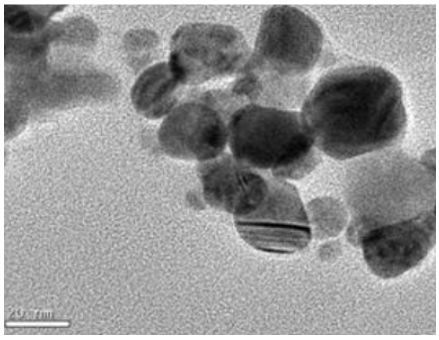

Figure 4: TEM Micrographs Showing the Relatively Spherical Shaped AgNps with the mean Size of 30 nm.

(Figures from Bhaskar *et al*., 2014)