Phytosynthesis of Silver Nanoparticles and Their Role as Antimicrobials

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Abstract

Bionanotechnology is an interdisciplinary field involving biotechnology, nanotechnology, chemistry, physics, and material science. Nanoparticles exhibit novel properties depending on the size, shape, and morphology which enable them to interact with plants, animals, and microorganisms. Some researchers have utilized the chemical and physical methods for the synthesis of metal nanoparticles; however, environment-friendly synthesis of metal nanoparticles is becoming popular in recent times. Also, the biological synthesis method is cost effective, and the raw material is available in abundance. Phytosynthesis of nanoparticles is also explained as the green chemistry approach for the synthesis of metal nanoparticles. It has emerged as a promising field of research in the field of bionanotechnology. Various studies have showcased diverse aspects of phytosynthesis of metal nanoparticles that include methods of synthesis, mechanism, and the applications offered by the biosynthesized particles. Among the nanoparticles researched till date, silver nanoparticles have gained significant position owing to their inherent characteristic as an antimicrobial agent. Although the antimicrobial potential of silver metal has been recognized since centuries, after the discovery of silver in the nanoparticle form, it has gained tremendous impetus due to the exceptional rise in its antimicrobial property. Phytosynthesized silver nanoparticles show efficient antimicrobial activity and are used in different biomedical applications. In the present chapter, we highlight the biosynthesis of nanoparticles, plants as a system for synthesis process,

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biomaterials used, and mechanism of formation of nanoparticles and silver nanoparticles as an efficient antimicrobial agent against bacteria, fungi, and viruses.

Keywords

Bionanotechnology · Nanoparticles · Plants · Phytosynthesis · Silver · Antimicrobial agent

16.1 Introduction

The term nanotechnology is related to the science and engineering involved in the synthesis, design, and characterization of materials at the nanoscale level (1–100 nm) (Rai et al. 2008). Nanotechnology offers application in different fields of science and technology ranging from devices to materials with at least one dimension at the nanometer level (De Morais et al. 2014). Bionanotechnology is a multidisciplinary field of science that involves intersection of biotechnology, nanotechnology, physics, chemistry, and material science for the development of different applications in the field of science and technology (Rai and Yadav 2013; Soman and Ray 2017). Bionanotechnology includes the synthesis of nanoparticles based on biologically available sources like plants, microorganisms, algae, and planktons (Siddiqui et al. 2018). Nanoparticles are of extreme scientific interest as they fill the gap between bulk materials and the atomic materials (Rauwel et al. 2015). The shape, size, and morphology of nanoparticles facilitate their application in medicine, drug delivery, sensors, and catalyst (Santoshkumar et al. 2017). There are number of synthesis methodologies developed for the fabrication of nanoparticles involving physical, chemical, and biological methods (Rai et al. 2008). However, the biological process is the most sought-after synthesis methodology applied for the synthesis of nanoparticles (Rai and Yadav 2013). As for the chemical synthesis process, the chemicals that are employed are hazardous in nature, while for the physical synthesis process, high energy is required which is not economic in nature (Saravanan et al. 2021). For the biological synthesis process, a variety of plant species and microorganisms like bacteria, fungi, yeast, and actinomycetes are harnessed for the synthesis of silver, gold, copper, platinum, palladium, and silica nanoparticles (Prasad et al. 2011; Santoshkumar et al. 2017). Among the wide variety of nanoparticles studied, silver nanoparticles are known for their potential antimicrobial activity (Fierascu et al. 2020). Silver in its ion form also depicted efficient antibacterial property, but silver in the form of nanoparticles shows exponential properties against a large number of microorganisms (Tang and Zheng 2018). With the major increase in the number of drug-resistant bacteria, fungi, and viruses due to the high usage of antibiotics, rising pollution, and changes in the environmental conditions, multiple infectious diseases are rising (Chang et al. 2019). To overcome this situation, scientists are working tirelessly to develop new drugs and treatment measures to fight rising cases of infection (Soman and Ray 2017). Silver nanoparticles function as efficient growth inhibitors and work by disrupting the cell wall and cell organelles of the microbial cell (Siddiqui et al. 2018). These properties of silver nanoparticles gained insight for its use in a number of commercial applications (Soman and Ray 2017). Silver nanoparticles are extensively used in applications like food packaging, antimicrobial topical agent, pharmaceutical products, and biomedical materials (Singh et al. 2018). The present book chapter also reviews biological synthesis of silver nanoparticles using plant as an efficient fabrication system. Also, the biological synthesis of silver nanoparticles and its antimicrobial application as antibacterial, antifungal, and antiviral agent will be discussed. Toxicity studies of biologically synthesized silver nanoparticles will also be explained in the chapter.

16.2 Methods of Synthesis

There are a number of methods employed for the synthesis of metal nanoparticles including physical, chemical, biological, and enzymatic method (Singh et al. 2018). A number of physical and chemical methods are employed for the synthesis of metal nanoparticles; however, these methods prove to be quite expensive and potentially harmful to the environment (Siddiqui et al. 2018). Utilization of a number of toxic chemicals and synthesis and elevated temperature and pressure conditions often result in hazardous by-products which in turn affect the micro and bio-flora of the environment (Saravanan et al. 2021). Biologically inspired synthesis methodologies have evolved with a new branch of nanotechnology, i.e., bionanotechnology which includes synthesis of nanoparticles using microorganisms like bacteria, fungi, viruses, yeast, actinomycetes, plant parts (leaf, stem, roots, fruits, seeds, peel, and biomass), and enzymes (Bilal et al. 2017; Saravanan et al. 2021). Majorly two approaches are in practice for the synthesis process; top-down and bottom-up approaches are involved for the synthesis of metal nanoparticles (Fig. 16.1).

16.3 Biological Synthesis Methods

Biological synthesis of nanoparticles is gaining great interest in research and development due to its application in different fields of science and technology (Priyadarshini and Mahalingam 2017). Owing to their environment-friendly approach, regulation and control of size and shape, use of safer solvents, and easy clean up, researchers are giving impetus on synthesis of nanoparticles using biological entities like bacteria, fungi, yeast, virus, algae, plants, enzymes, and bioactive compounds (Rai et al. 2008; Chand et al. 2020). Compared to the numerous physical and chemical methods used for the synthesis process, biological agents offer a single-step reduction method which needs comparatively low energy for the initiation of the synthesis process. Thus, biological synthesis methods are a cost-effective process (Rai and Yadav 2013).

Phytonanotechnology and phytosynthesis process has acquired considerable attention as a potential technique for synthesis of metal nanoparticles with multi-functional properties (Bilal et al. 2017). The single-step synthesis methodology using plant extracts has gained tremendous attention due to the eco-friendly

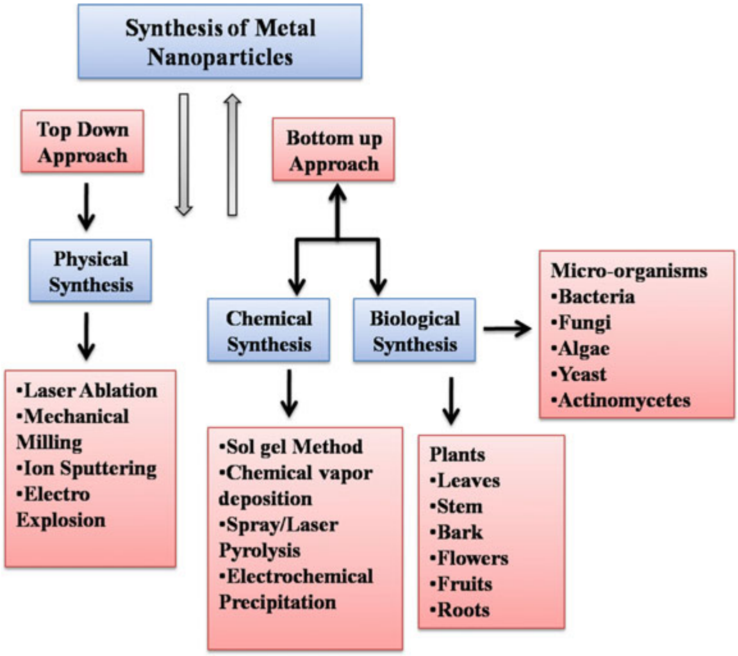

Fig. 16.1 Methods of synthesis of nanoparticles

approach, cost-efficient technology, and rapid results (Bilal et al. 2017). Although the potential of plant extracts to reduce metal ions has been recognized since the 1900s, the knowledge has been applied since the last 35 years for the reduction of metal salts; in the recent decade, the use of plant extracts for the synthesis of nanoparticles has been introduced (Mittal et al. 2014). Plant contain numerous bioactive compounds including phenols, steroids, flavonoids, poly phenols, ascorbic acid, terpenoids, and reductases which function as reducing agents for the synthesis of metal nanoparticles (Rai and Yadav 2013; Santoshkumar et al. 2017). Plant-induced synthesis of metal nanoparticles proves as an important biosynthesis procedure as the plant extract acts as both reducing and capping agent for the synthesis of metal nanoparticles. Synthesis of nanoparticles using the plant system can be followed by both the intracellular and extracellular methods (Bilal et al. 2017). The intracellular synthesis of nanoparticles can be performed by growing the plants in metal-rich organic media, metal-rich soil, or metal-rich hydroponic system, while the extracellular synthesis of nanoparticles is executed by preparing the extract of plant parts (leaves, stem, bark, seeds) through boiling or crushing the plant sample (Rauwel et al. 2015; Kuppusamy et al. 2016).

Incorporation of green synthesis procedures for nanofabrication of nanoparticles has decreased the use of lethal chemical agents and has also ensured noteworthy measures to assure social and environmental well-being. Plant-mediated synthesis protocols depend on various reaction constraints like temperature, pH, pressure, and

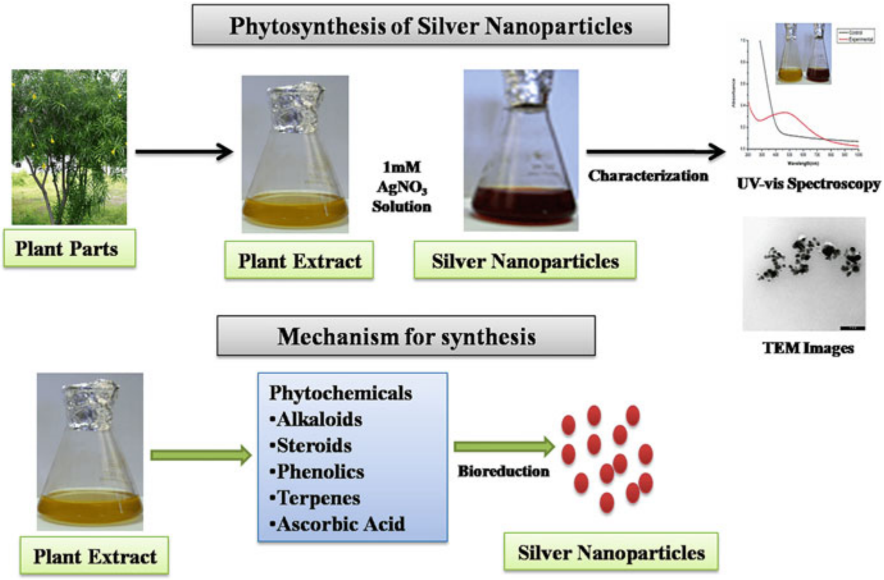

Fig. 16.2 Phytosynthesis of silver nanoparticles and mechanism of synthesis

solvent concentration (Chand et al. 2020). Due to the availability of a variety of phytochemicals like aldehydes, ketones, flavonoids, terpenoids, and phenolic compounds, a wide array of plant material is available for the synthesis process (Rajeshkumar and Bharath 2017) (Fig. 16.2). Thus, eco-friendly synthesis of metal nanoparticles using plant extracts serves as a novel substrate for bulk synthesis of nanoparticles. Nanoparticles synthesized using the plant system offer different applications in healthcare and commercial products.

16.4 Silver Nanoparticles

Silver metal ions have been known in the nineteenth century for its medicinal property and antimicrobial applications (Rai et al. 2009). Silver ions and silver-based compounds are recognized to depict antimicrobial activity against 16 major bacterial organisms (Dakal et al. 2016). Silver ions are mostly utilized in their nitrate form for the induction of antimicrobial activity. However, due to rising increase of antibiotics, the use of silver ion solution as a topical antimicrobial agent decreased (Rai et al. 2009). Silver-based compounds in the form of silver nanoparticles are employed for a number of physical, biological, and medicinal applications (Prabhu and Poulose 2012). Silver nanoparticles can be synthesized through the physical and chemical methods; however, due to the feasibility and environment-friendly nature of the process, biological methods are preferred for the fabrication of silver nanoparticles (Rai et al. 2009). Silver nanoparticles offer applications in diverse fields as catalyst, sensors, food packaging material, composites, bactericidal, and therapeutic agent (Chand et al. 2020).

16.5 Mechanism of Action

The exact mechanism of action of silver nanoparticles has not been postulated yet; however, several theories have been proposed by several researchers based on the microbicidal effect of silver nanoparticles on different microorganisms (Vasquez-Munoz et al. 2019). Silver nanoparticles possess the ability to get adhered to bacterial cell wall and subsequently penetrate inside the bacterial cell membrane (Pulit-Prociak and Banach 2016). Silver nanoparticles cause structural changes inside the cell membrane thereby increasing its permeability which in turn leads to the formation of pits on the cell surface and allows accumulation of silver nanoparticles on the cell surface (Singh et al. 2020). Higher accumulation of silver nanoparticles on the cell surface finally leads to cell death (Prabhu and Poulose 2012).

Another mechanism for antimicrobial action of silver nanoparticles is related to the interaction of silver nanoparticles with the thiol groups. It is supposed that silver nanoparticles release silver ions inside the cells, and these silver ions react with the thiol groups of the essential bacterial enzymes and inactivate them. The bacterial cells uptake silver ions from silver nanoparticles which slow down functioning of bacterial cells resulting in cell damage (Feng et al. 2008). Generation of reactive oxygen species is also a possible hypothesis for antimicrobial activity of silver nanoparticles (Morones et al. 2005). The silver ions react with the respiratory enzymes and inhibit their activity thereby resulting in the generation of reactive oxygen species that itself attack and damage the cells (Fig. 16.3). Liao et al. (2019) also the supported the theory of generation of reactive oxygen species; for the study, the authors chose multidrug-resistant bacteria *Pseudomonas aeruginosa* and checked the effect of silver nanoparticles on its growth. It was observed that silver nanoparticles depicted significant effect on the growth of bacteria in a time- and concentration-dependent manner. The bacterial cell wall became fragile and ruptured after interaction with silver ions resulting in spilling of bacterial cell constituents. Also, due to the rise in oxidative stress, response levels of catalase, peroxidase, and superoxide dismutase increased, while silver nanoparticles inhibited the activity of catalase and peroxidase leading to excessive generation of reactive oxygen species which conferred in impairment of DNA and ribosomes and decline in synthesis of macromolecules following cell death.

Bacterial DNA is majorly composed of sulfur and phosphorus groups; sulfur and phosphorus are soft bases, while silver is a soft acid; hence the interaction of silver ions with bacterial DNA causes hurdles in DNA replication thus resulting in cell death (Shrivastava et al. 2007). Ahmad et al. (2020) also suggested that positively charged silver ions play an important role in the exhibition of antimicrobial activity of silver nanoparticles. Silver ions interact with the negatively charged nucleosides; this electrostatic interaction of negatively charged bacterial cells and positively

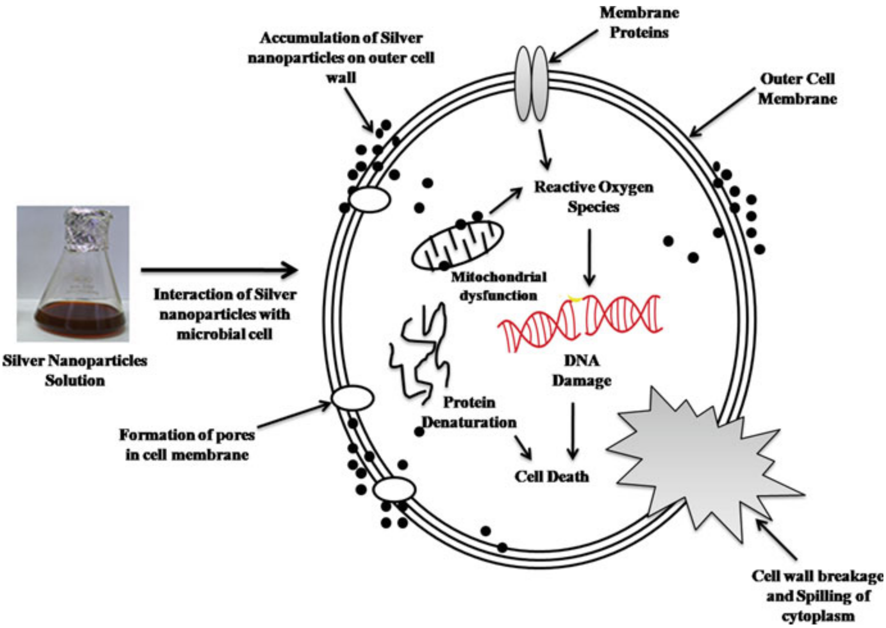

Fig. 16.3 Mechanism of action of silver nanoparticles on bacterial cell

charged silver ions leads to the disruption of cell wall and increase in the permeability of cell membrane, thus leading to cell disruption and cell death.

Signal transduction in microbial cells is affected by silver nanoparticles via the alternation of tyrosine phosphorylation. Silver nanoparticles also inhibit biofilm formation by affecting its growth; increased membrane permeability results in interaction of silver ions with cellular biomolecules causing damage to the microbial cells. Silver ions also bind with bacterial DNA thereby affecting DNA replication and cell division which leads to cell apoptosis and inhibition of cell growth (Singh et al. 2020). Similarly, silver nanoparticles while interacting with viruses get attached to the outer surface of the virus coat leading to disruption of glycoproteins, and also silver nanoparticles bind with viral DNA/RNA and trigger cell apoptosis, thereby blocking replication process (Singh et al. 2020).

16.6 Applications of Silver Nanoparticles as an Antimicrobial Agent

Infectious diseases are a serious threat to global health. Microbial infections often lead to serious medical conditions. Silver has been recognized for its microbicidal properties since centuries. However, silver nanoparticles have emerged as the new generation of antimicrobial agent with diverse applications (Chang et al. 2019).

16.6.1 Antibacterial Agent

Silver nanoparticles are highly acclaimed for their antibacterial activity. Silver ions in the form of silver nitrate solution have been used since centuries as a topical antibacterial agent. Silver nanoparticles are used in burn and wound infections, wound dressings, and antibacterial fabrics and for coating surgical instruments, catheters, etc. (Verma and Maheshwari 2019; Ahmad et al. 2020).

One of the practical applications of the food industry is to ensure the shelf life of the packaged food products to prevent them from spoilage. Microbial contamination of food products is a major issue for food industry. Prevention of food spoilage and maintaining the quality of food products are essential parts of food industry. Traditionally used food packaging materials do not provide efficient shelf life, but with the introduction of silver nanoparticles, development of nanocomposite material has been initiated. Impregnation of silver nanoparticles on biopolymer material has led to advancement of food packaging systems (Simbine et al. 2019).

With the rising areas of research, silver nanoparticles are used in dentistry for performing restorative dentistry. Silver nanoparticles along with polymers are used which act as adhesive and act against biofilm-forming bacteria. Silver nanoparticles due to their antibacterial properties are also used in root canal irrigation and denture implants and as root canal sealers or cement (Niska et al. 2016; Salas-Orozco et al. 2019). Chang et al. (2019) developed nanohybrids of albumin-conjugated silver-diamond and analyzed its antibacterial activity. The authors reported that the silver nanohybrids showed efficient antibacterial activity against gram-positive and gram-negative bacteria.

16.6.2 Antifungal Agent

Skin infections require use of antifungal agents like ketoconazole, itraconazole, Nystatin, and amphotericin. Mallmann et al. (2015) tested the antifungal activity of biologically synthesized silver nanoparticles against *Candida albicans* and *Candida tropicalis*. Silver nanoparticles showed substantial activity against *C. albicans* and *C. tropicalis* which was found to be equal to the activity showed by amphotericin B. Thus, it was suggested that silver nanoparticles can be efficiently used as a topical antifungal agent. Mussin et al. (2019) reported antifungal activity of silver nanoparticles against human skin microbiota *Malassezia furfur*. The authors checked synergistic activity of silver nanoparticles and ketoconazole and also prepared an antimicrobial gel with combination of silver nanoparticles and ketoconazole. The results of the study depicted that silver nanoparticles enhanced the antifungal activity of ketoconazole against *M. furfur*.

Apart from being used as a topical antifungal agent, silver nanoparticles can also be used for the management of plant diseases. Elgorban et al. (2016) used different concentrations of silver nanoparticles solution against six different *Rhizoctonia solani* anastomosis groups that infect cotton plants. The results of the study showed that silver nanoparticles strongly inhibit the activity of all six groups of *R. solani*.

Thus, silver nanoparticles can be used as a topical antifungal agent in diverse applications.

16.6.3 Antiviral Agent

The antiviral potential of biosynthesized silver nanoparticles is a field which has not been much investigated. Haggag et al. (2019) biosynthesized silver nanoparticles using the aerial part extracts of *Lampranthus coccineus* and *Malephora lutea*. The biosynthesized silver nanoparticles were checked for their antiviral activity against HAV-10 (hepatitis A) virus, HSV-1 (herpes simplex) virus, and CoxB4 (Coxsackie) virus. The results of the study showed that nano hexane extract of *L. coccineus* depicted potential activity against HAV-10, HSV-1, and CoxB4 virus, while extract of *M. lutea* showed significant activity against HAV-10 and CoxB4 virus thus confirming that biosynthesized silver nanoparticles can also be used as an efficient antiviral agent. Dung et al. (2020) reported the antiviral activity of silver nanoparticles against swine fever virus which a highly contagious disease. It was suggested that silver nanoparticles inhibited the activity of the virus and can be used as a disinfectant against the viral infection.

With the rise of the global pandemic COVID-19, scientist across the world started searching of probable treatment strategies that could be used to deal with the rising infections of coronavirus. Jeremiah et al. (2020) studied the effect of different concentrations of silver nanoparticles to inhibit the activity of SARS-CoV-2. It was observed that silver nanoparticles show antiviral activity in a concentration-dependent manner. Silver nanoparticles with the size range of 1–10 nm were found be highly effective. Silver nanoparticle-coated fabric masks, air filters, and polycotton fabrics can be efficiently used for protection against coronavirus. Silver nanoparticles bind with the spike glycoproteins of coronavirus and inhibit its binding with the cells, and also silver ions decrease the environmental pH of respiratory epithelium making it more acidic and difficult for the virus to reside (Jeremiah et al. 2020).

16.7 Toxicity of Silver Nanoparticles

Researchers throughout the world have centered their attention on the synthesis and application of silver nanoparticles focusing on their antibacterial and antifungal activities and optoelectronic properties. However, the large-scale use of metal nanoparticles in several applications has ensured their release into the environment raising concerns about the negative effect of nanoparticles on plants-soil and the aquatic bio-life and also on human health. Based on several studies, it is confirmed that silver nanoparticles have the tendency to get accumulated. Plants and soil can absorb metal nanoparticles thus paving way for their entrance into the food chain (Dobrucka et al. 2019). Hence, extensive research is needed for nano-toxicological studies, and their potential health impact cannot be neglected.

Sehnal et al. (2019) performed a study on the effect of silver nanoparticles on the growth of *Zea mays* (maize) plants. Silver nanoparticles and silver ions in the form of silver nitrate solution were tested for growth inhibition of aboveground plant parts. In the study it was observed that the effect of silver nanoparticles depends not only on the concentration of nanoparticles but also on the size of nanoparticles; smaller size leads to higher toxic effects. Plant cell walls allow entrance of nanoparticles through cell membranes and epidermal layers through the roots to reach the stem and leaves. As the pore size of cell wall is a few nanometers in size, smaller nanoparticles depict higher effect of plants. Also, the silver ions released from silver nanoparticles generate reactive oxygen species leading to cellular toxicity. The phytotoxic effect of silver nanoparticles was observed on the nucleus, mitochondria, and chloroplast of maize plants.

Dobrucka et al. (2019) studied the phytotoxic effect of biologically synthesized silver nanoparticles using plant extract of *Veronica officinalis*. For the study, biologically synthesized silver nanoparticles were checked for their phytotoxic effect on different stages of plant (*Linum flavum* and *Lepidium sativum*) development. The authors proposed that cell walls have small pore size of 5–20 nm and nanoparticles after entering the cell wall increase the pore size and form larger pores. After entering the cell wall, silver nanoparticles interact with the cytoplasmic membrane which becomes convex and traps nanoparticles in bubbles and allows their entry into inside the cell. However, in the present study, the biologically synthesized silver nanoparticles were around 40 nm in size, spherical, and agglomerated; hence, in the phytotoxic study, initially the silver nanoparticles stimulated the growth of seedlings (*L. sativum*), then inhibited the growth, and finally in later stages again stimulated the growth. For *L. flavum* seedlings, silver nanoparticles stimulated the growth of plants. Hence, it can be concluded that silver nanoparticles at a smaller size ($\leq$10 nm) have higher phytotoxic effects.

Ferdous and Nemmar (2020) studied the effect of silver nanoparticles on human health and its bio-distribution to local organs. The authors concluded that toxic effect of silver nanoparticles highly depends on the shape, size, and concentration of nanoparticles. Human exposure to nanoparticles comes through inhalation, oral, dermal, or intravenous implantation which assures its translocation and accumulation to various organs thereby depicting toxic effects. Toxic effects on nanoparticles on local and remote organs of the body are based on the duration of exposure, particle size of nanoparticles, route of administration, dose value, and the end concentration of the nanoparticles. The mechanism of action of silver nanoparticles on the human body is still a matter of research before concluding with the results.

Thus, with the increasing use of silver nanoparticles in diverse fields of medicine and technology, there is still great scope for the study of toxic effects of silver nanoparticles on the environment and the human health. As silver nanoparticles possess the tendency of accumulation, it is very likely that they enter the food chain and can adversely affect plant system, aquatic animals, and humans.

16.8 Conclusion

Silver nanoparticles provide a satisfactory answer to the growing concern of antibiotic resistance in microorganisms. As due to the growing number of infections, the human population is facing the number of effective antibiotics are depleting. Silver nanoparticles can be easily synthesized using the physical, chemical, and biological synthesis methods; however, much attention is given towards the synthesis of nanoparticles using the biological system as it offers clean, eco-friendly, and cost-effective synthesis process. Silver nanoparticles depict efficient antimicrobial activity and can be used as an effective antibacterial, antifungal, and antiviral agent both individually and in combination with antibiotics. With the rising global concern towards COVID-19, we should harness the multiple applications offered by silver nanoparticles. The present review also supports the use of silver nanoparticles as an effective antimicrobial agent.

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