



## Fabrication of Silver Nanoparticles Against Fungal Pathogens

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The use of silver nanoparticles (AgNPs) against various pathogens is now being well recognized in the agriculture and health sector. Nanoparticles have been shown to exhibit various novel properties and these properties, on other hand, rely upon the size, shape, and morphology of these particles. Moreover, these physical characteristics enable them to interact with microbes, plants, and animals. Smaller-sized particles have shown more toxicity than larger-sized nanoparticles. AgNPs have shown growth inhibition of many fungi like *Aspergillus fumigates*, *A. niger*, *A. flavus*, *Trichophyton rubrum*, *Candida albicans*, and *Penicillium* species. According to the current hypothesis, AgNPs act by producing reactive oxygen species and free radicals, which cause protein denaturation, nucleic acid and proton pump damage, lipid peroxidation, and cell wall damage. Therefore, they alter the cell membrane permeability, causing cell death. This mini-review summarizes the use of silver nanoparticles against fungal pathogens and fungal biofilm in the agricultural sector.

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## INTRODUCTION

Nanotechnology is an emerging area of scientific research that has been extremely well-known in recent decades (Mohseniazar et al., 2011). It has a wide range of applications in microbiology and biotechnology. It can assist in development, design, and manipulation of nanostructures to extremely small dimensions and with large surface area to volume ratio. As a result of their exceptional properties, nanoparticles attracted the attention of researchers in a variety of disciplines. Morphology, size, and chemical properties of NPs are used to classify nanoparticles and hence they are classified according to their physical and chemical properties; there are several well-known classes of nanoparticles (Khan et al., 2019) like the organic, inorganic, and carbon-based.

Nanoparticles or polymers of organic nature include dendrimers, micelles, liposomes, and ferritin. Micelles and liposomes have a hollow core, also known as a nanocapsule, and they are sensitive to thermal and electromagnetic radiation. Due to their efficiency, these are widely used in the biomedical field, for example, drug delivery systems (Tiwari et al., 2008). Metal- and metal oxide-based nanoparticles are generally categorized as inorganic nanoparticles. Metal-based nanoparticles are synthesized using destructive or constructive processes; the most commonly used metals are aluminum (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn). These nanoparticles have unique characteristics, including sizes ranging from 10 to 100 nm, high surface area to volume ratios, pore sizes, surface charge and surface charge density, crystalline and amorphous structures, spherical and cylindrical shapes, and environmental sensitivity (Salavati-Niasari et al., 2008). Metal oxide-based nanoparticles are

made to alter the properties of metal-based nanoparticles. The primary reason for the development of metal oxide nanoparticles is to improve their reactivity and efficiency. The most often synthesized oxides are aluminum oxide ( $Al_2O_3$ ), cerium oxide ( $CeO_2$ ), iron oxide ( $Fe_2O_3$ ), magnetite ( $Fe_3O_4$ ), silicon dioxide ( $SiO_2$ ), titanium oxide ( $TiO_2$ ), and zinc oxide (ZnO). These nanoparticles have superior properties when compared to their metal counterparts (Tai et al., 2007; Ealia and Saravanakumar, 2017). Carbon-based nanoparticles are made completely of carbon. They can be classified into fullerenes, graphene, carbon nanotubes (CNTs), carbon nanofibers and carbon black, and sometimes activated carbon in nano size (Bhaviripudi et al., 2007; Ealia and Saravanakumar, 2017).

Crop losses as results of phyto pathogenesis account for about 16 percent of the total crop production worldwide (Fontana et al., 2021). The losses as a result of these pathogens are undisputable as they may be amplified further depending upon the predisposing factors, pathogen virulence, and environment. Agricultural crops suffer significant losses owing to pest damage and plant diseases, despite the fact that weeds are the leading cause of crop loss on a global scale. The global yearly production tonnage percentage lost to various pests at the start of the twenty-first century has been approximated as follows in rounded (approximate) figures: Animal pests accounted for 18% of crop production tonnage losses; microbiological illnesses accounted for 16% (and fungi were responsible for 70–80% of these losses); and weeds accounted for 34%, for a total of 68 percent annual crop production tonnage loss (Moore et al., 2020).

Silver nanoparticles (AgNPs) due to their antimicrobial properties have got marvelous attention (Ahmad et al., 2003; Wong and Liu, 2010; Foldbjerg et al., 2015; Guilger-Casagrande and Lima, 2019). AgNPs have got a good number of applications in controlling development of microorganisms (Wright et al., 1999; Silver, 2003; Nithya and Ragunathan, 2012; Zhao et al., 2018; Feroze et al., 2020; Sathiyaseelan et al., 2020). There are still a large number of potential applications for silver nanoparticles in various fields of health and agriculture. AgNPs synthesized from fungi are well applied in agriculture, showing good potentials against plantpathogenic insects, fungi, bacteria, and viruses. The novel properties of silver nanoparticles in shape, size, and morphology have helped them to interact with different microorganisms, animals, and plants. Nanoparticles are more toxic in smaller size and even at a lesser concentration (Singh et al., 2015; Moradi et al., 2021). Silver nanoparticles are the most commercialized medical nanomaterial, widely used in medical product coatings, medical diagnostics, and sensors (Wijnhoven et al., 2009; Lin et al., 2011; Li et al., 2012; Sintubin et al., 2012; Burduşel et al., 2018). In commercial products, nanoparticles are primarily used in goods and self-care products that contain metals and metal oxides. The majority of global production is made up of nanoparticles of zinc oxide, titanium oxide, and silicon dioxide, with annual production rates of 5.50 tonnes, 3.00 tonnes, and 5,500 tonnes, respectively. Several estimates of global AgNPs production have been published in recent years. As of 2025, it is expected that AgNPs production will reach approximately 800 tonnes. As compared to other nanoparticles, AgNPs are thought to have a higher marketing value, and they are widely advertised in



consumer products. Nanoparticles have proven to offer fascinating, demanding, and promising features suitable for many biomedical applications; silver nanoparticles (AgNPs) have become one of the most examined and explored nanotechnology-derived nanostructures in recent years. Modern biomedical potential of AgNPs is focused on therapeutically enhanced personalized healthcare practice, which has attracted a great deal of attention. As a result of their unique properties, AgNPs are proving to be a valuable tool for the development of novel antimicrobial agents as well as drug delivery formulations, detection platforms, and diagnostic platforms (Burduşel et al., 2018). Synthesis of AgNPs by using microorganisms has gained huge attention over chemical and physical methods since the biological method is ecofriendly (Thakkar et al., 2010; Ghosh et al., 2012; Gaidhani et al., 2014). Various plant diseases caused by fungi, bacteria, virus, and pests can be tackled by use of nanotechnology, which provides a good management alternative (Alghuthaymi et al., 2015).

AgNPs are formulated according to a wide range of variables, such as temperature, pH, media, and AgNO3 concentration (Figure 1). Other considerations, such as methods of preparation, solvent types, and media, depend on the physical characteristics of nanoparticles (Husen and Siddiqi, 2014; Siddiqi and Husen, 2016; 2017). Effectiveness of AgNPs not only depends on their physical characteristics, like sizes, shapes, and coating agents, but also on the type of pathogen against which they are formulated. Many pathogens are more prone to AgNPs than other nanoparticles. Several studies reported that bacterial pathogens were more susceptible than fungal pathogens to AgNPs. It was studied that the differences in antimicrobial activity of bacterial species and fungal species were due to differences in organization of their cells and cell structure. The bacteria, being among prokaryotic organisms, have less complexity in cell structure, therefore incapable to fight against toxicity of AgNPs. The fungi, however, have much

more complex cell structure and better detoxification system, to effectively withstand the action of higher AgNPs concentrations (Panáček et al., 2009; Jalal et al., 2018). AgNPs surround the pathogen and inhibit its pivotal functioning by entering the cell. AgNPs can be effective at cellular and subcellular levels (Jayasree et al., 2006; Brayner, 2008; Panda et al., 2011; Ibrahim et al., 2021). AgNPs have broad-spectrum modes of action against different microorganisms, with a significant inhibitory effect on both Gram-positive and Gram-negative bacteria (Gogoi et al., 2006; Jo et al., 2009; Kim and Ryu, 2013).

### CHEMICALLY VS GREEN-SYNTHESIZED SILVER NANOPARTICLES

Chemical approach for synthesis of AgNPs is simple convenient and results in high yield. But this method is highly expensive and needs an additional step to prevent aggregation of particles (Zhang et al., 2016). Moreover, chemically synthesized AgNPs result in toxic effects of nanoparticles or by-products released to the environment (Gade et al., 2008). The chemical method requires involvement of toxic chemicals as reducing and capping agents, which leads to adsorption of these toxic chemicals on nanoparticles and results in adverse effects in application section (Kummara et al., 2016; Roy et al., 2019). On the contrary, biogenic synthesis of nanoparticles, especially using plant extract, utilizes less toxic reducing and stabilizing agents. The reaction can take place in ambient conditions without harsh or rigid reaction limitations. Plant extract-derived nanoparticles are of low or no cytotoxicity. Therefore, plant extract-mediated green synthesis of nanoparticles is considered ecofriendly, cost-effective, and safe and is a viable alternative for microbiological applications (Garibo et al., 2020). A previous study compared the antifungal activities of AgNPs synthesized by green and chemical methods (Tyagi et al., 2020). This study reported 100% inhibition against both Corynespora cassiicola and A. solani, and 85% inhibition against Fusarium spp., for chemically synthesized AgNPs; in contrast, greensynthesized AgNPs showed 90% inhibition against C. cassiicola, 95% inhibition against A. solani, and 90% inhibition against Fusarium spp. The antifungal activity for both types of nanoparticles depends on its dose and targeted fungal species. Moreover, toxicities for both types of AgNPs were analyzed against Drosophila melanogaster. Chemically synthesized AgNPs were toxic and their toxicity was dose-dependent, whereas greensynthesized AgNPs were not toxic. For four pathogens including C. albicans, the minimum inhibitory concentrations of greensynthesized AgNPs were from 0.06 µg/ml to 0.25 µg/ml, lower than those of chemically synthesized AgNPs from 2.5 µg/ml to 5.0 µg/ml, indicating a higher antifungal potency of green NPs than chemically synthesized NPs (Garibo et al., 2020).

## SILVER NANOPARTICLES AGAINST FUNGAL PATHOGENS

As an alternative to conventional paths, a green synthesis of nanoparticles has evolved. Several green sources were identified,

including microorganisms, viruses, natural polysaccharide reducing agents, polyols, and plant extracts. Green nanoparticle synthesis was done using a broad range of biological agents such as bacteria, fungi, algae, plant extracts, and a number of beneficial microorganisms (Narayanan and Sakthivel, 2010). Different plants for the formation of silver nanoparticles were previously investigated (Pulit-Prociak and Banach, 2016). Phyllanthus urinaria, Pouzolzia zeylanica, and Scoparia dulcis are herbs of great importance in the world, and have anti-bacteria, anti-inflammatory, anti-allergic, antioxidant, and many other potential activities (Hossain et al., 2017; Chen et al., 2018; Frezza et al., 2020). Under moderate conditions, three kinds of silver nanoparticles were actively biosynthesized (P.uri.AgNPs, P.zey.AgNPs, and S.dul.AgNPs), simply by applying silver (I) to these herbal plant minerals. P.uri.AgNPs, P.zey.AgNPs, and S.dul.AgNPs show antifungal capability against Aspergillus niger, A. flavus, and Fusarium oxysporum. This green approach is the most straightforward and widely scalable method for producing biomedical and agricultural antifungal silver nanoparticles (Nguyen et al., 2020). In addition, effective roles of AgNPs against Bipolaris and Magnapthe grisea have been reported previously (Morones et al., 2005; Panacek et al., 2006; Panáček et al., 2009). Therefore, silver nanoparticles have earned great attention in nanobiotechnological research, owing to their physical, chemical, and biological properties.

The leaf extract formulation of AgNPs using Acalphya indica have showed significant antifungal activity against pathogens such as Alternaria alternata, Botrytis cinerea, Curvularia lunata, Macrophomina phaseolina, Rhizoctonia solani, and Sclerotinia sclerotiorum (Krishnaraj et al., 2012; Medda et al., 2015). Green-synthesized AgNPs using leaf extract of Aloe vera showed a better antifungal activity against Aspergillus than Rhizopus (Medda et al., 2015). Microscopic observations showed that these synthesized nanoparticles had detrimental effects on fungal hyphae and conidial germination.

AgNPs synthesized using leaf extract of Brassica oleracea efficiently controlled the development of two pathogenic fungal pathogens, Aspergillus and Pneumocystis, at 50 µg/ml concentrations, with its inhibitory effect comparable to that of fluconazole (Kuppusamy et al., 2015). AgNPs synthesized from Cassia roxburghii leaf extract showed an excellent antifungal activity against five human fungal pathogens, A. fumigates, A. niger, A. flavus, Candida albicans, Penicillium sp., and three plant fungal pathogens, Curvularia sp., Rhizoctonia solani, and F. oxysporum (Balashanmugam et al., 2016). Amphotericin B was very effective against human fungal pathogens and moderately effective against plant fungal pathogens.

Some studies have reported antifungal action of AgNPs formulated using root extract of *Diospyros sylvatica* (Elumalai and Velmurugan, 2015; Pethakamsetty et al., 2017). These nanoparticles showed maximal activity against *Pencillium notatum* and *A. niger*, moderate activity toward *Saccharomyces ceriviseae* and *A. flavus*, and mild toward *C. albicans*. AgNPs synthesized from root extract of *Diospyros paniculata* showed significant antifungal potential against various pathogens with maximum activity toward *A. flavus*, *P. notatum*, and *S. ceriviseae* and moderate activity toward *A. niger* and *C. albicans* (Rao et al., 2016).

## SILVER NANOPARTICLES AGAINST FUNGAL BIOFILMS

Many bacteria and fungi primarily emerge in cell crowds called biofilms. Biofilms can form on both biotic and abiotic surfaces, and have distinct properties from free-floating (planktonic) cells. Biofilms can be produced in a laboratory environment where they are commonly examined. More than one type of microorganisms (bacteria, protozoa, and fungi) in natural environments may grow in a synthrophic consortium (Lohse et al., 2018; Motaung et al., 2020). While fungal biofilms have been linked to animal and plant diseases, bacteria and yeast biofilms have received a lot of attention. It has been highlighted by mounting evidence that many fungal species are increasingly posing a threat to global food production, biodiversity, and human health. Pathogenic fungi cause significant yield losses in large calorie on commodity crops such as wheat, rice, sugarcane, corn, potato, and soybean around the world. There is a paucity of literature on plant-associated fungal biofilms, suggesting that the degree to which these consortia of microbial cells underpin plant health is underappreciated (Fanning and Mitchell, 2012; Motaung et al., 2020).

Biofilm formations are prevalent, virulent, or resistant factors of some opportunistic pathogens (bacteria or yeasts). Biofilm can be defined as a multidimensional assembly of microbes surrounded by an extracellular matrix (ECM) formed either on abiotic or biotic substrate (Fanning and Mitchell, 2012). The concept of plant-associated biofilms is mainly attributable to free-floating microbial cells, also known as planktonic cells, which are mainly responsible for commencing biofilm formation via initial attachment. Plant-associated fungal biofilms are generally thought to configure interactions across and among plant populations; butits-related research lags far behind as compared to the research on human-associated biofilms. Such deficit could limit the research avenues for plant improvement programs and disease management (Hassani et al., 2018; Motaung et al., 2020). The knowledge of plant-associated fungal biofilms could be pivotal for gaining information on various ecological roles such as medically important fungi, secondary metabolite production, plant beneficial functions, and climate change (Morris and Monier, 2003; Harding et al., 2017; Villa et al., 2017). Such functions could further be complimented with nanotechnology, wherein, nano-based approaches could be employed to plan tpathogens, thus giving a huge scope for agriculture exploration. One such directed approach is of silver nanoparticles (AgNPs), mainly attributable for their strong antimicrobial and anti-biofilm activity (Ali et al., 2020). As compared to plant-associated conditions, the use of AgNPs is highly widespread and multidirectional in oncology, nosocomial infections, and pulmonary diseases. The applicability of AgNPs to control plant-associated diseases is quite relative. Owing to their exceedingly small scale, AgNPs have tiny areas, high reactivity, and fast penetration of the biofilm materials and cell membranes with huge surface areas (Figure 2).

Jo et al. have examined the antifungal activity of various silver ions and nanoparticle on two plant-pathogenic fungi, *Bipolaris* 



sorokiniana and Magnaporthe grisea. They found that silver ions and nanoparticles had a significant inhibiting effect on the microbial colony formation of aforementioned pathogens via in vitro petri dish assays. In addition, growth chamber inoculation assays have concluded that silver ions and nanoparticles significantly reduce these two fungal diseases on perennial ryegrass (Lolium perenne) upon application at 3 h before spore inoculation (Jo et al., 2009). Ibrahim et al. have documented the use of green-synthesized AgNPs from garlic plants (Allium sativum) against Fusarium graminearum (head blight pathogen) on wheat crops. They have reported that AgNPs show strong inhibition on mycelium growth, spore germination, germ tube development, and mycotoxin production of F. graminearum (Ibrahim et al., 2020). Kaur et al. have reported the biosynthesis of silver nanoparticles (AgNPs) from isolated rhizospheric microflora of chickpea and evaluated their potential



role in controlling wilt disease caused by F. oxysporum f. sp. ciceri (FOC) of chickpea. AgNPs showed a very high antifungal activity (95%) against FOC in vitro at the concentration of 100 µg/ml, and chickpea seeds coated with AgNPs showed high germinability (Kaur et al., 2018). Mishra et al. have obtained extracellularly synthesized AgNPs from the culture supernatant of the Serratia bacterium and demonstrated their efficacy for the management of Bipolaris sorokiniana, a causative agent of spot blotch disease on wheat. These AgNPs have exhibited strong antifungal activity with accountable inhibition of conidial germination as determined from a leaf bioassay (Fanning and Mitchell, 2012). Nanoparticles have gotten a lot of attention in the last decade because of their unusual properties. Since these particles are reactive, they can quickly penetrate the matrix. Non-toxic and environmentally safe methods are used in the synthesis of green NP. These AgNPs inhibit biofilm formation and cause the majority of P. aeruginosa biofilms to detach. Plant extracts are used to bioreduce metal ions for NP biosynthesis, which is a costeffective, fast, bacteria-free, and easy-to-scale-up operation (Qayyum and Khan, 2016). Although several studies have shown the anti-biofilm efficacy of metal NPs, their modes of action and toxicity remained to be elucidated.

## POSSIBLE MODES OF ACTION

Fungal cell wall plays an important role in maintaining cell homeostasis (Gow et al., 2017; Latgé, 2007). The surrounding environment of the fungal cell can play a positive or negative role that determines the survival rate of fungal cell. This cell wall contains a hard, durable, and tensile scaffold that is 40 percent of the total cell volume. Multitudes of proteins and carbohydrates support this system. The outer layer of the fungal cell wall contains mannosylated glycoproteins with modified N- and O-linked oligosaccharides (Shibata et al., 1995; Hobson et al., 2004). The inner layer contains chitin and glucan with 50-60% dry weight being  $\beta$ -(1–3)-glucan. The inner cell wall is pivotal in maintaining the cell environment by resisting the pressure created by the cell membrane and cytoplasm (Gow et al., 2017; Ghosh et al., 2012). Advances in recent studies on fungal cell wall composition, structure, and role in drug resistance have opened doors for new targets against fungal pathogens, and also helped in better understanding of mechanism by which antifungal resistance is developed. AgNPs might play a crucial role in breaking down such resistance. AgNPs cause cell wall disintegration, surface protein damage, nucleic acid damage by production and accumulation of ROS and free radicals, and blockage of proton pumps (Figure 3). It has hypothesized that AgNPs lead to accumulation of silver ions, which blocks respiration by efflux of intracellular ions and thus damages the electron transport system (Du et al., 2012). Smaller size to large surface ratio of nanoparticles is attributed for antifungal activity. AgNPs with smaller size can penetrate easily through cell boundaries. The toxicity of AgNPs is partially attributed through production of reactive oxygen species (ROS), which leads to apoptosis. It has been hypothesized that the in vitro toxicity of AgNPs is owing to either the mutual effect of Ag ions and AgNPs or their separate effect (Beer et al., 2012; Cronholm et al., 2013; Kim and Ryu, 2013). The exact mechanisms and modes of action of AgNPs need to be further explored.

# POTENTIALS OF SILVER NANOPARTICLE IN AGRICULTURE

In agriculture, there is huge loss annually due to bacterial and fungal pathogens. One way to control such loss is the use of fungicides and pesticides, but these chemicals have detrimental effects on the environment. A large number of companies produce water-soluble nano-fertilizers, nano-pesticides, and nano herbicides of 100–250 nm and intelligent plant-nutrient delivery systems in agriculture. Nano-fertilizers should synch with the production of biomass to prevent a nutrient shortage (Prasad, 2014). The alternative to this is the synthesis and use of AgNPs as antifungal and antimicrobial agents, which is safer than the synthetic fungicides.

Silver nanoparticles have widely been reported to cause disease control in many agricultural crops; their efficiency has been seen to be controlled by the particle size and concentrations in the formulation (Steinfeld et al., 2015). In many cases, the silver nanoparticles have been seen to be more effective against the phytopathogens in the plant tissues itself than the in vitro conditions (Villamizar-Gallardo et al., 2016). Abdelmalek and Salaheldin (2016) reported promising fungicidal activity of AgNPs against Alternaria alternata, Penicillium digitatum, and Alternaria citri at 150 ppm compared to synthetic fungicides, difenoconazole, and iprodione at the same concentration (Abdelmalek and Salaheldin, 2016). Human health on exposure to silver nanoparticles and other nanomaterials as agriculture products is of major concerns. The direct contact of these nanomaterials used as nutritional ingredients may risk human health. The ROS (reactive oxidative species) production serves as the toxicological mechanisms leading to cellular damage and even death. ROS overproduction can cause damage to neuron, DNA damage, autophagy, severe age-related diseases, mutagenesis, and carcinogenesis in humans. Allergic reactions are also unfortunate outcomes upon the food nano-products exposure. Furthermore, the nanoparticles accumulation in human body and plant seeds (edible parts) may cause serious problems at long-term interactions and high concentrations. Legislation and regulation are most important in order to regulate the manufacturing of nanomaterials, their processing, application, and disposal (He et al., 2019). In agriculture and other sectors, the use of silver-based nano-emulsions have widely been questioned because of the apprehensions of lethal health effects on humans and other biological entities, the deliberate flow of nanoparticles into the different ecological niches could bring about the irreversible environmental degradation, and accumulation of these substances in the food chain (Kah, 2015). These emulsions not only target the non-parasitic/ friendly entities but accumulate in the foods itself. The apprehensions in context to agricultural laborers getting into contact with these harmful xenobiotics could put their life at risk (Iavicoli et al., 2017). Although large work is carried on effects of AgNPs on bacteria, not much attention has been yet received on their role on antifungal effects (Kim et al., 2008; Roe et al., 2008). Antifungal potential of Ag-SiO<sub>2</sub> has been reported against Botrytis cinereal (Oh et al., 2006). The mutual effect of fluconazole and AgNPs were tested for their antifungal activity

against Phoma glomerata, P. herbarum, F. semitectum, Trichoderma sp., and C. albicans (Gajbhiye et al., 2009). AgNPs were also reported to decrease disease development of plant fungal pathogens including B. sorokiniana, Magnaportheas as well as sclerotium-forming phytopathogenic fungi Grisea, gloeosporioides, A. niger, and Sclerotium cepivorum (Park et al., 2006; Kim et al., 2009; Min et al., 2009; Choudhury et al., 2010). AgNPs also exhibit a significant antifungal activity against F. oxysporum and the unknown ambrosia fungus Raffaelea sp. that causes death of large oak trees in Korea (Wijnhoven et al., 2009; Musarrat et al., 2010). AgNPs were also demonstrated to be effective against plant fungal pathogens, A. alternata, S. sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, B. cinerea, and Curvularia lunata (Krishnaraj et al., 2012; Alghuthaymi et al., 2015). AgNPs prepared by using Fusarium solani from wheat proved to be very effective against different pathogens of wheat, barley, and maize (Abd El-Aziz et al., 2015). AgNPs synthesized from the fungus Aspergilus versicolor showed concentration-dependent activity against S. sclerotiorum and B. cinereaon strawberry plant (Elgorban A. et al., 2016). The fungus *Epicoccum nigrum* can be used to synthesize AgNPs against *C*. albicans and F. solani (Qian et al., 2013). Potential roles of the fungus Guignardia mangiferae-derived AgNPs were tested to control plant pathogens Colletotrichum sp., R. solani, and Curvularia lunata (Balakumaran et al., 2015). A combination of A. alternata-derivedAgNPs and fluconazole was evaluated against the phytopathogenic fungi P. glomerata, P. herbarum, and F. semitectum, as well as the human pathogenic fungus C. albicans, with the highest activity to C. albicans (Gajbhiye et al., 2009).

Silver nanoparticles play a significant role in control of fungal pathogens in many crops (Al-Zubaidi et al., 2019). Antifungal activity of silver nanoparticles derived from *Agaricus bisporus* against various fungal strains like *Sclerotium rolfsi*, *A. niger*, and *Rhizoctonia solani* which are known to be the causal agents of stem, collar, and root rot disease, respectively, in groundnut (Roy et al., 2013). Rao and Savitramma examined the effectiveness of naturally orchestrated silver nanoparticles from *Svensonia hyderabadensis* leaf extracts against *F. oxysporum*, *Curvularia lunata*, *A. niger*, and *Rhizopus arrhizus* (Rao and Savithramma, 2011).

Silver nanoparticles synthesized from turnip leaf extraction demonstrated a wide range of antifungal action against wood-degrading fungi including *Gloeophyllum abietinum*, *G. trabeum*, *Chaetomium globosum*, and *Phanerochaete sordida* (Narayanan and Park, 2014). AgNPs were also synthesized successfully with leaf extracts from *C. roxburghii* and could be used as an antifungal agent to treat various pathogenic diseases of humans and plants (Rai et al., 2021; Balashanmugam et al., 2016; **Table 1**).

#### **BIOSAFETY OF AgNPs**

Despite the numerous benefits of nanoscale materials, their possible health risks must be considered due to their uncontrollable use, discharge into the natural environment, and hazardous consequences. Aside from their numerous

#### TABLE 1 | AgNPs formulations with effective concentrations against fungi using different sources

Synthesis source	Target organism	Effective concentration	References
Penicillium chrysogenum and	Trichophyton rubrum	P.chrysogenum0.5 µg/ml and A. oryzae	Pereira et al. (2014)
Aspergillus oryzae		> 7.5 µg/ml	
Trichophyton rubrum, T.	C. albicans	4 μg/ml	Moazeni et al. (2012)
mentagrophytes and			
Microsporumcanis			
Pleurotuscornucopiae var.	Antifungal Candida spp.	20, 40, and 60 mg/well	Owaid et al. (2015)
citrinopileatus			
Penicillium italicum	Multidrug-resistant <i>S. aureus</i> , <i>Shewanellaputrefaciens</i> , and <i>C. albicans</i>	25 µl/disk	Nayak et al. (2018)
Arthrodermafulvum	Candida sp. and Aspergillus sp	0.125–4.00 µg/ml	Xue et al. (2016)
Fusarium oxysporum	Candida spp. and Cryptococcus spp	Candida spp. 0.84–1.68 µg/ml and	Ishida et al. (2014)
		Cryptococcus spp. 0.42 0.84 µg/ml	
Trichoderma harzianum	Sclerotinia sclerotiorum	0.15 × 1,012 and 0.31 × 1,012 NPs/mL	Guilger et al. (2017)
Trichoderma harzianum SYA.F4	Helminthosporiumsp., Alternaria alternata, Phytophthora	100 µg/ml	El-Moslamy et al.
	<i>arenaria,</i> and <i>Botrytis</i> sp		(2017)
Arthrodermafulvum	<i>Fusarium</i> sp	0.125–4.00 µg/ml	Xue et al. (2016)
Aspergillus versicolor	S. sclerotiorum and Botrytis cinerea	150 ppm	Elgorban et al. (2016b)
Epicoccum nigrum	Fusarium solani, Sporothrixschenckii, Cryptococcus neoformans, Aspergillus flavus, and Aspergillus fumigatus	0.125–1.00 μg/ml	Qian et al. (2013)
Guignardiamangiferae	Colletotrichum sp., Rhizoctonia solani, and Curvularia lunata	1 mg/ml	Balakumaran et al. (2015)
Fusarium solani	wheat, barley and corn associated fungi	1, 2, and 4%	Mosselhy et al. (2015)
Alternaria alternata	Phoma glomerata, Phomaherbarum, and Fusarium semitectum	20 µl/disk	Gajbhiye et al. (2009)
Acalphya indica	Alternaria alternata, Botrytis cinerea, Curvularia lunata, Macrophomina phaseolina, Rhizoctonia solani and Sclerotinia sclerotiorum	5 mg/10 µl	Krishnaraj et al. (2012)
Aloa vera	Aspergillus sp. Rhizopus sp	100 µl 1 M	Medda et al. (2015)
Amaranthus retroflexus	Macrophomina phaseolina, Alternaria alternata and	50, 100, 200, and 400 µg/ml	Bahrami-Teimoori et al.
Teucrium polium I	F oxysporum	100, 150, 300, 600, and 900 ul	Ghoiavand et al. (2020)
Bacillus sp. (MB353)	Asperaillus niger Asperaillus fumigatus. Eusarium soleni	10 and 50 µg/ml	Khan et al. (2020)
Lactobacillus reuteri E81	Botortis cinerea. Eusarium ovusnorum Asperaillus	1.5 and 10 mg ml <sup>-1</sup>	Vilmaz et al. $(2020)$
	parasiticus Alternaria alternata, Aspergillus niger and Penicillium chusococum	r, s and to fight	1 ii 1 iaz et al. (2020)
AaNPs (Procured)	Fusarium solani	2 4 6 and 8 mg/l	Shen et al. $(2020)$
Ethanolic propolis extract	Candida krusei. Candida paransilosis. Candida tropicalis	>16.98 mg/l	Kischkel et al. (2020)
	Candida albicans, Candida glabrata, Fusarium oxysporum, Trichophyton interdigitale, Trichophyton rubrum, Microsporumcanis	2 TO.30 HIGH	NSOINE Et al. (2020)

commercial and medical applications, NPs and other nanomaterials are linked to a number of toxicities. To deal with these hazardous effects appropriately, you will need some basic understanding. NPs enter the environment through water, soil, and the air during a variety of human activities. Instead, NPs are actively injected or dumped into soil and aquatic systems. Small size, high reactivity, and high capacity are advantages of magnetic nanoparticles (NPs), but they can also be disadvantages because they can have toxic and detrimental effects on cells, which are rare in micron-sized counterparts (Xu et al., 2020). Commercial and biomedical applications of nanosilver include its use in cosmetics and textile engineering, as well as a bactericidal agent in dressings and disinfectants for wounds or surgical instruments. As a result, AgNPs interactions with terrestrial and aquatic environments, as well as human exposure and toxicity, have increased. A number of factors influence the cytotoxic effects of AgNPs, including size, shape, coating, dose, and cell type

(Tang and Xi, 2008; Khlebtsov and Dykman, 2011; El-Sheikh et al., 2013; Xu et al., 2020).

There is a lack of appropriate and standard characterization procedures that may be adopted for research evaluating the toxicity of AgNPs in order to compare the results of different investigations utilizing similar NPs. The data on the impact of AgNPs exposure on animal models of increased vulnerability, such as hypertension, diabetes, and asthma, are scarce. The cytotoxicity of AgNPs is determined by their size, shape, and concentration. Wang et al. (2015) employed TEM and SR-TXM combined with 3D tomographic imaging to explore AgNPs uptake, accumulation, degradation, chemical change, and elimination at the cellular level. According to the findings, the chemical transformation of AgNPs (Ag0-to-AgO-to-AgS) may produce cellular metabolic alterations. The possible cytotoxicity, long-term health impacts, and particular mechanisms of AgNPs are still unknown. Furthermore, AgNPs can cause acute lung injury, the severity of which is determined by particle

accumulation and clearance. Akinori et al. employed mouse models to investigate the lung toxicity of nanoparticles. Ultrafine particles may pass across the air-blood barrier due to the narrow space between alveolar epithelial cells, producing bronchiolar epithelial cell vacuolation and necrosis, resulting in transitory acute lung inflammation and tissue damage. Because ultrafine particles promote oxidative stress and cell death, this is the case. Size-dependent lung toxicity was also observed for nanoparticles, meaning that smaller particles were more capable of causing lung inflammation and tissue damage than larger particles (Kaewamatawong et al., 2005; Kaewamatawong et al., 2006). There has been some discussion of AgNPs' potential toxicities in multiple organ systems including the skin, kidneys, respiratory system, hepatobiliary, and immune systems. AgNPs' biocompatibility and potential cytotoxicity must be evaluated in more detail, which could lead to the development of safer and biocompatible AgNPs-based agents.

Before mass manufacture and use of NPs in the food business, precise and standard tests should be used to assess the long-term consequences of acute and chronic exposure to various NPs found in food systems.

#### CONCLUSION AND FUTURE PROSPECTIVE

Agriculture forms the backbone of economy in most of the countries. AgNPs have been studied comprehensively from the last few decades, and use of AgNPs in agriculture provides alternative protection mean against pests, which could reduce our dependence on environmentally detrimental fungicides and pesticides. Extracts from fruits and some medicinal plants serve as an easy and cheap means for AgNPs biosynthesis. Yet, for large-scale production of AgNPs, there is a need for a more reliable route, which is environmentally sound as well as economically viable. Although the mechanism of AgNPs synthesis from plant extracts is still not clear, but some studies mention the formation and mechanism of nanoparticles from a diverse range of plant species successfully (Simchi et al., 2007; Mittal et al., 2013; Al-

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Rashed et al., 2019). AgNPs are extensively used as antimicrobial agents and biosensors in medicine, healthcare, and agriculture sectors. Precise modes of action of AgNPs against fungal pathogens need to be elucidated with experiments. AgNPs can be modified and engineered in order to enhance its stability, efficacy, and biosafety. Nanoparticles fabricated and coated with cappings derived from fungi have exhibited great biological activity. Mycosynthesized AgNPs have been reported to disintegrate membrane integrity in fungal cells. Different formulations of mycosynthesized AgNPs must be tested for their biological activities in various fields. Although the role of silver nanoparticles against various fungal pathogens has been established, there are large number of fungal pathogens responsible for huge loss in agricultural productivity which need to be tested against AgNPs. Future research should focus on the discovery of potential candidates and mechanisms involved in their actions, taking into consideration their effects on the living and non-living components of the environment before their large-scale production. Keeping in view of their toxic effects, rational strategies with minimum risk of toxicity need to be designed. Work must be done to promote the development of nanobiosensors and nano-based delivery systems, which are user friendly and easy to carry out for precise analysis of soil, plants, and water. Efforts should be made for their use in plant disease management on account of being a cost-effective and ecofriendly alternative. There are many studies that endorse NPs as antibiofilm agents, but there are still some know-hows about their modes of operation. Using NPs as anti-biofilm agents to manage biofilm-mediated pathogens can help increase plant life and human health in the future.

#### **AUTHOR CONTRIBUTIONS**

Conceptualization, SM and LJ; software SM; validation, formal analysis, investigation, data curation; SM, IZ, TRB, SAP, ZAB, AMK, and LJ; writing—original draft preparation, SM, IZ, and LJ; review and editing TRB, SAP, ZAB, AMK, and LJ. supervision LJ.

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