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Properties, effects and chemical and biological method of synthesis of silver nanoparticles

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Abstract

The silver nanoparticles (AgNPs) have gained significant attention due to their remarkable properties. Due to the electrical and catalytic properties of silver nanoparticles it is greatly exploited commercially. However, silver nanoparticles adversely affect the environment and can have hazardous, detrimental toxic effects on mammals, non-mammals and plants. The properties of silver nanoparticles greatly depend on the synthesis process conditions such as process type, temperature, pH, concentration of precursor, reducing agents and capping agents used. In this review we have discussed the bottom-up approach which is categorized into chemical synthesis and green synthesis. The chemical synthesis is a conventional method which is used to synthesize silver nanoparticles. However, it does possess major disadvantages as compared to the green synthesis. The green synthesis has gained a significant interest among the researchers as an alternative route to effectively synthesize silver nanoparticles with varying morphology. Green synthesis methods such as viral-biotemplates synthesis using TMV has gained specific attention as it is used in the synthesis of 1D silver nanostructure. The advantages and disadvantages of bottom-up method of silver nanoparticle synthesis is also discussed. This article also provides a critical review on the properties and effects of silver nanostructures.

Keywords: Silver; Silver nanoparticles; Toxicity in mammals; Toxicity in non-mammals; Toxicity in plants; Green synthesis

1. Introduction

Metallic Silver (Ag) is the 67th abundant among the elements and is a durable transition element. Due to its rarity and attractive metallic luster silver has been used as jewelry, currency coins and other valuable items. In ancient times silver utensils were used to keep the water and wine clean. Silver finds its applications in medicine, as due to its antimicrobial activity silver is used as antibacterial, antiviral, and antifungal. In the 17th and 18th century Silve Nitrate ($AgNO_3$) was applied to treat ulcers [1]. 1% Silver Nitrate was introduced as an eye solution to prevent conjunctivitis in small children [2]. Silver Sulfadiazine in its tropical form is used to treat burn wounds [3].

Besides all these applications, exposure to high concentration of silver for prolonged periods may result in deposition of silver in the body, which may further cause irreversible discoloration of skin and eyes, this condition is called Argyria [4]. Due to the hazards and high cost associated with silver its medical interest started diminishing around the mid-1900s. Antibiotics like Penicillin and Cephalosporin started to replace the medicinal interest gained by silver due to its low cost and low risk associated with its usage. The interest of silver as medicinal drug was revived because of the large-scale increase in the number of multiple drug-resistant bacteria which was the result of exploitation of antibacterials. Silver performed excellent due to its antimicrobial properties and when enhanced using Nanotechnology the limitation of silver in its pure state were overcome. These nano synthesized silver materials were having smaller size (in nm range)

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which enhanced its surface area, that in turn improves its reactivity. AgNPs showed biocidal action by slowly releasing Ag^+ that results in enhanced drug delivery and bioavailability. By using different mechanisms like interaction with Thiol groups in protein and enzymes, inducing Oxidative stress by generating ROS and inhibition of DNA replication; making it more difficult for the bacteria to produce drug resistant strains [5].

Due to their properties such as electrical properties [12-14], catalytic properties [15-18], antimicrobial properties [24-30], surface plasmon resonance [14] and surface-enhanced reman scattering [19-23] the silver nanoparticles find wide spectrum of application. However, the AgNPs exhibits toxicity to mammals [39-42], non-mammals [44-49] and plants [50-55].

Generally, all silver nanoparticles including silver nanoparticles are synthesized by two approaches [57] namely top-down where nanoparticles are synthesized from the bulk material and bottom-up approach in which the nucleation sites are formed initially, and then grown into nanoparticles. The top-down method that consist of physical methods for the synthesis of AgNPs is not the scope of this review. This review only focuses on the bottom-up approach for the synthesis of silver nanostructures. The bottom-up method can be further categorized into Chemical synthesis which consist of Chemical reduction [56-59], Sol-Gel Method [60-67], Chemical Vapor deposition method [68-76], Reverse micelle process [77-83] , Wet chemical synthesis [84-94] and the Biological synthesis (Green synthesis). The biological synthesis consists of two categories, Plant mediated synthesis of AgNPs [104-123] and Microbial-mediated synthesis which consist of Bacteriogenic synthesis [131-138], Algae-mediated synthesis [143-151], Fungi-mediated synthesis [153-158] and Virus-mediated synthesis [162-168] for the synthesis of AgNPs.

Various studies have focused on the synthesis of AgNPs without considering the cost and hazards associated with it. For example, Hydrazine and $NaBH_4$ which are commonly used as a strong reducing agent in many chemical methods of synthesizing AgNPs [85], is very toxic, carcinogenic chemical [86-88]. Due to the disadvantages associated with the chemical method green synthesis has observed more attention in recent years as the method is less detrimental and more cost-effective. However, there are still concerns regarding the stability, size distribution and morphology of the AgNPs produced by green synthesis [119], some biological methods cannot serve as a feasible option for industrial use as they are time consuming [157].

In this review, first we explain the properties and effect and in the second half of the article chemical and green synthesis of silver nanoparticles is discussed stating the application, advantages and disadvantages of the methods used to synthesize AgNPs.

2. Properties of Silver Nanoparticles

2.1. Electrical Properties

Silver exhibits high thermal and electrical conductivity along with low contact resonance, making it a preferred choice in field of electronic. Silver nanoparticles find its application in Thin-Film transistor electrodes [12], conductive inks for printed circuit boards [13], data storage device and battery-based intercalation materials [14].

2.2. Catalytic Properties

Silver nanoparticles owing to their small size possess a high surface area, which translates into enhanced surface energy and numerous reactive sites. These features the AgNPs as a promising material in catalytic processes. AgNPs are effective in catalyzing CO and benzene oxidation [15], reduction of 4-nitrophenol in presence of $NaBH_4$ [16]. Reduction of Rhodamine B (RhB) [17] and reducing 4-nitrophenol to 4-aminophenol [18].

Catalyzing CO:

$$\boldsymbol{CO} + \frac{\mathbf{1}}{\mathbf{2}}\boldsymbol{O_2} \xrightarrow{\boldsymbol{AgNPs}} CO_2$$

This reaction has application in air purity and reducing toxic CO emissions.

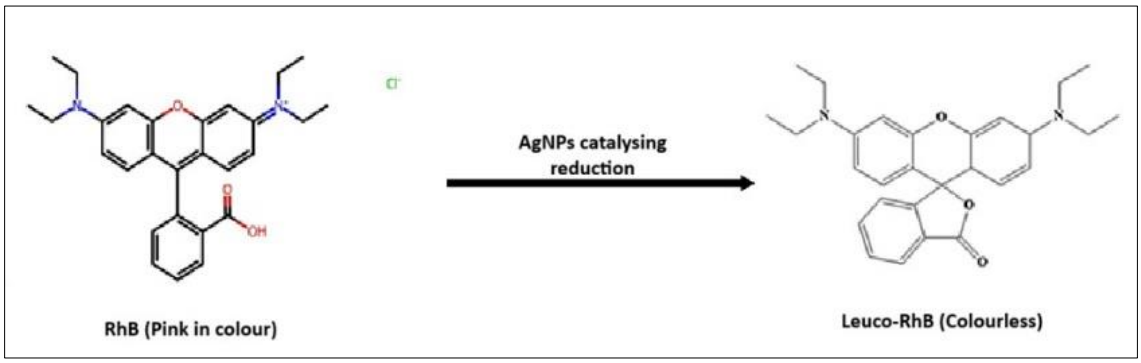
Catalyzing Benzene Oxidation:

$$\mathbf{C_6H_6 + [O]} \xrightarrow{\boldsymbol{AgNPs}} \mathbf{C_6H_5OH}$$

Oxidizing agents such as O_2 or H_2O_2

This reaction finds its application in Phenol production for chemical industry and degrading benzene in wastewater and polluted air.

Catalyzing the reduction of RhB



This reaction can be used to reduce the pollution caused by dye industries

2.3. Surface Plasmon Resonance (SPR)

Silver nanoparticles (AgNPs) show unique properties distinct from their bulk metal counterpart. One such characteristic is to show Surface plasmon Resonance under irradiation of light. This phenomenon induces SPR peaks in UV- visible spectrum. Typically, the size, shape and dispersion of the nanoparticles influenced the width and position of SPR peaks [14].

2.4. Surface-enhanced Raman scattering (SERS)

A key application of AgNPs is their role in Surface-enhanced Raman scattering (SERS). AgNPs allows the detection and identification of single molecules by enhancing the efficiency of SERS by as much as 10^{14} to 10^{15} folds [19]. Due to these unique properties AgNPs have found its application in various fields, particularly sensing and imaging technologies. They are utilized in detecting DNA, where their plasmonic properties enable high sensitivity [20]. Additionally, AgNPs are employed in selective colorimetric sensing for biomolecules such as Cysteine [21], monitoring Purine nucleosides phosphorylase activity [22], and detecting environmental contaminants like Mercury [23].

2.5. Antimicrobial Properties

Silver when undergone nano synthesis showed excellent performance in antibacterial applications as it inhibits the growth of Gram-Positive and Gram-negative bacteria that includes *Pseudomonas Aeruginosa*, *Escherichia Coli*, and *Staphylococcus Aures* [24-25]. A study revealed that antimicrobial activity of many antibiotics was enhanced in the presence of silver nanoparticles (AgNPs) [26]. AgNPs is also used as antifungal and can kill fungal strains including *Candida tropicalis, Aspergillus Fumigatus, Mucor* and *Saccharomyces Cerevisiae* [27]. Nano silver synthesized in Hepes buffer ($C_8H_{18}N_2O_4S$) at pH 6.8 to 8.2 could inhibit HIV-1 replication and exhibit a much higher anti-HIV activity (98%) than gold nanoparticles (6-20%) [28]. Due to its antiviral properties, it is also used to inhibit Hepatitis B virus [29] and Herpes simplex virus [30].

3. Effects of Silver Nanoparticles

3.1. Mechanism of Toxicity

Numerous studies have been conducted to fully elucidate the mechanism of biocidal action of AgNPs, however no firm conclusion can be drawn so far. The antibacterial activity of AgNPs is complex process, and numerous modes of action are proposed, which involves:

- **Generation of Reactive oxygen Species (ROS):** AgNPs are believed to induce the ROS production, ROS are unstable molecules that are derived from oxygen metabolism [31,32]. In cellular processes, normally ROS levels are regulated by antioxidant defenses. However, excessive ROS production overwhelms these defenses, leading

to oxidative stress. This oxidative stress may result in damaging vital cellular components, including lipids, proteins and nucleic acids, which disrupts cellular homeostasis [5,33].

- **Attachment to the Cell membrane and Disruption of Membrane Integrity:** AgNPs directly interacts with cell membrane leading to structural damage. This interaction compromises the integrity of the cell membrane, making it more permeable and susceptible to further damage. It is a critical factor in antibacterial efficacy of AgNPs[34].
- **Alteration in Membrane Permeability:** Changes in the permeability of cellular membrane is another consequence of AgNPs exposure. Changes like this can interfere with the cells ability to maintain a stable internal environment, which leads to a cascade of dysfunctions that can potentially impair cellular survival [35].
- **Interaction with proteins and Disruption of their regular functions:** AgNPs may bind to proteins, altering their structure and function. Proteins plays a vital role in a wide range of cellular processes, and their disruption can have significant downstream effects. For example, AgNPs may inhibit enzymes or other proteins that are essential for cellular metabolism and repair [36,37].
- **DNA Damage and Disruption of Replication:** Another important mechanism involves the interaction of AgNPs with DNA, leading to damaging and hindering replication process. DNA damage is particularly detrimental as it impairs the cell's ability to divide and sustain itself that contributes to antimicrobial action of AgNPs [38].

3.2. Toxicity to Mammals

To date the available data on the effect of AgNPs in mammals in vivo is very less. The existing results have portrayed that AgNPs can cause toxicity to test animal model. This was studied by experimenting on Sprague-Dawley rats. In the first experiment Sprague-Dawley rats showed no significant changes in lungs and nasal cavity at a high dose of 1.32 X 10^6 particles cm^{-3} AgNPs in an inhalation chamber for 4 weeks. However, Goblet cells containing neutral mucin was affected and hence they increased in size and number. This suggest that AgNPs affects the neutral mucin in respiratory system [39]. In the second one, lung inflammation was reported. In this Sprague-Dawley rats undergoes inhalation exposure at a dose of 2.9 X 10^6 particles cm^{-3} for 6 hours per day this was done for 90 days (13 weeks). After 13 weeks, inflammation in the lungs was reported in rats, also the lung functional test showed significant decrease in tidal volume and minute volume. This experiment indicates that AgNPs may cause lung damage and affect their normal function [40]. No significant changes were observed in body weight, hematology and blood biochemistry for both male and female *Sprague-Dawley* rats after 4 weeks (28 days of exposure at high dose of 1.32 X 10^6 particles cm^{-3}, implying that AgNPs having concentration near sliver dust ($1X10^6$ μ cm^{-3}) did not produce any remarkable health effects on Sprague-Dawley rats [41].

A notable change in the values of alkaline Phosphatase and Cholesterol in both male and female Sprague-Dawley rats after 28 days of 30 mg kg^{-1} dose mixed with their diet was, was observed and demonstrated in an oral toxicity study. No genetic toxicity in the bone marrow of rat was observed [42]. This is summarized in Table 1.

To remove the toxic effect of AgNPs, a modified Tollens process involving reduction of the cation $[Ag(NH_3)_2]^+$ can be used for synthesis of AgNPs to reduce the toxicity of AgNPs, this is traditionally done by reducing silver ions (Ag^+) to metallic silver (Ag) using reducing agents like Glucose and Ammonia. However, this can only reduce the toxicity of AgNPs to a certain limit.[43]. The above explanation is summarized in Table 1.

Table 1 Toxicity on mammals

Organism	Dose concentration	Exposure method	Effect Measured	Ref
Sprague-Dawley rats	Low dose of 1.74 X 10^4 particles cm^{-3} ; medium dose of1.27 X 10^5 particles cm^{-3} ; high dose of 1.32 X 10^6 particles cm^{-3} of AgNPs size 13-15 nm	Inhalation exposure for 6hrs a day, 5 times a week for 4 weeks	Size and number of Goblet cells containing neutral mucin increased. However, no remarkable changes in nasal and lungs canal.	[39]
Sprague-Dawley rats	Low dose of 0.7 X 10^6 particles cm^{-3}; medium-dose of 1.4 X 10^6 particles cm^{-3}; high dose of 2.9 X 10^6 particles cm^{-3} of AgNPs size 18 nm	Inhalation exposure for 6 hrs a day, 5 times a week for 13 weeks	Lung inflammation and decrease in tidal volume and minute volume.	[40]

Sprague-Dawley rats	Low dose of 1.74 X 10^6 particles cm^{-3} ; medium dose of 1.27 X 10^6 particles cm^{-3} ; high dose of 1.32 X 10^6 particles cm^{-3} of AgNPs size 12-16 nm	Inhalation exposure for 6 hrs per day,5 times a week for 4 weeks	Both male and female rats show no significant changes in the body weight, hematology and blood biochemical values.	[41]
Sprague-Dawley rats	Low dose of 30 mg kg^{-1}; medium dose of 300 mg kg^{-1}; high dose of 1000 mg kg^{-1} of AgNps size 60 nm	Ingestion exposure in which the AgNPs are mixed with diet for 28 weeks	Gender related difference in accumulation of AgNPs in kidneys; significant dose dependent changes in alkaline phosphate, cholesterol values.	[42]

3.3. Toxicity in non-mammals

Numerous non-mammals are been considered and used to test the adverse effects of AgNPs. It is not a surprise that majority of these tested and adversely affected non-mammals are aquatic organisms as a large quantity of AgNPs are released from fabrics and textile would flow into aquatic system. Few non-aquatic non-mammals such as Fruit Fly (Drosophila *melanogaster*) are studied.

Zebrafish has been used as a correlative and predicted model in many studies to evaluate thre effect of AgNPs [44]. A study, in which embryos of Zebrafish were exposed to a dose concentration of 0.04-0.71 nM for 120 hours. It was noted that single AgNPs of size 11.6±3.5 nm could transport into the Zebrafish embryos through Chorion pore canal, AgNPs were detected in each development stage. At a concentration of 0.19 nM development abnormality could be triggered [45]. Another study consisted of synthesizing four different size of nanoparticles of size 3 nm, 10 nm, 50 nm, and 100 nm respectively, which were then tested on Zebrafish embryos to test its toxicity, only a few differences were observed between them. It was reported that AgNPs induced 100% mortality when exposed for 120 hours at 250 μM. At a dose of 100μM, variety of embryonic morphological deformations were reported [46]. In another study the author reported that larger AgNPs of size 41.6±9.1 nm were more toxic and produced sever deformation of Zebrafish than smaller AgNPs of size 11.6±3.5 nm [47].

Drosophila melanogaster commonly known as Fruit Fly has been used as a model organism in a great number of toxicity test due to its ease of manipulating and cultivating it. One such study on the toxicity of AgNPs on *Drosophila melanogaster* reported acute and chronic toxicity effect. By reporting acute toxicity, half of the flies tested failed to finish their development cycle when exposed to a concentration of 20 mg L^{-1}. The fertility of Drosophila was decreased significantly after long time exposure to 5 mg L^{-1} AgNPs. However, due to adaptation the fecundity of Drosophila was regenerated [48]. In other study of the Larvae of Drosophila were exposed to a concentration of silver nanoparticles at 50 and 100 μg mL^{-1}, which resulted in DNA damage and apoptosis related toxicity [49]. This is summarized in Table 2.

Table 2 Toxicity in non-mammals

Organism	**Dose concentration**	**Exposure time**	**Effect Measured**	**Ref**
Zebrafish embryos	Concentration of 0.04-0.71 nM of AgNPs size 11.6±3.5 nm	120 hours	Development abnormality is reported.	[45]
Zebrafish embryos	Concentration of 0.25,2.5,25,100 & 250μM	120 hours	100% mortality is reported, malformation of embryonic morphology.	[46]
Zebrafish embryos	Dose concentration of 0.02 nM to 0.7 nM of AgNPs size of 41.6±9.1 nm	120 hours	Development of abnormality and mortality dependent on dose.	[47]
Drosophila melanogaster	In this the concentration for Acute toxicity is 10-100 mgL^{-1} Ag and Chronic toxicity at concentration of 5mgL^{-1} of AgNPs size 3μm in solid dispersion	Ingestion exposure time of 10 days of AgNPs prepared in solid dispersion which are then added to culture medium	Acute toxicity resulted in 50% of flies did not finish their development cycle. Chronic toxicity influences the fertility by adhering it	[48]
Drosophila melanogaster	Exposed to the concentration of 50, 100 μmL of AgNPs size 10 nm.	Ingestion exposure of 24 and 48 hours	Introduction of Oxidative stress as a result of generation of ROS	[49]

3.4. Toxicity in Plants

Once the plants were exposed to AgNPs, remarkable changes were noted in the morphology of plants, commonly used parameters for assessing the phytotoxicity of AgNPs in plants are growth potential, seed germination, root growth and reduced biomass leaf area. Many plant species were been tested to study to Phytochemical nature of AgNPs. One of which is *Arabidopsis thaliana* also known as Mouse Ear Cress. In a literature *Arabidopsis thaliana* was exposed to AgNPs of size 41 nm at a concentration of 100-500 mg/L, this resulted in reduced root length, leaf expansion and photosynthesis efficiency and disruption of plasma membrane's K^+ efflux and Ca^{2+} influx, it also resulted in induced ROS accumulation [50]. In another experiment conducted *Arabidopsis thaliana* was exposed to 0.2 µgL concentration of AgNPs of size 10,20,40 and 80 nm which caused inhibition of development of root hair and repressed the transcriptional responses to microbial pathogen which results in increased bacterial colonization [51]. *Arabidopsis thalian* when exposed to concentration of 75-300 μgL^{-1} showed prolonged vegetative and shortened reproducible growth. It also decreased germination rate of offspring [52]. In a study of *Lolium multiflorum* plant, the plant was exposed to the concentration of 1-40 mgL^{-1} of AgNPs of size 6nm, root biomass decreased from 18.6±1.3 mg to 4.7±0.7 mg also the root length decreased from 7±0.6 cm to 0.7±0.08 cm [53].

When *Arabidopsis thalian* was exposed to a concentration of 67-535 μgL^{-1} this resulted in inhibition of seedling root elongation [54]. Inhibition of root growth, disruption of Thylakoid membrane structure and decrease in chlorophyll content was observed when Arabidopsis was exposed to s series of concentrations of 0.2, 0.5 and 3 mgL^{-1} [55]. This data is been summarized in Table 3.

Table 3 Toxicity in Plants

Organism	Dose concentration	Exposure time	Effect Measured	Ref
Arabidopsis thaliana	It is exposed to concentration of 100-5000 mgL^{-1} of AgNPs size 41 nm	3-7 days	Induce ROS accumulation, Root length, leaf expansion and photosynthetic efficiency is reduced. Ca^{2+} is induced in cytoplasm. Inhibits K^+ efflux and Ca^{2+} influx currents in plasma membrane.	[50]
Arabidopsis thaliana	Is exposed to concentration of 0.2 μgL^{-1} of AgNPs size 10,20,40&80	7-20 days	Root hair development is induced and transcriptional responses to microbial pathogens is repressed.	[51]
Arabidopsis thaliana	Concentration of 75-300 $\mu gL^{-1}$of AgNPs size 20 nm	21-45 days	Decreases germination rate of offspring and reproductive growth.	[52]
Lolium multiflorum	Exposed to the concentration of 1-40 mgL^{-1} of AgNPs size 6nm	7-21 days	Root and shoot length is decreased.	[53]
Arabidopsis thaliana	Exposed to concentration of 67-535 μgL^{-1} of AgNPs size 20,40 and 80 nm.	24-96 hours	Inhibit seedling root elongation and AgNPs were aggregated at plasmodesmata.	[54]
Arabidopsis thaliana	Exposed to concentration of 0.2,0.5 and 3 mgL^{-1} of AgNPs size 10 nm.	7-21 days	Disruption if the Thylakoid membrane structure is disrupted and chlorophyll content is decreased. Inhibition of root growth.	[55]

4. Synthesis of Silver nanoparticles

4.1. Chemical Synthesis

4.1.1. Chemical reduction

The Chemical reduction method, also known as conventional chemical synthesis is the most common approach of synthesizing AgNPs [56]. This is generally carried out in the presence of precursor such as $AgNO_3$. Different reducing agents such as sodium citrate, sodium borohydride, hydrogen, ethylene glycol or dimethylformamide (DMF) are used for the reduction of silver ions (Ag^+). These reducing agents reduces silver ion (Ag^+) to metallic silver (Ag) in aqueous or non-aqueous solution. This can cause agglomeration forming oligomeric clusters. Stabilizer such as

polyvinylpyrrolidone (PVP) or polyvinyl alcohol (PVA) [57]. It is important to use stabilizer as it stabilizes dispersive AgNPs during preparation, avoiding agglomeration and oligomeric clusters [58].

Recently, recovery and synthesis of AgNPs from electronic waste have become a vital issue among the scholars, as silver is used to coat medical appliances. For this, initially 10 g of electronic waste is crushed to form a homogeneous powder which then was placed in an over for 10 minutes at 500°C, by this any possible contaminations would be removed. After which Nitric acid (HNO_3) solution is added to dissolve the silver content from the homogeneous powder. Thereafter, liquid containing silver salt was centrifuged at 6000rpm. $AgNO_3$ is collected and dried for 24 hours at 60°C under a vacuum of 10 millibars. From the obtained dried $AgNO_3$, 5g was added to 100 ml of ethanol (has reductive role) which is then stirred for 30 minutes. 5% PVP is added dropwise as a stabilizer to avoid agglomeration. Finally, the system was mixed at a specific temperature and time. Usually, to investigate the effects the system is timed as 30,60,90,120 minutes and the samples were synthesized at temperatures 20,30,40 and 60 °C. The AgNPs obtained at 60°C for 60 minutes were of size 400 to 450 nm which were more significant as compared to those obtained at 30 minutes and 90 minutes [59]. This method is simple and cost-effective and produces uniformly sized nanoparticles under controlled condition. However, it does require careful control of reaction parameter to prevent agglomeration [57].

4.1.2. Sol-Gel Method

One of the methods that has gained prominence in the synthesis of silver nanoparticles is the sol-gel process, due to its versatility in producing nanoparticles in various forms such as complex, metal oxides, inorganic nanocomponents and chalcogenides [61]. This process involves preparation of gel-like solution by mixing the silver precursor (such as silver nitrate) with a metal complex (containing elements like calcium, titanium or strontium), this mixture is added to solvents such as alcohol or water [60,61]. The solution obtained as a result of mixing, undergoes a chemical reaction that often involves controlled heating which promotes nucleation and growth of AgNPs. Reaction conditions such as temperature and solvent type often plays a crucial role in determining the size and shape of AgNPs [60,63]. The AgNPs synthesized by depositing them within the thin films of metal oxides, such as TiO_2, SiO_2, ZrO_2. These films are often heated at high temperature (e.g., The heating temperature for SiO_2 is 600°C and for TiO_2 the heating temperature is 500°C). The average nanoparticle size obtained is 10 nm [63]. AgNPs can be synthesized using hydrolytic sol-gel process at 400°C,600°C and 800°C, where the average particle size is 20nm and the silver nanoparticles are crystalline in shape [62]. The sol-gel technique is also performed at low temperatures (100°C) to produce silver-doped hydroxyapatite nanorods with an average diameter of 25 nm hexagonal cross sectional [60]. High temperature (400°C to 800°C) are typically used to achieve crystalline nanoparticles, lower temperatures can also be used with specific modifications. Besides temperature and gel composition, solvent plays an important role in determining the shape and size of the AgNPs [64]. Generally, organic solvents are preferred, due to their oxygen supplying characteristic for metal, which helps to control particle uniformity and size distribution [64]. The process enables customization of nanoparticles as it allows for a wide range of precursors and additives [67]. Additionally, the sol-gel process can synthesize AgNPs in hot aqueous environment under high pressure and also at low temperatures [61]. However, producing thick nanoparticle-doped films without defects like cracks is difficult [65]. Often the sol-gel process is associated with costly precursors and reproducibility issues, particularly in large scale production [66]. The film quality is highly dependent on environmental conditions such as humidity and temperature [65].

4.1.3. Chemical Vapor Deposition Technique

The Chemical Vapor Deposition is one of the techniques to synthesize nanoparticles. It allows the synthesis of nanoparticles on the surface of 3D substrate [68]. The deposition process takes place in three steps, initially volatile precursor is introduced to the reactor chamber by a carrier gas (like H_2, Ar or N_2). This process requires volatile silver containing precursors that decomposes thermally to produce silver. Commonly, silver nitrate, silver acetate, or silver organometallic compounds like silver (I) penta-fluoro-propionate is used as a precursor in CVD methods. After this step, the precursor vapors are adsorbed on the substrate surface and intermediate products are formed, followed by formation of layers. Lastly, decomposition of products occurs on the heated substrate, which is followed by nucleation and growth of layer [68]. The factors that affect the quality of the nanoparticle produced are the methods used for precursor delivery, pressure in reactor chamber, chemical properties of carrier gas, rate of deposition, substrate surface temperature and duration of deposition [68,69]. The type of precursor used seems to be the most significant factor in this method. The CVD precursor should have the following characteristics: it should be appropriately volatile to achieve highest concentration of the precursor in vapor form, the precursor should be thermally stable to avoid premature degradation during transportation of vapors by carrier gas (like H_2, Ar or N_2), it should be able to thermally decompose on the surface of the substrate leading to the deposition of desired materials, it should be inexpensive and simple to synthesize and have very low toxicity [70].

Usually, two types of compounds are used as precursors for CVD, namely silver nitrate ($AgNO_3$) and silver (1) complex such as perfluorinated carboxylates. $AgNO_3$ is widely used as precursor in techniques such as Flame Assisted CVD and Atmospheric Pressure CVD [70-72]. The Flame Assisted CVD (FACVD) enables the deposition of metallic silver layer of thickness 60-90 nm [70], when the substrate surface is heated this technique facilitates the deposition of silver metallic layer having thickness of 60-250 nm [71]. The FACVD method allows the formation of nanocomposite coating of Ag metal oxides (e.g., TiO_2 and SiO_2) in deposition process on large substrate area [70,73]. Atmospheric Pressure CVD is used to produce nanocomposite coating on textile surface which is composed of silicon with incorporated AgNPs [72]. Silver(I)acetate and silver (I) trifluoroacetate cannot be used as a precursor in conventional CVD methods such as Metal Organic CVD (MOCVD) and Plasma-Enhanced CVD (PECVD) due to their weak volatility, however continuous films of pure silver were deposited by these compounds with the help of Laser-Inducted CVD (LICVD) [68,74]. Silver(I)penta-fluoro-propionate is considered as a good organic Ag CVD precursor since layers of AgNPs of diameter 20-60 nm were obtained on the surface of Si (111) substrate after 5 minutes of CVD process at 563K [75]. The CVD method enables scalability, purity, uniformity and control over the size and shape of nanoparticles. However, the process is costly [76].

4.1.4. Reverse Micelle Process

Reverse micelle process is a specific type of microemulsion technique, which is prominently used for the synthesis of silver nanoparticles. In this method surfactants such as sucrose fatty acid in organic solvent such as hexane or toluene produces reverse micelles [79]. Surfactants, reducing agents and organic solvents are used to enhance the stability, size and overall morphology of AgNPs [77]. There is a water pool inside the microemulsion which is a water phase that consist of reactants. The water phase serves as nanoreactors where silver ions (Ag^+) are reduced to silver atoms (Ag) which then forms AgNPs. Hydrazine [83], ascorbic acid [77], sodium borohydride ($NaBH_4$) [80] and glucose [81] are some of the regularly used reducing agents in this method. It was observed that hydrazine hydrate ($N_2H_4.H_2O$) can yield much smaller AgNPs with higher dispersion as compared to sodium borohydride ($NaBH_4$) which is a strong reducing agent [82]. So, the size distribution of AgNPs in controlled by the strength of reducing agent [77]. The size and distribution of AgNPs obtained also depends on the type of solvent and reducing agents used in the synthesis process [77]. Silver nanoparticles were synthesized in sodium dioctyl sulfosuccinate (AOT) reverse micelle in this ascorbic acid was used as a reducing agent. AgNPs were obtained with an average size of 6nm [77]. AOT microemulsion is generally used for for the synthesis of AgNPs [77]. AgNPs were prepared by using octadecyl amine (ODA) as solvent and sodium borohydride (strong reducing agent) as a reductant, this resulted in the AgNPs of average size 3.38 nm [78]. This method enables precise control over nanoparticle size and distribution as it provides many choices on the type of surfactants and solvent used [77]. It does not need any specialized equipment and extreme temperature or pressure conditions [79]. Due to the surfactant stabilization agglomeration tendency is low, it is scalable and could be used in large scale synthesis [79]. The AgNPs produced with AOT microemulsion may have poor surface plasmon characteristic [77].

4.1.5. Wet Chemical Synthesis

Wet chemical synthesis is one of the most commonly used methods for synthesizing silver nanoparticles; most for synthesizing AgNPs still rely on wet chemical reduction using a chemical reducing agent. In the conventional wet chemical synthesis of AgNPs strong reducing agents such as glucose, sodium borohydride, hydrazine and dimethyl formamide (DMF) is used [85]. PVP coated silver nanoparticles were synthesized by reduction with glucose in the presence of PVP at 90°C for up to 7000 minutes under ambient light this resulted in AgNPs of size 200 nm [84]. The wet chemical method can successfully offer narrow size distribution of AgNPs. Besides all these advantages reductants used in this method have reported to posses' toxicity which might have adverse effect on human body. Hydrazine derivative compounds such as hydrazine hydrate is known to be carcinogenic and can cause irreparable damage to the vital organs such as lungs [86,87]. It has been ranked as a potential carcinogen that has threshold limit as low as 10 ppb by Environmental Protection Agency [86,88]. The nanoparticles obtained from hydrazine may contain remanent of hydrazine, making it unsuitable for biomedical uses. DMF has also reported to cause damage to liver [84]. $NaBH_4$ which is also a strong reducing agent have adverse effect on lungs and may cause serious lung related problems [90]. Another wet chemical method used for the preparation of AgNPs is polyol method. In this method ethylene glycol is used as the solvent and reducing agent and PVP as surface stabilizer, this method is performed at 120°C-160°Cin the presence of salt mediator [91-93]. The polyol process is widely accepted method for the synthesis of AgNPs as it is non-hazardous and utilizes natural compounds to synthesize AgNPs at room temperature, in this method only one reagent is used as both reducing and capping agent [92]. However, there are many conditions such as the temperature (above 120°C) and concentration of precursor (0.1M or less), PVP to AgNO3 ratio plays an important role in size, morphology and yield of AgNPs, which limits their scalability [94].

4.2. Green synthesis of silver nanoparticles

The concept of green synthesis of silver nanoparticles is a ecofriendly approach to AgNPs production. The method utilized in the green synthesis of AgNPs are non-toxic, biological and non-hazardous which ensures minimum environmental toxicity and hazards related to human health [57,85,97,99]. The green synthesis aligns closely with chemical synthesis in terms of its principle, but avoids the use of hazardous toxic chemicals making it safer option [57]. The primary motivation behind green synthesis is to minimize the adverse effects of traditional chemical synthesis that often involves use of harmful chemical reducing agents such as $NaBH_4$, N,N-DMF and hydrazine derivatives . The green synthesis synthesizes AgNPs by reducing silver ions (Ag^+) to metallic silver (Ag) by using biological species or bio-based compounds [98]. This process is also known as biological synthesis as the bio-based compounds are often derived from microorganisms or plant extracts, which acts as reducing agent, replacing conventionally used toxic reducing agents [57]. While the biological synthesis plays a main role in green synthesis, it is not completely restricted to biological methods, there are several physical methods such as laser irradiation [98], microwave irradiation [100,101], ionizing radiation [98,103] and pulse radiolysis [98] have also been employed to synthesize AgNPs without the use of chemical reducing agents like hydrazine and $NaBH_4$ [98]. However, these methods consume a huge amount of energy, which pose a challenge in terms of their cost effectivity and sustainability. Green synthesis presents itself as an alternative to conventional chemical and physical methods [96]. This is because while chemical synthesis often involves the use of toxic solvent and reducing agents and physical methods require sustainable energy input, the green synthesis strikes a balance by synthesizing AgNPs using natural reducing agents [57,102] and energy efficient techniques [57,85], thus making it ideal for mass production [95] and more economically feasible [57,95].

4.2.1. Synthesis of silver nanoparticles using Plant extracts.

Synthesis of silver nanoparticles using plant extract is an extremely cost-effective method of synthesis and hence can be a valuable alternative for large scale production of AgNPs [122]. In this, the plant extract is used as reducing agent and stabilizing agent for the synthesis of AgNPs which rules out toxicity caused by conventional chemicals used to synthesis AgNPs [123]. Due to their non-pathogenic characteristics and biocompatibility, AgNPs synthesized by plant extract are ideal for biomedical applications [104]. The plant extract consists of phenolic compounds such as alkaloids and flavonoids which provide the reagent with unique reducing and capping properties, these compounds are also soluble in water [105,106]. Plants naturally detoxify the ground water by removing impurities such as heavy metals [107]. Redox potential is one of the most significant factors in the detoxifying process [108], this can be used to utilize plants for reduction of metal cations (Ag^+) and synthesize AgNPs. This process may be considered as in vivo and in vitro synthesis process [119]. The in vivo synthesis can be defined as the synthesis of AgNPs inside the plant on the other hand in vitro synthesis can be defined as the synthesis of AgNPs by the plant extract

Torresday et al. [109] synthesized AgNPs by using in vivo synthesis process by using *Alfalfa Sprouts*. The obtained nanoparticles were of diameter 2-20 nm and were spherical in shape. This study reported that Ag was absorbed from the agar medium by the root hair of the plant and was transferred to the shoot. This study also reported the synthesis and nucleation of AgNPs within the plant tissue. In several studies it was reported that AgNPs could also be synthesized by using Brassica Juncea, in which AgNPs were reported to be in the plant biomass [111,112]. Flavones, terpenoids, polyphenols and catechins are phytochemical in plants that facilitates AgNPs synthesis [110,113]. Synthesizing AgNPs in plant phytochemicals can be advantageous as the water solubility of the phytochemicals simplify the process [114]. Plant parts such as roots, fruits, seeds and other aerial parts can be used for extraction of phytochemicals, which contain polyphenols that are strong antioxidants and have redox potential [114,119,121]. Makarov et al. [115], proposed a hypothesis which stated that when flavonoids are used as reducing agents it undergoes tautomerization that releases hydrogen, transforming flavonoids into keto-form. This leads to the reduction of silver ion (Ag^+) to metallic silver (Ag). Functional groups such as hydroxy (-OH) groups are known to reduce Ag^+ [114]. The size and morphology of AgNPs can be altered by selecting the plant source [120,116].

In recent times, using plant extract as reducing, stabilizing and capping agent for the synthesis of AgNPs has grown torrentially. AgNPs was synthesized by Johnson et al. [117] by using *Odontosoria Chinensis* extract, which resulted in the formation of spherical AgNPs of diameter 22.3-48.2 nm. Sivakumar et al. [118] used *Parthenium hysterophorus* extract to to synthesize spherical AgNPs having average size of 10.3±1.7 nm. In this the hydroxy group was responsible for the reduction of Ag^+ and formation of AgNPs. The obtained AgNPs portrayed antibacterial activity and anti-cancer activity. This process is highly dependent on the plant extract composition and parameters such as pH, temperature and concentration ratio. The process is simple, cost-effective, environmentally friendly and had low reaction time. However, the mechanism for affecting the synthesis of process is unknown [57,119]. The examples of different plant extracts to synthesize AgNPs is given in Table 4.

Table 4 Examples of different plant extracts used to synthesize silver nanoparticles

Reducing agent	Solvent used	Morphology and dimension	Functional groups responsible for reducing Ag^+	Reaction time and temperature	Applications of synthesized AgNPs	Ref
Elephantopus Scaber	Distilled water	Spherical of size 37.86 nm	O-H and C=0(plant constituent) and C=C (aromatic ring)	The reaction is carried out at 40°C for 30 minutes	Anticancer and antimicrobial	[101]
Origanum vulgare extract	Deionised water	Spherical of size 2-25 nm	Phytomolecues	The reaction is carried out at 90°C for 2 hours	Antimicrobial	[121]
Camelia Sinensis (powder)	Deionised water	Spherical of size 34.68±4.95 nm	Polyphenols	The reaction is carried out at 60°C for 15 minutes	Antibacterial	[124]
Blackberry fruit extract	Deionized water	Spherical of size 12-50 nm	C=O group and O-H group	The reaction is carried out at 25°C for 48 hours	Antioxidant	[125]
Lignin	Milli-Q water	Spherical of size 7.3±2.2 nm	Phenolic hydroxy group	The reaction is carried out at 85°C for 30 minutes	Antimicrobial and Green catalysis in hydrogen fuel cells	[127]
Coffea Arabica extract	Deionized water	Spherical of size 20-30 nm	Phenolic groups	The reaction is carried out at room temperature for 2 hours	Antibacterial	[126]
Banana peel extract	Distilled water	Spherical of size 23.7 nm	Amide, carboxylic and hydroxy group	The reaction is carried out at 100°C for 76 hours	Antibacterial	[128]
Ginger	Deionized water	Spherical of size 10 to 18 nm	Flavonoids	The reaction is carried out at room temperature for 2hours	Antimicrobial and antioxidant	[130]
Turmeric extract	Milli-Q water	Spherical of size 18±0.5 nm	Hydroxy groups which are present in the Curcumin powder	The reaction is carried out at room temperature for 24 hours	Antimicrobial	[129]

4.2.2. Microbial Mediated synthesis of Silver Nanoparticles

The microbial synthesis is a branch of green synthesis under biological synthesis, in this synthesis of AgNPs the nanoparticles are synthesized with the help of Microbes. The microbes such as bacteria, fungi, algae and virus are employed to synthesis AgNPs.

Bacteriogenic Synthesis of AgNPs

Bacteria is used for the synthesis of silver nanoparticles. In this method the reactive response of bacteria to silver results in the synthesis of AgNPs. This method uses Ag resistant strains for the synthesis of AgNPs [57]. A study reported the synthesis of highly stable AgNPs of size 40 nm by reduction of silver ions (Ag^+) to metallic silver (Ag) by using a culture supernatant of *Bacillus licheniformis* which is an antibacterial strain [131]. Another study suggested the synthesis of silver nanocrystals of size 50 nm by using *Bacillus Licheniformis* [132]. The antibacterial strains accumulate silver atoms on the cell wall [57]. The AgNPs synthesized by bacteria-mediated synthesis is either by extracellular process (using cell culture supernatant) [135] or intracellular process (biomass) [136]. Generally, the AgNPs obtained by bacteriogenic synthesis are spherical of size ranging from 5nm to 200nm. In a study, Saifuddin et al. [133] synthesized AgNPs of size 5nm to 50nm with the help of culture supernatant of *B. subtilis* combines with microwave radiation. The microwave radiation increased the rate of reaction and reduced aggregation.

The result of FTIR or Fourier Transform Infrared spectroscopy addressed that the functional groups such as carboxylic acid and hydroxylic group, coupled with primary amides and secondary amides which are present in the biomass and cell culture supernatant are responsible for the synthesis and stabilization of AgNPs [138].

By using cell culture supernatant, synthesis rate of some bacterial strains such as *Enterobacter cloacae*, *E. coli* and *K. Pneumonia* [137]. The AgNPs obtained were formed within 5 minutes of silver ions (Ag^+) contacting the cell filtrate, it is reported that the nitro reductant enzymes are responsible for the reduction of silver ion to metallic silver which is accumulated on the cell wall [57,137]. A study reported that visible-light emission can significantly enhance the synthesis of AgNPs of size 1-6 nm by using culture supernatant of *K. pneumonia* [134]. The obtained AgNPs can be used in biosensor, drug delivery and as antimicrobial agent [136-138]. However, the process is slow as compared to the other biological methods used to synthesize silver nanoparticles [57,135]. The examples of different bacterial strains used in the synthesis of AgNPs are summarized in Table 5.

Algae-Mediated synthesis of Silver Nanoparticles.

The synthesis of silver nanoparticles by marine based organisms like algae and microalgae is emerging as a promising method to prepare AgNPs due to their non-toxic and environmentally friendly nature. Marine algae such as *Chaetoceros calcitrans, Chlorella salina and Tetraselmis gracilis* can reduce silver ions to metallic silver, thereby synthesizing AgNPs [137]. Single cell microalgae are used extensively for the synthesis of AgNPs as it enables the formation of homogeneous suspension that can be used directly in the synthesis of AgNP [143]. The microalgal synthesis of AgNPs is generally carried out by mixing microalgal biomass with the silver solution of $AgNO_3$ which results in the synthesis of AgNPs [152]. The synthesis process in cell wall deficient microalgae is intracellular or extracellular when the synthesis occurs outside the cell, which may be due to the presence of biomolecules [143,146]. The AgNPs obtained are spherical [144,148,149] with an average size ranging from 4.3 nm [144] to 35 nm [147]. The AgNPs are used as antioxidants and antibacterial [145,150]. This method synthesizes small size AgNPs with uniform morphology using non-hazardous reagents. However, the production rate is significantly low. [150,151]. The examples of the Algae used in the synthesis of AgNPs is summarized in table 5.

Fungi-Mediated synthesis of Silver Nanoparticles

Fungi species due to their bioaccumulation capacity, intercellular uptake and high binding capacity have demonstrated remarkable potential for synthesis of AgNPs [157]. Fungi synthesis is usually followed by enzymatic process, which affects the formation of AgNPs [153]. Similar to bacteria, in fungi amide, carbonyl and hydroxy groups are responsible for the stabilization and synthesis of AgNPs [155,156]. By using *Fusarium oxysporum* AgNPs of size 5-50 nm were synthesized by extracellular process. This study reported no signs of flocculation of AgNPs even a month after the reaction was carried out [153]. The stabilization of AgNPs by protein ensure long term stability [154]. The obtained AgNPs were generally spherical with few triangular in shape [154]. A study by Naqvi et al. [158] demonstrated that AgNPs prepared by using *A. flavus* significantly enhanced the biocidal effectiveness against drug resistant bacterial strains. However, the process is long as compared to other green synthesis methods [156,157]. The examples of the Fungi species which are used in the green synthesis of AgNPs is summarized in table 5.

Virus Mediated Synthesis

Viruses, generally the Plant based viruses are used as biotemplates for the synthesis of silver nanoparticles. This method is rarely used for the synthesis of AgNPs as compared to other chemical and green methods [161,162-164]. Plant based viruses such as *Cowpea Mosaic Virus, Red Clover Necrotic Mosaic Virus and Tobacco Mosaic Virus (TMV)*are used to synthesis AgNPs [165]. Among, these viruses the TMV is the most studied and applied widely, considering the fact that very limited studies are carried out on virus-mediated green synthesis as compared to other methods. In a study, Dujardin et al. [162] used TMV as biotemplate to synthesis the AgNPs of size 2-4 nm. The AgNPs obtained were coated on the inner surface of the cylindrical TMV channel. The synthesis process is mediated by amino acid [164]. The AgNPs obtained by viral template is used in target imaging and to enhance the drug delivery system [165]. Due to the rod shape of some plant viruses like TMV, the AgNPs obtained from plant-based virus biotemplate is used to synthesize 1D AgNPs [163,164]. The 1D nanoparticles synthesized by using TMV biotemplate is advantageous as green reagents can act as both reducing and capping agents, which may significantly reduce the overall material cost [167]. The synthesis is carried out at relatively low temperature or even at room temperature [168] which may reduce the energy consumption making it a feasible option for large scale production of 1D silver nanoparticles. Beside all these advantages the biotemplates often lack strong metal-binding sites [163] and the preparation of viral biotemplates is often time consuming [166]. As the information on the synthesis of AgNPs through viral biotemplate is limited, further research is required to explore their full potential.

Tables 5 Examples of the microbes used in the green synthesis of AgNPs

Microbe	Solvent used	Morphology and dimension	Functional groups responsible for reducing Ag^+	Reaction time and temperature	Applications of synthesized AgNPs	Ref
			Bacteria			
Bacillus krulwichiae	Distilled water	Spherical of size 25.88±10.49 nm	Ester, carboxyl, hydroxy, aldehyde group	The reaction is carried out at room temperature for 24 hours	Antimicrobial and antibiofilm	[139]
Enterobacter cloacae	Deionised water	Spherical of size 12-30 nm	Primary and secondary amides	The reaction is carried out at room temperature for 72 hours	Antibacterial	[140]
Bacillus cellulosilyticus	Distilled water	Spherical of size 23.99±8.43 nm	amide, hydroxy and carbonyl	The reaction is carried out at room temperature for 24 hours	Antibiofilm and antimicrobial	[139]
Staphylococcus aureus	Milli-Q water	Varid morphology of size 160-180 nm	Extracellular enzymes	The reaction is carried out for 5 minutes	Antimicrobial	[141]
Cyanobacteria	Milli-Q water	Spherical of size 60-80 nm	N.A.	The reaction is carried out at 30-60°C for 1 hours	Decolourization of Dye	[142]
			Algae			
Chlamydomonas reinhardtii	Deionised water	Spherical of size 5.6±2.4 nm	Carbohydrate and amine	The reaction is carried out at room temperature for 192 hours	Antimicrobial and anticancer. It is also used in biosensing	[144]
Chlorococcum humicola	Sterile Distilled water	Spherical of size 2-16 nm	C-N bond which corresponds to aromatic group	The reaction is carried out at room temperature for 48 hrs	Antibacterial activity	[148]
Spirulina	Sterilized Double distilled water	Spherical of size 5-50 nm	O-H, COOH, primary and secondary amine	The reaction is carried out at room temperature for 3,6,9 and 12 hrs	Antibacterial activity	[149]
			Fungi			
Penicillium aculeatum	Milli-Q water	Spherical of size 4-55nm	N-H, C=O and -C-N	The reaction is carried out at room temperature for 4 weeks	Antibacterial	[159]
Rhizopus stolonifer	Deionized water	Spherical of size 2.86±0.3nm	Carbonyl group	The reaction is carried out at 40°C for 48 hours	Antimicrobial and used as a green catalyst in organic pollutant degradation	[160]
			Virus			
Tobacco Mosaic Virus (TMV)	N.A.	Spherical of size 2nm	Hydroxy, thiol and carboxyl groups	The reaction is carried out at 50°C for 1 hour	Antibacterial and catalytic activity. Used to synthesize 1-D AgNPs	[161, 164]

5. Conclusion

The unique properties of AgNPs are ubiquitously used in growing number of applications such as in renewable energy, electronics, waste water treatment, medicine, clinical equipment and biosensors. However, the use of AgNPs in such a wide spectrum of applications make it susceptible to be released into the environment, which may adversely affect human health and rise environmental concerns. The article focuses on the properties, the potential risk to organisms and bottom-up methods to synthesis silver nanoparticles. Besides the efforts to understand the hazards and environmental concerns related to the AgNPs, the information available is still limited with results that are uncertain or even controversial, hence explanatory research is needed. Once released in the environment AgNPs due to their large surface are and high surface energy could undergo oxidation, aggregation, chlorination and sulfurization that may cause detrimental effects on the environment. Hence, environmental transformation related to AgNPs toxicity should be studied and investigated. The lack of study related to the mechanism of toxicity of AgNPs, leads to poor understanding of the cause of toxicity i.e., if the toxicity is caused by silver nanoparticles or silver ions. Therefore, efforts should be made to discern this question so that the toxicity can be controlled or reduced. The conventional synthesis methods such as chemical synthesis often involves the use of hazardous chemicals such as Hydrazine, DMF and $NaBH_4$ that may cause lethal effects on humans. As an alternative to chemical method green synthesis is used as the reagents used in the green synthesis are natural compounds such as polyphenols, flavonoids, alkaloids and functional groups such as hydroxy, carboxylic and amide groups which acts as strong reducing and capping agents. However, these methods may be time consuming. Green synthesis methods such as plant virus biotemplate mediated synthesis are less studied and better understanding is needed as viral template methods such as TMV- biotemplate synthesis is can used to synthesis 1D silver nanostructures.

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