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Mycosynthesis of silver nanoparticles by endophytic Fungi: Mechanism, characterization techniques and their applications

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ABSTRACT

Fungi are attractive as reducing and stabilizing agents in the biogenic synthesis of silver nanoparticles due to the production of considerable amounts of proteins, high yields, simplicity of handling, and low toxicity of the residues. Nanotechnology is one of the prospective technologies that could be utilized to address the recent issues as agrichemical production lines, nanotechnology production lines can also have drawbacks. As a result, a brand-new branch of nanotechnology called as "green nanotechnology" was developed, integrating biological ideas with physical and chemical procedures to produce nanosized particles with specific uses that are ecologically friendly. Biochemical synthesis methods utilizing microorganisms and plants are being investigated and developed in an effort to create nanoparticles (NPs) in a sustainable and eco-friendly manner. Biological synthesis has grown in favor as a possible alternative to the drawbacks related to physical and chemical methods of synthesis. The current analysis discusses the potential for enhancing plant health and disease resistance using AgNPs produced by endophytic fungi, which will contribute to a greater level of agricultural sustainability. Future research should focus on understanding this important method, it is advised.

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Introduction

With regard to applications and synthesis processes, nanotechnology has developed into a multidisciplinary field in recent years with contributions from numerous scientific disciplines, including physics, chemistry, materials science, biology, medicine, mathematics, technology, and engineering (Rahman et al. 2019). Nanotechnology is an area of study that focuses on the creation and use of nanoparticles (1 to 100 nm or 1.0 × 10^{-9}) (Taniguchi 1974; Thakkar et al. 2010; Naik 2020). The term "Nano" refers to a measurement that is 1 billionth of a meter in size and is derived from the Greek word "Nanos," which meaning "dwarf" (Thakkar et al. 2010, Pantidos & Horsfall 2014).

Nanoparticles can be created in a variety of methods from a variety of materials, but they can generally be divided into three groups: chemical, physical, and biological processes. Among them, biological methods hold the most promise for producing nanoparticles (Sandhu et al. 2017). When utilized in physical processes, high temperatures, radiations, and pressure have the potential to cause serious harm to living things and the environment (Alzahrani et al. 2015). Chemical processes are employed because they can swiftly manufacture a large number of uniform nanoparticles with control over their size and distribution (He et al. 2015).

On the other hand, chemical processes employ risky materials, require a lot of energy, and produce deadly pollutants that constitute a significant threat to the environment. Additionally, due to the frequent use of potentially harmful chemicals and radiation-damaging radiation in physical and chemical processes, physical-chemical methods for producing nanoparticles had low

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production rates, demanded high costs, took a long time, and released harmful compounds into the atmosphere (Mallick et al. 2004; Naik 2020). As a result, a brand-new field called "Nanobiotechnology" has evolved, fusing biotechnology and nanotechnology to provide techniques for producing biosynthetic nanoparticles that are ecologically friendly, simple, inexpensive, don't require a lot of energy because they don't generate any toxic waste and environmentally beneficial (Kalishwaralal et al. 2008; Ovais et al. 2018b; Shah et al. 2018).

Among the various kinds of metal NPs such as gold (Au), silver (Ag), zinc (Zn), Palladium (Pd), and Platinum (Pt), silver nanoparticles (Ag NPs) have attracted the greatest interest due to their distinctive properties, including high conductivity, robust chemical stability, and catalytic powers (Diantoro et al. 2018). Given that AgNPs also possess antibacterial properties and are efficient against fungus and other prokaryotic pathogens even at extremely low concentrations, they can be used as nanomedicines, implants, gadgets, and dressing materials (Kumar et al. 2016; Ranjani et al. 2020). AgNPs produced through biological means are more biocompatible than NPs created through chemical means, making them better suitable for use in medical applications (Abdelghany et al. 2018). Numerous biological species, such as bacteria, fungi, algae, and plants, are used to create NPs (Khalil et al. 2018; Rahman et al. 2019; Fadiji et al. 2022).

Myconanotechnology, a combined field of mycology and nanotechnology, describes research on nanoparticles produced by fungi (Rai et al. 2009). The idea of using fungi is based on their capacity for tolerance and metal bioaccumulation, which make the biosynthesis of nanoparticles produced by fungi considered to be a significant branch. Fungi are also capable of producing biogenic Ag NPs, and because their biomass is simpler to handle, they are preferred over bacteria and other microorganisms (Ranjani et al. 2020).

The method of silver ion bioreduction by fungi is essentially found to be nitrate reductase-mediated synthesis. Several researchers advocated for the use of NADH-dependent reductase in the extracellular creation of nanoparticles (Ahmad et al. 2003; Kumar et al. 2007; Gade et al. 2008; Ingle et al. 2008). Many papers have been published on the use of medicinal plants for the production of silver nanoparticles (AgNPs), including *Ocimum sanctum* (Ahmad et al. 2010), *olive leaf* (Mustafa et al. 2013), *Rumex dentatus* (El-Shahaby et al. 2013) and *Raphanus sativus* var. *aegyptiacus* (Ali et al. 2015).

Stone et al. (2000) estimated that endophytic fungi inhabiting medicinal plants may number over a million species. Fungi reside within the living tissues of medicinal plants as endophytes (Dreyfuss & Chapela 1994). A bacteria or fungus that spends at least some of its life cycle inside a plant without obviously causing disease is known as an endophyte (Rahman et al. 2017). Endophytes are essential to plants for their chemical growth and development, which they obtain from the environment, soil, water, and organic materials (Ranjani et al. 2020). Endophytes have a substantial impact on nutrient absorption. Endophytic fungus from medicinal plants is a potential source of antioxidants (Strobel et al. 1997). Thus, there is a symbiotic relationship between plants and endophytes in which the partnership will benefit both the plant and the endophytes through plant development and protective mechanisms (Ranjani et al. 2020).

There are more than (300,000) distinct plant species on Earth, and a large number of them include endophytic microbes. The stability and prevention of NP aggregation during synthesis are connected with high levels of proteins and other biomolecules generated by endophytic fungus (Netala et al. 2016a). It is easy to use in the creation of AgNPs and has several potential applications (Kathiresan et al. 2009; Rahman et al. 2019) Recent research has shown that these nanoparticles are powerful antibacterial agents for preventing the spread of infectious diseases caused by a variety of harmful bacteria that are antibiotic resistant (Kim et al. 2007).

Nanotechnology

Nanotechnology is one of the most active research areas and a potent tool for battling germs (Abd-Ellatif et al. 2022). The appropriate size of nanoparticles (NPs) can be the foundation for nanotechnology (Liz-Marzan & Kamat 2003). The terms "NPs" can also refer to nanomaterials, "nanoscale materials," "nanosized materials," "nanosized particles," "nanoscale objects," and "nanostructured materials." The noble metal-based NPs that are now being studied most successfully include Ag, Au, Pd, and Pt. These particles exhibit unique characteristics at nanoscales between one and one hundred nm. The variations in their features are a result of their small sizes and high surface area to volume ratio, which favors quantum processes. Here are a few of their exceptional qualities that make them useful in many industries.

1. High tensile strength
2. Improved thermal and electrical conductivity
3. Increased surface-to-volume ratio
4. High reactivity

Metallic nanoparticles with tremendous promise for biomedical applications include Ag, Au, Zn, Pd, and Pt nanoparticles (Bhattacharya & Mukherjee 2008; Hirst et al. 2009). Although numerous studies have demonstrated that metal nanoparticles have bioactive properties, silver nanoparticles currently hold a bigger significance than any other metallic nanoparticle due to their extensive use as therapeutic agents in the medical industry. Its outstanding antibacterial and anti-inflammatory qualities have been found to be beneficial in the treatment of a number of ailments (Sandhu et al. 2017).

Silver nanoparticle (AgNPs)

White, brilliant, soft, and having a high thermal and electrical conductivity, silver is a transition metal. Although metallic silver itself is insoluble in water, metallic compounds like AgNO3 and Silver chloride are. Metals in the nanoscale size range have different physical properties from ions and bulk materials. They exhibit extraordinary qualities like increased catalytic activity as a result of their morphologies with particularly active aspects (Yacaman et al. 2001).

In addition to being present in sutures, coins, vessels, foils, solutions, and colloids used in lotions, creams, and other goods, silver has a long history of therapeutic and medicinal usage (Rahman et al. 2019). Silver is the most important medical treatment agent for infectious disorders and surgical infections (Rahman et al. 2019).

Biosynthesis of (AgNPs)

There are numerous ways to synthesize NPs, but they can generally be divided into three categories: chemical, physical, and biological (Sandhu et al. 2017). Since they do not call for the employment of poisonous chemicals that can flow with generated waste lines, the biological procedures among them are regarded as being safe (Mallick et al. 2004). Chemical processes produce or use highly toxic chemicals and other hazardous substances, which restricts their application in biomedicine. The physical techniques demand a lot of energy. Because they are safe for the environment and are energy-efficient, biological approaches are gaining popularity. In addition, the cost and poor production rate of the physical and chemical processes for synthesizing nanoparticles (Guilger-Casagrande & Lima 2019). In large-scale manufacturing, polydispersity and stability are issues, particularly when the reduction occurs in aqueous circumstances. The biological methods have many advantages, such as not needing the use of hazardous chemicals, high temperatures, or pressures, and being easy to scale up at a big scale (Mallick et al. 2004).

Biological techniques make use of microbes that can take up and accumulate metals (Sandhu et al. 2017). The biological systems that are most frequently utilised for this purpose are herbal extracts, microalgae, fungus and bacteria. Biosynthesis methods come under the bottom-up approach where in the AgNPs are produced by reduction via, using fungi which they have an advantage over other organism in that certain of the proteins and enzymes such as (nitrate reductase) they produce can be used for fast and sustainable synthesis of nanoparticles (Alghuthaymi et al. 2015). The mechanism of biosynthesis of nanoparticles using fungi may be intracellular or extracellular. When using intracellular synthesis, the metal precursor is added to the mycelial culture and is internalized in the biomass (Sandhu et al. 2017).

As a result, after the synthesis, the nanoparticles must be extracted. This may be done by using centrifugation, filtering, and chemical treatment to break the biomass and extract the nanoparticles (Molnár et al. 2018). When the metal precursor is introduced to an aqueous filtrate that solely has fungal biomolecules, extracellular synthesis results in the formation of free nanoparticles. The final method is the most often used because no additional actions are needed to remove the nanoparticles from the cells (Sabri et al. 2016; Costa Silva et al. 2017). However, the dispersion of nanoparticles needs to be filtered to remove fungal remnants and other contaminants. Simple filtering, membrane filtration, gel filtration, dialysis, and ultracentrifugation are a few techniques that can be used to achieve this (Ashrafi et al. 2013; Qidwai et al. 2018; Yahyaei & Pourali 2019) The experimental method for producing silver nanoparticles frequently stays the same with only small alterations depending on the species of fungus being used (Figure1).

Endophytic Fungi

Endophytic fungi are microscopic fungus that live inside plants, with out causing illness or injury to the host (Azevedo et al. 2000). They are found in almost all classes of vascular plants and grasses that have been studied thus far (Zhang et al. 2006). In addition to fungi, several other groups of living things, such as bacteria, actinomycetes, and mycoplasma, have been referred to be plant endophytes (Bandara et al. 2006). Over a century has passed since the discovery of their existence. The Greek words "endon," which means "within," and "phyton," which means "plant," are the origin of the word "endophyte"(Sandhu et al. 2017).

Endophytic fungi are of tremendous interest to applied microbiology, and numerous studies are conducted to see whether they might affect how plants develop and survive (Toju et al. 2016; Hiruma et al. 2018). These investigations show that a number of root endophytic fungi promote plant growth by reducing infections or enhancing the nutritional status of the rhizosphere (Rodriguez et al. 2009; Newsham 2011) Numerous factors are crucial, including the host (plant), compatibility, fungus strains, and soil type (Hiruma et al. 2018; Svenningsen et al. 2018).

The bioactive compounds that endophytic fungus create potentially take the place of those that plants produce. Numerous antibacterial, anticancer, antioxidant, and immunomodulating properties are displayed by these compounds (Ding et al. 2008; Netala et al. 2016a). Endophytes have received the greatest attention because it has also been partially reported that they can synthesize nanoparticles (Musarrat et al. 2010; Sogra & Balakrishnan 2014). A critical stage in the application of nanotechnology is the development of a dependable, environmentally friendly process for the green synthesis of nanoparticles. One method that has a lot of potential is based on endophytes, which are microfungi that host plants (Abdel-Azeem et al. 2020).

Endophytic fungi and silver nanoparticles

Due to the growing issue of antibiotic-resistant microorganisms, it is imperative that new antimicrobial medications be developed. The use of silver nanoparticles is the most efficient strategy for reducing antibiotic resistance in microorganisms (Klasen 2000; Rahman et al. 2019). Due to its increased toxicity to a variety of microbes and decreased toxicity to mammalian cells, silver offers advantages over other metals (Rahman et al. 2019). Additionally, silver ions are known to be effective against a variety of microorganisms that are resistant to antibiotics (Sharma et al. 2013). Plants, bacteria, fungi, and algae have all been employed as "biofactories" to produce nanoparticles; each of these biological resources has benefits and drawbacks (Baker et al. 2013; Iravani et al. 2014).

Plant diversity disturbance is a serious problem regarding using plants (Baker & Satish 2012; Baker et al. 2015). Microbes are a desirable alternative because they are an abundant and reliable source of secondary metabolites that may transform a metal into its corresponding nanoparticles (Staniek et al. 2008). Endophytic fungal flora has been characterised by many researchers as a source of many bioactive substances with potential action. However, endophytic fungi have only recently been employed to produce nanoparticles by a small number of scientists (Sandhu et al. 2017). Fungi have several benefits over bacteria in the synthesis of nanoparticles, since most fungi are simple to handle, require few nutrients, have strong wall-binding capacities, and can take up metals intracellularly (Dias et al. 2002; Sanghi & Verma 2009).

Bacterial broth can be more simply filtered than fungal broth using a filter press, saving you money on the specialist equipment that could be needed for other procedures. In light of this, fungi are preferred to other biological systems for the large-scale synthesis of nanoparticles.

The first indication that silver nanoparticles have been synthesized is obtained by detecting a shift in color from light yellow to brown in the reaction mixture. Rahi & Parmar (2014) were able to establish the existence of silver nanoparticles When 10 g of *Penicillium* sp. (Link) biomass were exposed to a 1 mM silver nitrate solution. After being shaken for three days at 28±2°C, the reaction mixture's colour changed from transparent to brown. Similar to this, three endophytic fungus, *Aspergillus tamarii* PFL2 (Kita), *Aspergillus niger* PFR6 (Tiegh), and *Penicillium ochrochloron* PFR8 (Biourge), isolated from *Potentilla fulgens* L., were used in the production of silver nanoparticles (Devi & Joshi 2015).

In Table (1) provides a summary of several research on the synthesis of AgNPs from various endophytes fungi with regard to their sizes, morphologies, absorbance peaks, and prospective biological activities.

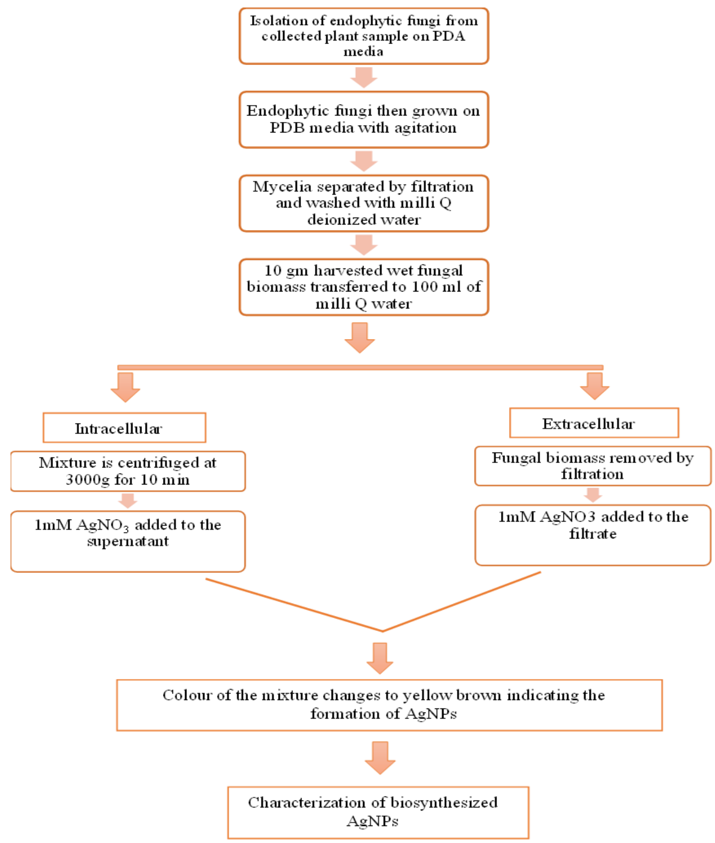

Fig 1. General methodology for biosynthesis AgNPs by using endophytic fungi (©Sara A. Gezaf)

The mechanism for producing AgNPs using endophytic fungi

Although it is clear that the specific mechanism of nanoparticle production has not yet been identified, study experts have made a number of recommendations. Nanoparticle production is mostly carried out by microorganisms using both extracellular and intracellular enzymes (Ovais et al. 2018a; Rahman et al. 2019) In reaction to environmental challenges including exposure to harmful compounds (metallic ions), varying temperatures, predators, etc., some fungi are able to create extracellular metabolites and enzymes, according to Mehra & Winge's (1991) hypothesis. Even though the fungus cannot survive without these metabolites and enzymes, they have a propensity to convert silver ions into nanoparticles.

Table 1 Different taxa of endophytic fungi a source of AgNPs with special reference to their sizes, morphologies, absorbance peaks, and prospective biological activities.

Host plant	Endophytic fungi	Absorbance peak	range	Activity	Reference
Raphanus sativus L.	*Alternaria* sp. Nees	426 nm	4-30 nm	Antibacterial (*Bacillus subtilis, Escherichia coli, Serratia marcescens*)	Singh et al. (2017)
Calotropis procera (Aiton) Dryand.	*Aspergillus terreus* Thom	410- 425 nm	16.54 nm	Antibacterial	Rani et al. (2017)
Simarouba glauca L.	*A. niger* Tiegh	400 nm	41.9 nm	Antioxidant, antibacterial	Hemashekar et al. (2017)
Casuarina junhuhniana Miq.	*Aspergillus tamarii* Kita	424 nm	31-40 nm	Antimicrobial, Germination study	Abdel-Aziz et al. (2018)
Azadirachta indica Juss.	*Aspergillus clavatus* Desm	415 nm	10-25 nm	Antimicrobial	Verma et al. (2010)
Centella asiatica L.	*A. versicolor* Tirab	429 nm	3-40 nm	Antifungal, Antibacterial	Netala et al. (2016b)
Potentilla fulgens L.	*Aspergillus tamarii* Kita PFL2 *Aspergillus niger* Tiegh PFR6	1- 419 nm 2- 430 nm	1- 3.5 ± 3 nm 2- 8.7 ± 6 nm	Antimicrobial growth kinetics,	Devi & Joshi (2015)
Sargassum wightii Grev.	*Cladosporium cladosporioides* (Fresen.) de Vries	440 nm	30-60 nm	Antioxidant, antibacterial	Joshi (2017)
Tanacetum sinaicum (Fresen.) Delile ex Bremer & Humphries	*Chaetomium globosum* Kunze	400 nm	-	-	Abu-Elsaoud et al. (2015)
Phellodendron amurense Rupr.	*Epicoccum nigrum* Link	250–750 nm	1-2 nm	Antifungal	Qian et al. (2013)
Withania somnifera L.	*Fusarium semitectum* Berk. & Ravenel	420 nm	10-20 nm	Antibacterial	Halkai et al. (2017)
Curcuma longa L.	*Penicillium* sp. Link	425 nm	25-30 nm	Antibacterial	Singh et al. (2013b)
Chetomorpha antennina (Bory) Kuetz.	*Penicillium polonicum* Zaleski	430 nm	10-15 nm	Antibacterial, Killing Kinetics assay	Neethu et al. (2018)
Aloe vera (L.) Webb.	*Penicillium* sp. Link	380-680 nm	16.1-39.6 nm	Antibiotic enhancing activity, Antibacterial	Rahi & Parmar (2014)
Solanum lycoprasicum L.	*Trichoderma harzianum* Rifai	-	12.7 nm	Antifungal	El-Moslamy et al. (2017)
Sida acuta Burm.	*Syncephalis* Tiegh. & Le Monn.	380-680 nm	6.40-16.9 nm	Antimicrobial	Rahi et al. (2014)

To produce nanoparticles, as shown in Fig. 3, fungal mycelium is exposed to a metal salt solution. When exposed, fungus experiences osmotic stress from the salt solution, which prompts the fungus to create enzymes and metabolites in order to survive (Sandhu et al. 2017). The cell membrane may contain the enzymes created during this process, which can decrease Ag+ to Ag during intracellular production (Zhao et al. 2018). These materials, which are produced from fungi, can catalyse the transformation of hazardous metal ions into harmless metallic nanoparticles.

There are 3 steps found in the biosynthesis of nanoparticles by fungi:

Step 1: catching of metal ions near to the fungus cells

Step 2: Enzymes released by the cell which reduce silver ions

Step 3: Fungal peptides and proteins stabilise AgNPs

A range of anthraquinones and naphthoquinones (involved in NP synthesis), as well as enzymes and metabolites (Barabadi et al. 2017). Fungi have also been described as having exceptional redox properties and serving as an electron shuttle in the reduction of metal, as seen in Fig. 3. The stabilisation is performed, in accordance with Durán & Seabra (2012) and Zhao et al. (2018), through electrostatic contact between cysteine residues or free amine groups and carboxylate groups. This interaction further reduces the Ag+ to silver nuclei on mycelia by causing enzymes in the cell wall to produce AgNPs (Salunke et al. 2016). PH, concentration, temperature and exposure time to substrate are some of the variables that can affect how quickly and in what size nanoparticles are synthesised (Singh et al. 2013a; Alghuthaymi et al. 2015; Sandhu et al. 2017). Figure 3 showed a Schematic graph of mechanism of AgNPs synthesis by endophytic fungi and figure 4 displayed a schematic of the variables affecting the production of AgNPs utilising fungal extract.

Techniques for characterising and analysing biosynthesised AgNPs

After initial confirmation of the synthesis of AgNPs by observation of the colour shift of the reaction mixture from pale white to yellowish brown, several characterisation techniques are employed to establish the presence of AgNPs in the solution (Sunkar & Nachiyar 2012). It is the most crucial phase in the biosynthesis of AgNPs since it explains the existence of any biomolecules that might be linked to these particles in addition to the size and shape of the generated silver nanoparticles. Silver nanoparticles are characterised using a variety of molecular techniques, such as the following: 1- UV-visible spectroscopy; 2- X-ray diffraction technique (XRD); 3- Fourier transform infrared spectroscopy (FTIR); 4- Atomic force microscopy (AFM); 5- Scanning electron microscopy (SEM); 6- Transmission electron microscopy (TEM); and 7- Energy Dispersive X-ray Spectroscopy (EDX). More information about these techniques is covered in the following pragaraphs (Table 2).

UV-visible spectroscopic analysis

For the initial characterization of created nanoparticles, UV-visible spectroscopy is a very beneficial and trustworthy method that is also utilised to demonstrate the production and stability of AgNPs (Sastry et al. 1998).

AgNPs have unusual optical properties that greatly affect how they interact with certain light wavelengths (Zhang et al. 2016). Additionally, UV-vis spectroscopy may characterise the particles in colloidal suspensions without a calibration because it is quick, simple, sensitive, selective for different types of NPs, and only needs a short period of time for measurement (Tomaszewska et al. 2013).

According to various studies, characterisation is best done in the absorbance band at about 200-800 nm wavelengths when the particle size is between 2-100 nm (Das et al. 2009). The valence and conduction bands are relatively close to one another in silver nanoparticles. The free motion of the electrons inside these bands produces the surface plasmon resonance absorption bands. Depending on the size of the particles, the chemical environment, and the dielectric medium, AgNPs absorb differently (Zhang et al. 2016). The finding of this peak attributed to a surface plasmon for several metal nanoparticles with diameters ranging from 2 to 100 nm has been frequently reported (Almatroudi et al. 2020). Over the course of more than a year, AgNPs made via biological methods were monitored, and an SPR peak at the same wavelength was found using UV-vis spectroscopy.

This technique determines the presence of silver nanoparticles by measuring the absorbance of bio-reduced solution at wavelengths between 200 and 800 nm. When silver nanoparticles (brown solution containing reduced silver nitrate in aqueous extracts of fungus) are exposed to light, a high absorption peak is thought to develop in the wavelength region between 390 and 420 nm, whereas the control does not (pale white solution containing silver nitrate in deionized Milli-Q water). This indicates how endophytic fungus water extracts have less silver nitrate (Kleemann 1993).

X-Ray diffraction analysis (XRD)

Crystalline metallic nanoparticles are employed in X-ray diffraction (XRD), the most popular non-destructive analytical technique, to track the structure of the crystals and analyse both molecular and crystal structures (Vijayaraghavan & Ashok 2017). It is feasible to confirm the creation of nanoparticles with crystalline structure using the ensuing diffraction (Alexander & Klug 1950). The intensity of the refracted radiation is measured with a goniometer when crystalline or powdered materials are placed on a sample holder and exposed to X-rays of a specific wavelength (Aziz et al. 2015). The particle size can

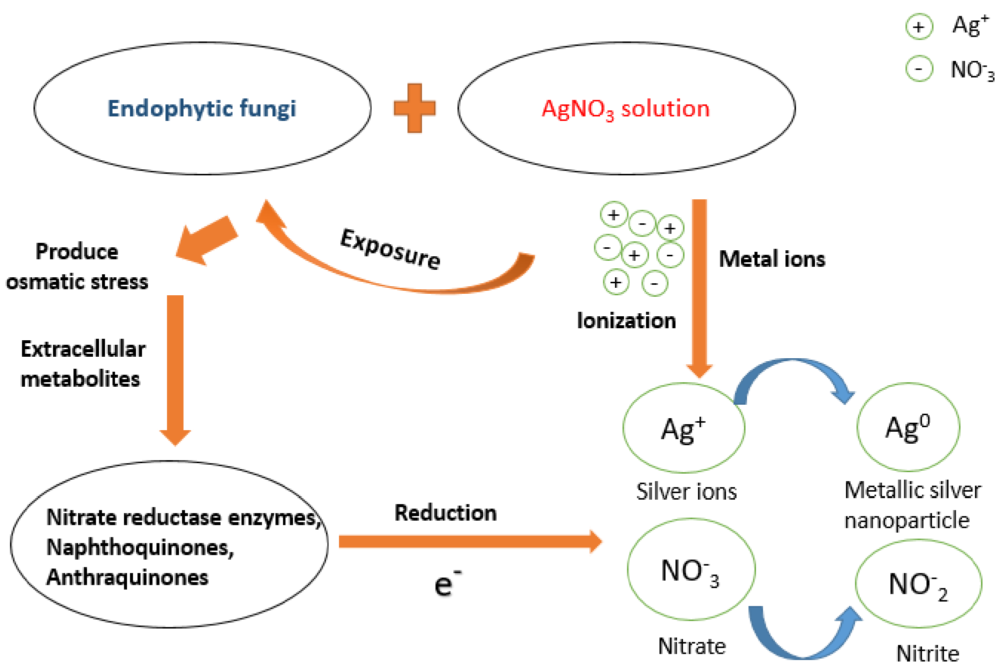

Fig 2. Schematic graph of AgNPs synthesis by endophytic fungi (©Sara A. Gezaf)

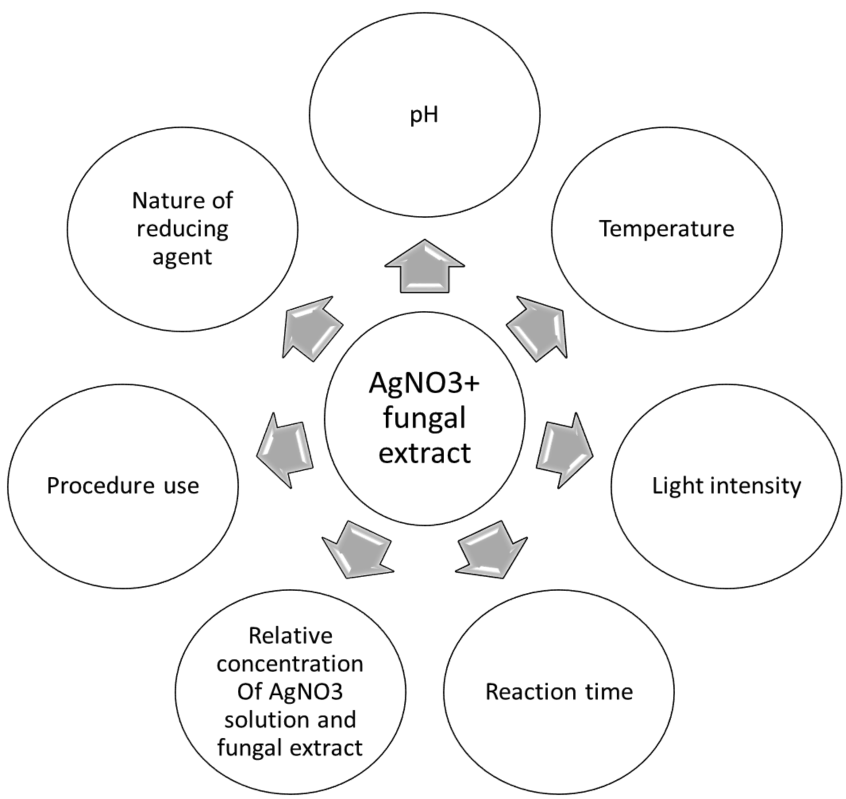

Fig 3. Factors influencing biosynthesis of AgNPs (©Sara A. Gezaf)

Table 2 Overview of characterization methods of silver nanoparticles and analyzed properties

Techniques used in characterization	Information obtained
UV–visible spectrophotometer	Size, concentration, aggregation state, optical characteristics, and hints on nanoparticle shape.
Dynamic Light Scattering Particle Size Distribution Analysis (DLS)	Agglomeration detection and hydrodynamic size.
Fourier transform infrared spectroscopy (FTIR)	Surface composition, ligand binding.
Transmission Electron Microscope (TEM)	Used to known nanoparticle size, size monodispersed, aggregation state, detect and localize/quantify nanoparticles in matrices, shape and study growth kinetics.
High Resolution Transmission Electron Microscopy (HRTEM)	Information from traditional TEM and the crystal structure of individual particles. Differentiate between amorphous, monocrystalline, and polycrystalline nanoparticles.
Liquid TEM	Show real-time of nanoparticle growth, investigate growth mechanisms, single particle motion, and production of super lattices.
Scannig Electron Microscope-High Resolution Transmission Electron Microscopy (SEM-HRSEM), Scannig Electron Microscope- Energy-dispersive X-ray Spectroscopy (SEM–EDX)	Rapid examination of elemental composition, give information about morphology, nanoparticle lateral dimension accuracy and dispersion of nanoparticles in cells and other matrices/supports.
EDS/EDX	Chemical characterization, examination of a sample using light-matter interactions, and analysis of X-rays in its particular case.
Atomic Force Microscopy (AFM)	Nanoparticle morphology, precision in lateral dimensions of nanoparticles, rapid examination of elemental composition.
X-ray Powder Diffraction (XRD)	Crystal structure, composition and grain size of crystalline.
X-Ray Absorption Spectroscopy (XAS)	X-ray absorption coefficient (element-specific)—chemical state of species, Debye–Waller factor sand also for non-crystalline nanoparticles.
DCS and Nanoparticle Tracking Analysis (NTA)	Nanoparticle size and size distribution.

be calculated from the XRD data by calculating the width of the Bragg reflection law using the formula: d = K / cos, where K is the Scherrer constant, is the X-ray wavelength, is the full width half maximum, and is the diffraction angle (half of Bragg angle) that corresponds to the lattice plane (Prathna et al. 2011). Large silver nanoparticle aggregates form over an extended incubation period (over 70 h). The crystalline nature of the silver nanoparticles in these aggregates, which is caused by clearly defined morphologies, is further supported by X-Ray Diffraction study (XRD). After being created by an endophytic fungus, silver nanoparticles' crystal structure was evaluated using XRD analysis (Verma et al. 2010). The silver diffraction facets that correspond to the peaks at 38.06 (111) and 44.1392 (200) show that the precipitate is entirely made of crystalline silver.

Fourier transform infrared spectroscopy (FTIR)

By detecting the stretching and bending of chemical bonds by absorbing energy using infrared spectroscopy, FTIR is an analytical technique used to identify certain types of chemical bonds or functional groups based on distinctive absorption patterns. This energy is located in the infrared (IR) portion of the electromagnetic spectrum (Khandel & Shahi 2018). By generating an identical absorption pattern that is different from that of free groups, FTIR identifies the functional groups attached to the metal nanoparticles surface and offers details on the surface chemistry of the nanoparticles. The FTIR method, which is practical, adaptable, straightforward, and non-invasive, can be used to determine the role of biological molecules in the conversion of silver nitrate to silver (Khandel & Shahi 2018).

The FTIR spectra of the silver nanoparticles generated from the endophytic fungus *Pestalotia* sp. (De Not.) Revealed peaks at 1651.4, 1542, 1387, and 1057 cm-1. While the bands at 1651.4 and 1542.4 correspond to the bonding vibrations of the amide I and amide II bands of proteins, respectively, the bands at 1387 and 1057 cm-1 are caused by the presence of C-N stretching vibrations of aromatic and aliphatic amines (Raheman et al. 2011). These results are consistent with those of Gole et al. (2001), who discovered that negatively charged carboxylate groups in free amine groups or cystein residues can electrostatically attract enzymes located in the cell walls of mycelia to nanoparticles. According to Basavaraja et al. (2008), proteins can coat metal nanoparticles, such as a cap over silver nanoparticles, to prevent the particles from aggregating and becoming stable in the medium. Proteins' amino acid residues and peptides have the strongest ability to bond with metal.

Electron microscopic analysis

The shape and size of nanoparticles, which are both important elements, play a huge role in determining how well they perform. These variables are affected by the type of microorganisms used, the pH of the medium, and other factors. As a result, a wide range of nanoparticles are produced by various fungus species. Electron microscopy can be used to measure and in large part determine the size and shape of nanoparticles. By using transmission electron microscopy (TEM) or scanning electron microscopy (SEM), it is accomplished (TEM). The size of nanoparticles has been the subject of numerous investigations using microscopic methods.

Scanning electron microscopy (SEM)

SEM is used to determine the shape and topology of metal nanoparticles. The surface picture of the specimen is produced in the SEM by scanning the surface with an accelerated electron beam. Backscattered and secondary electrons are collected by the detector and analysed to create pictures or images. The diameters of several nanoparticles at the micro (10^{-6}) and nano (10^{-9}) scales were determined using SEM (Khoshnevisan & Davoodi 2011). Using electron microscope examination, the morphology of the cell is evaluated before and after nanoparticle therapy. According to various research, the antibacterial activity of nanoparticles is used to predict observable changes in cell shape and the existence of nanoparticle perforations in the cell wall (Zhang et al. 2014). The smooth and undamaged architecture of control and silver nanoparticle-treated bacterial cells could be contrasted using SEM. The latter demonstrated apparent cell membrane morphological changes that led to a loss of membrane integrity (Roy et al. 2019).

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a useful, well-liked, and important method for characterising nanoparticles (Rajeshkumar & Bharath 2017). TEM offers information on the size and morphology of nanoparticles. The magnification of TEM is primarily responsible for determining the ratio of the distance between the objective lens and the specimen to that between the objective lens and its image plane (Williams & Carter 2009). TEM can provide superior spatial resolution and the ability to run more analytical procedures than SEM (Lin et al. 2014). According to Lin et al. (2014), the drawbacks of TEM include the requirement for a powerful vacuum, a narrow sample section, and the time-consuming nature of sample preparation. Thus, sample preparation is essential to producing the best pictures possible

Applications

There are numerous possible uses for the silver nanoparticles made by fungi in human health, agriculture, antimicrobial and pest control. It is unclear if fungus, bacteria, or plants that produce biogenic nanoparticles will have advantages or disadvantages in terms of their effects. However, the enormous amount of metabolites generated might make a fungus-based synthesis favourable in terms of production (Sandhu et al. 2017).

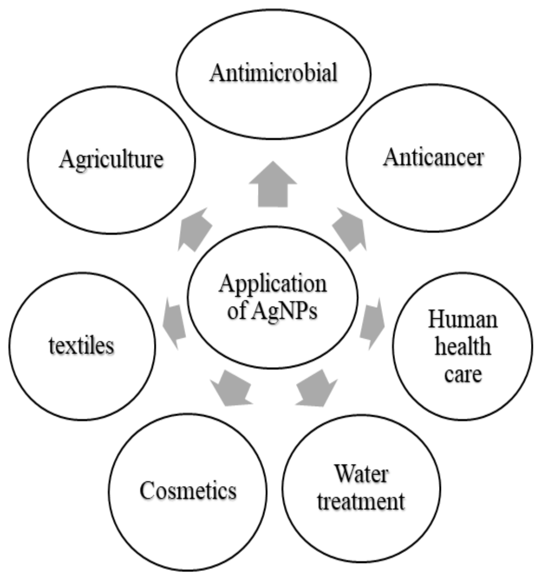

Fig 5. Schematic showing application of AgNPs (©Sara A. Gezaf)

Antimicrobial

AgNPs are effective against numerous bacterial, fungus, and human disease types, claim Shah et al. (2018). It is directed against both aerobic and anaerobic microbes. Since ancient times, AgNPs have been used in medical research. They are also recognized as an effective disinfectant and antibacterial agent with no side effects (Chan & Don 2012). They precipitate with cellular protein and stop the respiration of bacteria (Barreiro et al. 2007; Gravante et al. 2009). AgNP is more useful than other chemicals, hence interest in it has increased (Lok et al. 2007). In connection to pathogenic diseases, endophyte microorganisms are frequently examined for their antifungal and antibacterial characteristics.

Antibacterial

Many scientists are working to create brand-new, potent antimicrobial drugs to address the rise in bacterial resistance to various antibiotics, which will raise the cost of healthcare. Therefore, there is an urgent need for new bactericides (Maiti et al. 2014). AgNPs must therefore be developed in order to act as antibacterial agents. AgNPs are one of many possible nanomaterials that have a high surface-to-volume ratio and a crystalline surface structure, making them appear to be efficient antibacterial agents. The quantity of nanoparticles affects how antimicrobial they are (Amanulla et al. 2018). There are several shapes of AgNPs that are available, including spherical, rod-shaped, cubic, and truncated triangular geometries; the latter has the greatest surface to volume ratio (Singh et al. 2016). Various shapes have different crystal faces, and sharp-edged nanoparticles show higher antibacterial activity than those with spherical forms even at low concentrations (Soleimani et al. 2018).

Gram-positive and gram-negative human bacterial infections were assessed using AgNPs. Due to the thickness of the cell wall and the lipid polysaccharide in the cell wall membrane, which prevent nanoparticle penetration into the cell, AgNPs have a greater inhibitory effect on gram-negative bacteria than gram-positive bacteria (Yun'an Qing et al. 2018). Singh et al. (2017) use AgNPs to target a number of microorganisms, including those with strong antibacterial activity including *Serratia marcescens, Escherichia coli*, and *Staphylococcus aureus.* Additionally, looked at by Baker et al. (2015) were *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Xanthomonas axonopodis pv. Malvacearum, Xanthomonas oryzae pv. Oryzae,* and *Ralstonia solanacearum. Bacillus subtilis and Pseudomonas aeruginosa* are studied by Abdel-Aziz et al. (2018) *Salmonella flexneri, Proteus mirabilis,* and *Enterococcus faecalis* (Rani et al. 2017), *Salmonella paratyphi,* and *Bacillus cereus,* as well as *Salmonella typhi, Enterobactor aerogenes,* and *methicillin-resistant Staphylococcus aures*, are other bacteria that have been found in various studies.

Antifungal

Since there are now only a limited number of antifungal drugs accessible, treating fungus in immunocompromised people is a laborious process (Kim et al. 2007). Therefore, it is essential and inescapable to produce nontoxic, biocompatible, and environmentally friendly antifungal medications. AgNPs are a significant family of antifungal medications that are being utilised to treat a wide range of fungi-related disorders (Esteban-Tejeda et al. 2009). Only a few publications have been published on the AgNPs' antifungal action, which has gotten little attention. To examine the antifungal efficacy of bio-synthesised AgNPs. *Candida parapsilosis* Langeron & Talice., *Candida krusei* Berkhout., *Cryptococcus neoformans* Vuill., *Aspergillus flavus* Link., *Aspergillus fumigatus* Fresen., *Candida tropicalis* Berkhout, *Fusarium solani* Sacc. and *Sporothrix schenckii* Hektoen & Perkins were among the nine harmful strands of fungi examined by Qian et al. (2013). The significant antifungal action against these pathogenic strands is demonstrated by the miimum ihiatory cocentrationn (MIC) range of 0.125-1 g/ml. Devi & Joshi (2015) and Joshi (2017) investigated the anti-*Candida* *albicans* activity of AgNPs. It's noteworthy to note that indoor fungal species like *Chaetomium globosum* Kunze, *Aspergillus fumigatus* Fresen., *Cladosporium cladosporioides* Fresen., *Mortierella alpina* Peyronel and *Penicillium brevicompactum* Dierckx that are grown on agar media are also suppressed by AgNPs (Ogar et al. 2015).

Antiviral

When nanoparticles come as antiviral activity to preventing and controlling the spread of viral infections, it provides a good alternative to medications. In order to create potent antiviral medications that would restrict the actions of viruses, silver nanoparticles could be biosynthesized. The mechanism of antiviral activity is still not fully known. A viral infection starts when a virus's nucleic acids are delivered to the host cell and subsequently replicated there. Previous studies have demonstrated that the size of the Ag NPs is essential for the expression of antiviral activity, which is similar to the findings in bacteria. Suriyakalaa et al. (2013) looked explored bio-silver nanoparticles with strong anti-HIV activities at an early stage of the reverse transcription mechanism.

Metallic nanoparticles that have been biosynthesized have a number of gp120 binding sites that enable them to control viral activity. In contrast, another study found that when utilised against free *HIV* or cell-associated virus, bio-based nanoparticles are effective virucidal agents (Sun et al. 2005). Silver nanoparticles have been demonstrated to have antiviral activity against *HIV-1* at non-cytotoxic doses. These silver nanoparticles were investigated to determine their mode of antiviral action against *HIV-1* using a range of different in vitro assays (Lara et al. 2010). A different study found that the *monkeypox* virus was resistant to the antiviral properties of silver nanoparticles, whether they were covered in polysaccharides or not. This study shows that silver nanoparticles greatly reduce *monkeypox virus* infection in vitro. Rogers et al. (2008) demonstrated that Ag NPs' antiviral activity may be the subject of further reports in the future.

Agricultural applications

One of the most significant upcoming solutions for the food and agricultural industries is nanotechnology, which may also be used to control pests and phytopathogenic fungi in agriculture. The use of 100-250 nm Ag-NPs in agricultural nanofertilizers, nanopesticides with nanoherbicides, nanocoatings, and brilliant delivery systems for plant nutrients is widespread. These particles' effect is increased by the fact that they are more soluble in water (Prasad et al. 2014). By balancing the delivery of nutrients with how rapidly plants can absorb them, nanofertilizers can reduce the danger of groundwater contamination and nutrient losses (Prasad et al. 2010). Seed germination serves as the proper foundation for plant production, growth, and development (Hojjat 2015). Positive effects of AgNP on plant growth and seed germination are observed. Environmental variables and typically water-soluble compounds have an impact on germination. According to numerous studies, AgNP has a favourable impact on plant development and seed germination (Ghavam 2018). When seeds were exposed to various concentrations of AgNP suspension, low concentrations produced the highest rates of germination and medium concentrations produced the highest rates of biomass. It demonstrated that, when compared to the control, low concentrations can be used as bioinoculants with significant effects on growth and biomass (Abdel-Aziz et al. 2018).

Small studies have suggested that the biological synthesis of AgNPs has the potential to manage agricultural pests and phytopathogenic fungi. Some studies demonstrated the use of AgNPs produced by various fungal species for the control of agriculture and pests. For example, Elgorban et al. (2016) demonstrated the effectiveness of AgNPs produced by the fungus *Aspergillus versicolor* (Vuill.) against *Sclerotinia sclerotiorum* (Lib.) and *Botrytis cinerea* (Pers.) in strawberry plants.

Challenges in Nanoparticle Biosynthesis Using Endophytic Microbiomes

For usage in agriculture, nanoparticles that are cheap, safe for the environment, biocompatible, and non-toxic are preferred. Numerous studies have been done on the potential advantages of employing endophytes to create metal-based NPs for agricultural purposes. A significant obstacle is posed by the lack of knowledge regarding the precise mechanisms of action, selectivity, and toxicity of the applications. The full effect on soil microorganisms and the ecosystem in general has also not been fully studied. The effects and dosages of endophytic NPs that can be used to support crop development and health are still subject to much debate. There are no predetermined guidelines governing the application method or carriers used in the use of these NPs for agriculture. Additionally, there are still holes in the laws controlling the use of NPs in agriculture.

This situation demonstrates how little policymakers and practitioners know about how diverse nanomaterials interact with biological systems in particular, how they could accumulate and perhaps have negative environmental repercussions. Despite all the beneficial developments, not a single regulatory opinion is provided by any of the several regulatory organisations. However, there is still a significant promise for the future development of new sustainable farming methods thanks to the green production of nanoparticles employing endophytes and other bacteria.

Potential Futures

Nanotechnology has unquestionably had a huge impact on the food and agriculture industries. It is important to keep in mind that, despite prior contributions and the potential for NPs in future applications, the majority of the knowledge we currently possess is derived from laboratory tests. It is impossible to appreciate the practical applications of NP technology without knowing how it affects environmental toxicity. To advance our understanding now and develop fresh NP technology platforms for the future, extensive trials are necessary.

For the open government awareness project to inform the public about agricultural and food nanotechnology and its advantages, it is essential that a sizable database and accompanying materials be created to serve as logistical assistance for both farmers and the general public. Trials assessing the dangers of using endophytic NP-based products in the agriculture sector are also crucial. The lowest NP dosage must be determined using concentration-dependent soil tests.

Additionally, more research is needed to provide a better understanding of how nanoparticles (NPs) tend to bioaccumulate during field applications and how this influences nanotoxicity, as well as how trophic chain transmission affects the ecosystem. Studies examining how NPs affect the microbial populations in the soil and how they interact with soil systems. In order to demonstrate how endophytic nanoparticles are impacted by their environment, it is crucial to conduct pilot studies in natural settings, as well as analyses of the value chain for the biosynthesis of NPs using endophytes and comparisons with traditional, industrialized methods of production.

Conclusion

Recent studies have shown that biogenically synthesizing silver nanoparticles using fungi has a lot of advantages, and that these materials have a promising future in a range of medical and agricultural applications. Using cappings made of fungus, the nanoparticles are stabilized. Using biological organisms to produce silver nanoparticles is a promising technique. As a result, a brand-new branch of nanotechnology known as nanobiotechnology was created. Nanobiotechnology combines biological ideas with physical and chemical processes to produce microscopic particles that serve specific objectives. Biosynthesis is a promising alternative to hazardous chemical and physical processes for creating nanoparticles. The most frequent element affecting whether NPs are appropriate for usage in various fields, especially in agriculture and biomedical, is their size. However, research on the biosynthesis of nanopesticides has revealed that they are less harmful and effective in managing a variety of plant diseases. Depending on the type of fungus utilised, this capping may also exhibit biological activity that cooperates with the effect of the nanoparticle core. A range of bioactive substances that can be used in the creation of NPs can be found in endophytic microbiomes, particularly in fungus. Future studies are required to investigate the molecular processes by which endophytic microbes produce the NPs, which will also improve their size and shape. Future studies should focus on understanding the interactions between plants, different NPs, and the multiple plant diseases that are influenced by them.

Competing interests

The authors declare that they have no competing interests

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