Bioproduction of Silver Nanoparticles and Its Potential Applications in Agriculture

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2.1 Introduction

Nanotechnology is a new technology playing a vital role in different fields of science like medicine, engineering, pharmaceuticals, agriculture, and food industry (Gul et al. 2014). Agriculture is the backbone of most developing countries and it provides food for humans, directly and indirectly. The world's population will grow to an estimated 8 billion people by 2025 and 9 billion by 2050, and it is widely recognized that global agricultural productivity must increase to feed a rapidly growing world population (Jo et al. 2009). Nanotechnology provides new agrochemical agents and new delivery mechanisms to improve crop productivity, and it promises to reduce pesticide use. Nanotechnology can boost agricultural production, and its applications include (1) nanoformulations of agrochemicals for applying pesticides and fertilizers for crop improvement; (2) the application of nanosensors/nanobiosensors in crop protection for the identification of diseases and residues of agrochemicals; (3) nanodevices for the genetic manipulation of plants; (4) plant disease diagnostics; (5) animal health, animal breeding, and poultry production; and (6) postharvest management.

Precision farming techniques could be used to further improve crop yields but not damage soil and water, reduce nitrogen loss due to leaching and emissions, as well as enhance nutrients long-term incorporation by soil microorganisms. Nanotechnology uses include nanoparticle-mediated gene or DNA transfer in plants for the development of insect-resistant varieties, food processing and storage, nano feed additives, and increased product shelf life. Nanotechnology promises to accelerate the development of biomass-to-fuels production technologies. Experts feel that the potential benefits of nanotechnology for agriculture, food, fisheries, and aquaculture need to be balanced against concerns for the soil, water, and

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environment and the occupational health of workers. Raising awareness of nanotechnology in the agri-food sector, including feed and food ingredients, intelligent packaging, and quick detection systems, is one of the keys to influencing consumer acceptance. On the basis of only a handful of toxicological studies, concerns have arisen regarding the safety of nanomaterials, and researchers and companies will need to prove that these nanotechnologies do not have more of a negative impact on the environment (Sekhon 2014).

Nanotechnology is one of the emerging areas of research in the field of science. Nanoparticles show novel properties such as morphology of particles, size, and distribution (Kaviya et al. 2011). Metal nanoparticles have precise surface area due to its distinctive physicochemical characteristics that enhance antimicrobial, electronic, magnetic, catalytic, and optical properties (Catauro et al. 2005). Nanotechnology and nanobiotechnology are the emerging fields which have tremendous potentials to renovate agriculture and allied fields. Nanotechnology in the field of agriculture focuses currently on target farming that involves the use of nanoparticles with unique properties to boost crop and livestock productivity (Panpatte et al. 2016). Authors investigate in vitro growth of *Zea mays L.* using silver nanoparticles.

2.2 Problem

Streptocycline is an aminoglycoside antibiotic which has been extensively utilized in the treatment of bacterial diseases of humans and animals and also used to prevent bacterial pathogen for plant (Sundin and Bender 1993). Resistance was developed by microbes against these synthetic molecules that posed severe issue on the sustainability of them to aid in protection to humans, plants, and animals.

Rice is one of the most important cereal crops of the world providing more than half a million their daily nutrition intake. Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most severe diseases of rice. The disease increases with plant growth, peaking in the flowering stage, while symptoms are noted as early as at the tillering stage (Tagami and Mizukami 1962). It is also one of the oldest known diseases, first noted by farmers in Kyushu Province, Japan, around 1884 (Swing et al. 1990).

Development of resistance in *Xanthomonas oryzae* pv. *oryzae* against streptocycline has become an alarming situation for the crop pathologist to address this issue (Shetty and Rangaswami (1971) and Catauro et al. (2005)).

Also for resistance in several *Culicidae* species of mosquitoes worldwide, current control tools mainly rely on the employment of (i) synthetic or microbial pesticides, (ii) insecticide-treated bed nets, (iii) adult repellents, (iv) biological control agents against mosquito young instars (mainly fishes, amphibians, and copepods), (v) sterile insect technique (SIT), (vi) “boosted SIT,” (vii) symbiont-based methods, and (viii) transgenic mosquitoes. Currently, none of these single strategies are fully successful. Novel eco-friendly strategies to manage mosquito vectors are urgently needed (Benelli 2017).

2.3 Metal Nanoparticles

Metal nanoparticles have precise surface area due to their distinctive physicochemical characteristics that enhance antimicrobial, electronic, magnetic, catalytic, and optical properties (Catauro et al. 2005).

2.3.1 Silver Nanoparticles

Silver nanoparticle is a nontoxic, safe inorganic antimicrobial agent and is capable of killing about 650 types of microorganisms (Jo et al. 2009). Silver is renowned for possessing an inhibitory effect against various bacterial strains and microorganisms usually present in industrial and medical processes (Jiang et al. 2004). Silver nanoparticle is an alternative to chemically manufactured pesticides without toxicity problems (Jo et al. 2009). It is found that small concentration of silver nanoparticles increases the in vitro growth of *Zea mays L.* 40 ppm concentration of silver nanoparticles possessed significant increase in the growth of shoot and root of *Zea mays L.* Similarly 60 ppm concentration of silver nanoparticles possessed increasing germination in both dry and fresh weight of seeds when compared to other concentrations of nanoparticles (Sriram and Pandidurai 2017).

2.4 Source of Silver Nanoparticles

It is crucial to perform experiments with variable sets of parameters, including pH, temperature, bioagents, substrate concentrations, and reaction time (Quester et al. 2016). There is a growing interest in the development of alternative strategies in plant disease management to reduce dependency on synthetic chemicals. Synthesis of metallic nanoparticles has been carried out by three methods: [1] chemical, [2] physical, and [3] biological. Several biological methods for both intracellular and extracellular nanoparticle synthesis have been reported by means of [a] plants, [b] fungi, and [c] bacteria (Mukherjee et al. 2001). Biological method offers enhanced platform for nanoparticle synthesis because they are free from toxic chemicals and offer natural capping agents. Furthermore, the use of plant extracts diminishes the cost of microorganism isolation and maintenance (Singhal et al. 2011).

2.4.1 Biogenic Synthesis

Biogenic synthesis of silver nanoparticles (AgNPs) has attracted worldwide attention as it is cheap and nontoxic (Mankad et al. 2018). Different researchers have synthesized silver nanoparticles from different sources (bacteria, fungi, yeasts, algae, and plants).

2.4.1.1 Plant

Synthesis of nanoparticles using plant extracts is the mainly opted procedure for eco-friendly and green synthesis of nanoparticles that has benefits in that the plants are usually dispersed, easily accessible, and much safer to handle and act as a source of various metabolites. Also the plant-mediated fabrication of nanoparticles is cheap and single-step and does not require high pressure, energy, temperature, or the use of highly toxic chemicals (Ankamwar et al. 2005).

From Gloriosa superba seeds, seed powder was extracted with methanol. Then methanol seed extract was mixed with 1 M silver nitrate solution and incubated for a period of 15 h at room temperature. The change in color from yellowish brown to dark brown indicates the formation of silver nanoparticles (Saradhadevi 2017).

Sunlight-mediated silver nanoparticle was synthesized from *Azadirachta indica* A. *Juss* (Neem) leaf extract and sunlight. Leaf extract provides both reducing and capping agent, while sunlight served as catalyst for the synthesis process. The plant extract concentration used also plays important role in the conversion of $Ag+$ to Ag^0 (Mankad et al. 2018). In the latest years, a growing number of plant-borne compounds have been proposed for efficient and rapid extracellular synthesis of metal nanoparticles effective against mosquitoes at very low doses (i.e., 1–30 ppm) (Benelli 2017).

Synthesis of silver nanoparticles by plants such as *Ziziphora tenuior* (Sadeghi and Gholamhoseinpoor 2015), *Solanum trilobatum* (Logeswari et al. 2013), *Erythrina indica* (Sre et al. 2015), and *Spirogyra varians* (Salari et al. 2016), carnivorous plants such as *Drosera* sp. and *D. muscipula* (Banasiuk et al. 2017), leaf extract of *Acalypha indica* with high antibacterial activities (Krishnaraj et al. 2010), and *Sesuvium portulacastrum* reported with nanoparticle size ranging from 5 to 20 nm (Nabikhan et al. 2010) is crammed in literature as a substitute to the conventional methods. From the studies carried out by Kumar et al. (2015) for silver nanoparticles synthesized from neem leaf extract, the maximum peak was found at 435 nm.

2.4.1.2 Fungi

The silver has much higher antifungal activity than that of other metals. Eukaryotic microbes, such as fungi, are considered as an exceptional choice for synthesizing NPs because they produce a great amount of secreted enzymes and proteins responsible for the bio-reduction (Gade et al. 2013; Cilerdzˇ ic´ et al. 2014). In addition, stabilizing and capping properties of the agents produced by fungi offer important advantages in “green synthesis” of NPs, including AgNP (Gade et al. 2013; Tran et al. 2013; Quester et al. 2016).

Ganoderma applanatum is the most effective antibacterial and antifungal basidiomycete for AgNP synthesis among the seven investigated basidiomycetes (Jogaiah et al. 2018) as “green synthesis” of AgNPs, using extract as reducing and capping agent.

Several fungal species like *Aspergillus niger*, *Fusarium oxysporum*, *Neurospora crassa*, and *Penicillium* spp. have been reportedly used for the biosynthesis of

AgNPs; however, the antimicrobial activity of the synthesized AgNPs, especially in crop protection against pathogens, remains to be determined (Quester et al. 2016).

2.4.1.3 Bacteria

Prokaryotic bacteria have received the most attention in this area. One advantage of using bacteria for synthesis of nanoparticles is ease of handling and their genetic manipulation without much difficulty. Extracellular synthesis of nanoparticles using cell filtrate could be beneficial over intracellular synthesis. For example, cell-free culture supernatants (extract) of five psychrophilic bacteria *Pseudomonas antarctica*, *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis*, and *Arthrobacter gangotriensis* and three mesophilic bacteria *Bacillus indicus*, *E. coli*, and *Bacillus cecembensis* have been used to synthesize silver nanoparticles (Abo-State and Partila 2015). The extracellular biosynthesis of AgNPs by the four bacterial species, *Ochrobactrum* sp. (MAM-C9), *Achromobacter xylosoxidans* (MAM-29), *Pseudomonas aeruginosa* (MAM-42), and *Bacillus cereus* (MAM-I.11), was confirmed (Abo-State and Partila 2015).

2.5 Mechanism of Silver Nanoparticle Reduction

The reduction of Ag^{+} to Ag^{0} nanoparticles using neem leaf extract was due to the presence of phenolics, flavonoids, terpenoids (occurs through oxidation of aldehyde groups to carboxylic acids), alkaloids, lipids, proteins, and carbohydrates in the leaf extract (Mittal et al. 2013). Thus, leaf extract plays dual role (i) reducing agent and (ii) capping agent for stability of nanoparticles, it overcome post modification like aggregation of them (Kumar et al. 2015) and also responsible for the reduction of metals are enzymes like reductases in various microbes (Abo-State and Partila 2015). Function group plays a role in reduction of silver metal to silver nanoparticles, like OH, C=O, and others (Abo-State and Partila 2018).

2.6 Characterizations of Silver Nanoparticles

2.6.1 UV-Visible Spectrophotometry

The green synthesized AgNPs were characterized using change in color due to surface plasmon resonance and by UV-visible spectrophotometry. The peak ranges around 420 nm.

As far as different shapes in the UV-visible spectra above 600 nm are concerned, it represents different extents of aggregation (Desai et al. 2012); the excitation of surface plasmon results in vibration of bands corresponding to the absorption by colloidal silver nanoparticles in the region (400–450 nm). The intensity of color increases in proportion to time due to reduction of Ag+ (Das et al. 2017). The change in color is due to the excitation of surface plasmon resonance (SPR) in solution (Mulvaney 1996).

2.6.2 Dynamic Light Scattering

It is a quantitative analytical technique which measures the velocity of dispersed particle by measuring fluctuations of light scattering intensity due to Brownian movement of particles. The size of nanoparticles is influenced by silver nanoparticle concentration and incubation time (Abo-State and Partila 2015).

2.6.3 Zeta Potential (mV)

Zeta potential was carried out to study the stability of silver nanoparticles is it is very important for various applications. The values that fall in the negative side showed the effectiveness of the capping materials in stabilizing the nanoparticles by providing intensive negative charges that keep all the particles away from each other. The zeta potential for green synthesized silver nanoparticles ranges between 19.6 and 22.8 mV. The strong negative values for the AgNPs clearly suggest stability of nanoparticles at room temperature and therefore could be utilized effectively for its downstream applications like antimicrobial. Criteria of stability of NPs are measured when the values of zeta potential ranged from higher than +30 mV to −30 mV (Ciftci et al. 2013).

2.6.4 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR is a highly informative technique for revealing the biomolecules present in the sample which had played an important role in the formation and stabilization of nanoparticles. The FTIR spectrum of green synthesized silver nanoparticles by the neem leaf extract shows spectra at 3415, 1578, and 1384. A broad peak at 3415 cm^{-1} corresponds to stretching vibrations of hydroxyl (AOH) group, while a peak at 1384 cm^{-1} is attributed due to OAH bending vibrations of polyols present in leaf extract like flavanoids (Senthilkumar and Sivakumar 2014). Flavanoids and terpenoids absorbed on metal nanoparticle surfaces may be due to interaction of carbonyl groups or p-electrons in absence of optimum ligating agents (Gericke and Pinches 2006).

The spectral bands (1450–1600 cm^{-1}) show presence of proteins which are accountable for the reduction of metal ions or affinity for metal nanoparticles. From the study of the FTIR spectrum, carboxyl group was found adsorbed on the particle surface; hence, this confirms the presence of biomolecules like terpenoids and flavonoids which act as a capping agent for the synthesized nanoparticles. Capping of nanoparticles by protein stabilizes silver nanoparticles and prevents agglomeration in the medium (Lalitha et al. 2013). Over the years, stability of nanoparticles is one of the most important factors limiting usage of these nanoparticles. This issue is generally addressed through binding of stabilizing agents like citric acid (Hindi et al. 2009) and polyvinylpyrrolidone (Van der Zande et al. 2012).

However, natural coating by biomolecules present in leaf extract is one of the effective alternatives to overcome this post modification (Ali et al. 2015).

2.6.5 Atomic Force Microscopy (AFM)

It confirms the size and form of silver nanoparticles (Abo-State and Partila 2015).

2.6.6 X-Ray Diffraction (XRD)

It confirms the crystalline nature for silver nanoparticles and also its size by Scherrer's equation,

$$D = 0.89 \ \lambda / \beta \ \cos \ \theta,$$

where D is the average of particle size, β is the full width at half maximum of X-ray reflection in terms of 2 Θ in radians, and 2Θ is the position of the different peaks in the diffractograms (Abo-State and Partila 2017).

2.7 Application of Silver Nanoparticles

Nanoparticle technologies have begun to be deployed in agricultural applications, with diverse, powerful results. Of these, bioactive metal- and biopolymer-based chemistries have emerged as the first-generation nanoparticle technologies for use in agriculture, with crop responses reported from tests in greenhouse-controlled and field-based studies (Hendrickson et al. 2017).

Next-generation nanoparticle technologies are emerging incorporating variability in internal pore space, surface porosity, and surface chemical adsorptive properties that indicate a significantly improved capacity to adsorb, contain, and ultimately deliver phytonutrients and various agrochemicals into plant tissues (Hendrickson et al. 2017).

Both foliar and root zone applications have suggested that nanoparticle-inherent properties alone can trigger an array of beneficial responses in target crops. However, with the increasing structural and chemical diversity exhibited in such next-generation technologies, nanoparticle uptake, mobility, and even biodegradability are being tuned to address challenges in crop production (Hendrickson et al. 2017). This can open the possibility of loading such NPs with desired active chemistries, for uptake and some degree of translocation in apoplastic or cytoplasmic space. Some mobility of NPs has been reported in pumpkin; others have shown no mobility at all as in maize (Zhu et al. 2008; Birbaum et al. 2010).

Although fertilizers are very important for plant growth and development, most of the applied fertilizers are rendered unavailable due to many factors such as leaching, degradation by photolysis, hydrolysis, and decomposition. Hence it is necessary to minimize the nutrient losses in fertilization and increase the crop yield through exploitation of new applications with the help of nanotechnology and nanomaterials (Zhu et al. 2008; Birbaum et al. 2010).

The exposure of the snails and soil matrix to silver nanoparticles in a laboratory experiment reduced the activity and the viability of the land snail (20% of silver nanoparticle-treated snails died) as well as the frequency of fungal population in the surrounding soil (Ali et al. 2015).

Effect of silver nanoparticles with diameters of 20 nm on seeds of Fenugreek (*Trigonella foenum-graecum*) has been carried out (Hojjat 2015). Different concentrations of silver nanoparticles (0, 10, 20, 30, and 40 μg mL^{-1}) were used, and results showed maximum seed germination (76.11%), speed of germination (4.102), root length (76.94 mm), root fresh weight (2.783), and root dry weight (1.204) at a concentration of 10 μg mL^{-1}. These results revealed that application of silver nanoparticles could be used to significantly enhance seed germination potential, mean germination time, seed germination index, seed vigor index, seedling fresh weight, and dry weight (Kulkarni et al. 2012).

Due to high contamination of woody plants especially fruit trees and also adverse environmental effects of mercury chloride, the nano-silver solution can be used as a low-risk bactericide in micro-propagation of hybrid of almond · peach, root stock and can be an appropriate alternative to mercury chloride in the future (Arab et al. 2014).

Numerous experiments conducted by scientists evaluated the possibility of using nano-silver as a potential plant growth regulator for crops and also as a means to extend the postharvest longevity of cut flowers and ornamental foliage (Andżelika Byczyńska 2017). Silver NPs promote seed germination and seedling vigor of peanuts (Prasad et al. 2012). The synthesized AgNPs exhibit high antioxidant capacity, in vitro antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and in vivo antifungal properties against *Botrytis cinerea* and *Colletotrichum gloeosporioides* in tomato and strawberry leaflet assays, respectively (Jogaiah et al. 2018). It's found that *Ganoderma applanatum (G. applanatum*) can be efficiently used in synthesis of AgNPs with potent antimicrobial properties, which can be used for both clinical and agrochemical purposes (Jogaiah et al. 2018). Silver nanoparticles exhibited strong antifungal activity against *Bipolaris sorokiniana*, the spot blotch pathogen of wheat (Mishra et al. 2014). Further studies are needed to assess optimum levels of NP application as well as toxicity to nontarget plants (Lee et al. 2012).

Nanoparticles can provide both direct and indirect benefits, including reduced production costs through reduced agrochemicals applied, reduced application frequency, and reduced environmental impact, like antibacterial activity of green synthesized silver nanoparticles (AgNPs) that have shown good antibacterial activity against wide bacterial species which has drawn the attention of several researchers to evaluate and assess these nanoparticles for control of various diseases including crop diseases. Because of extremely smaller size, these particles could be effectively utilized in control of microbes without developing resistant microbes.

The zone of inhibition (mm) was found higher for most of the green synthesized AgNPs as compared to antibiotic streptocycline; it was found that the green synthesized AgNPs possess excellent antimicrobial activity even at lower concentration (Chhipa 2017; Kanhed et al. 2014; Khot et al. 2012; Torney et al. 2007).

Maximum and minimum zone of inhibition for *X. campestris* was found to be 24.8 ± 0.1 mm and 12.2 ± 0.1 mm, respectively, using red algae-mediated green synthesized AgNPs (Vadlapudi and Amanchy 2017).

The effectiveness of silver nanoparticles (AgNPs) was clarified in inhibition the fungus *Aspergillus flavus*. The maximum inhibition 100% fungal growth inhibition was at 200 and 175 ppm of silver nanoparticles, while 95% inhibition was at 150ppm. The addition of silver nanoparticles showed reduction in mycotoxins production by 95.5 to 81.1% also silver nanoparticles can change the metabolism and toxicity of molds in the case of high concentrations of silver nanoparticles used (Al-Othman et al. 2014).

Authors found silver nanoparticles inhibit microorganisms in several ways. So it can be used with relative safety factor for control of various pathogens, compared with fungicide manufacturers (Park et al. 2006). The previous studies have demonstrated that bulk silver in an oxygen-charged aqueous media will catalyze complete destructive oxidation of microorganisms (Davies and Etris 1997). In most cases, inhibition increased as the concentration of AgNPs increased. This could be due to the high density at which the solution was able to saturate and cohere to fungal hyphae and to deactivate plant pathogenic fungi.

Antimicrobial potentials of medicinal plant's extract in biomedical fields, in which the various plant parts such as bark, stem, leaf, fruit and seed examined against Gram-negative and Gram-positive bacteria, by using different solvents for extraction i.e. methanol, ethyl acetate, chloroform, acetone, n. hexane, butanol, petroleum ether and benzene. The extract was showed acting as antiviral, bactericidal, and fungicidal.

Antibacterial activity of synthesized silver nanoparticle was examined against plant pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo) and showed a good antimicrobial activity compared to 200 mg/l of streptocycline (Sriram and Pandidurai 2017).

The present dataset was provided to identify the antioxidant, antitumor, and apoptotic (in DLA cells) properties with the synthesized AgNPs. The result reveals the AgNPs exhibit antitumor and apoptotic activity in DLA cells and antioxidant properties. The results of the in vivo experiments increased the life span of liver cells in DLA-induced tumor mice and did not show any histopathological variations between control and DLA-induced mice animals. The HPTLC examination of the *Gloriosa superba* (L.) seed extract infers the presence of colchicine derivatives as a major alkaloid source (Saradhadevi 2017).

It was shown that AgNPs were capable to prevent the fungal spreading in the inoculated tomato and strawberry leaves without affecting the leaf morphological status. 40 ppm concentration of silver nanoparticles possessed significant increase in the growth of shoot and root of *Zea mays L.* Similarly 60 ppm concentration of silver nanoparticles possessed increasing germination in both dry and fresh weight of seeds (Sriram and Pandidurai 2017). Rawani et al. (2013) showed mosquitocidal silver nanoparticles synthesized using *Solanum nigrum* berry extracts were not toxic against two mosquito predators, *Toxorhynchites larvae* and *Diplonychus annulatum*, and *Chironomus circumdatus* larvae, exposed to lethal concentrations of dry nanoparticles calculated on *A. stephensi* and *C. quinquefasciatus* larvae (Kumar et al. 2015). Silver nanoparticles fabricated using the 2,7-bis[2-[diethylamino]-ethoxy]fluorence isolate from the *Melia azedarach* leaves did not show acute toxicity against *Mesocyclops pehpeiensis* copepods (Ramanibai and Velayutham 2015). Later on, Govindarajan et al. (2016) assessed the biotoxicity of *C. spinarum*-synthesized silver nanoparticles on the nontarget aquatic organisms *Anisops bouvieri*, *D. indicus*, and *G. affinis*. Toxicity testing revealed minimal toxicity, obtaining LC50 values in the range of 424–6402 lg/mL. Similarly, in Govindarajan et al. (Govindarajan et al. 2016), the *Malva sylvestris*-synthesized silver nanoparticles exhibited minimal biotoxicity against nontarget organisms *D. indicus* and *G. affinis*, as with LC50 values ranging from 813 to 10,459 lg/mL (Govindarajan and Benelli 2016). Cristata-fabricated silver nanoparticles tested on the nontarget organisms, *A. bouvieri*, *D. indicus*, and *G. affinis*, showed LC50 values ranging from 633 to 8595 lg/mL. Genotoxicity experiments testing neem cake-synthesized silver nanoparticles on *Carassius auratus* erythrocytes showed no significant damages at doses below 12 ppm, while when carbon nanoparticles were tested, *C. auratus* erythrocytes showed no significant damages at doses below 25 ppm (Murugan et al. 2016). Notably, sub-lethal doses of mangrove-fabricated silver nanoparticles did not reduce the predation efficiency of mosquito natural enemies, such as *Carassius auratus*, on *A. aegypti* mosquito larvae (Murugan et al. 2016).

Overall, extremely low doses of gold and silver nanoparticles may help to boost the control of *Anopheles*, *Aedes*, and *Culex* larval populations in copepod-, tadpole-, and fish-based control programs (Benelli 2017).

2.8 Mechanism of Action for Silver Nanoparticles

Nanoparticles (NPs) have garnered worldwide interest, due to their electrostatic attraction between positively charged NPs and negatively charged microbial cells and a large surface-to-volume ratio, resulting in improved physicochemical properties and enhanced antimicrobial activities of the NPs. The efficacy of silver nanoparticles is dependent on particle size and shape and decreases with increasing particle size. It has been found that truncated triangular particle shape showed greater "cidal" effect than spherical- and rod-shaped particles.

The mechanism of action for nano-silver on fungi, bacteria and virus is the same. Nano-silver disrupts the water balance of fungi and influences the catalytic decomposition of lipid-protein layers of viruses (Mroczek–Sosnowska et al. 2013).

Scanning electron microscope (SEM) analysis showed distinct structural changes in the cell membranes of *C. albicans* upon AgNP treatment (Balashanmugam et al. 2016).

Incorporation of silver nanoparticles in the cell membrane results in leakage of intracellular substances which eventually causes cell death. This is because silver ions cause the inactivation of cell wall thiol groups of fungal cell wall resulting in disruption of transmembrane, energy metabolism, and electron transport chain. Also nanoparrticle make mutations in fungal DNA, dissociation of the enzyme complexes that are essential for the respiratory chain (Velmurugan et al. 2009). Reduced membrane permeability and cell lysis are also other mechanisms (Velmurugan et al. 2009).

The author suggested morphological changes on treated fungi could occur. SEM examination of fungal hyphae treatment with silver nanoparticles has shown damage such as deformations in mycelial growth and the shape of hyphal walls and unusual bulges and ruptures (Al-Othman et al. 2014). Physical and chemical pressure and antifungal compounds have been reported to trigger necrosis or apoptosis-like cell death in fungi (Sharon et al. 2009). The high surface-area-to-volume ratio and nano-scale particle size allow NPs to have greater contact with soil colloids, mineral complexes, root tissues, and microorganisms in the rhizosphere. Zinc and copper oxide and silver-based NPs are also soluble in aqueous conditions, integrating easily with existing fertilization regimes. Inherent surface chemical properties (measured in zeta potential) can generate increased antimicrobial activity through disruption with neighboring membrane stability, possibly impacting other cellular processes (Wang et al. 2012). Some examples of the effects of nano-silver on some crops are shown in Table 2.1.

Table 2.1 Examples of biostimulant effects of nano-silver on crops (Andżelika Byczyńska 2017)

Effects	Method of application	Nano-silver type and concentration	Crop
Increased plant height	Sprayed on plant at seed growth stage	Nano-silver (20, 40, and 60 ppm)	Basil (*Ocimum basilicum*)
Improved dry weight of plant			
Enhanced seed yield			
Improved seed yield	Sprayed on plant at 125 days after cultivation	Nano-silver (20, 40, and 60 ppm)	Borage (*Borago officinalis*)
Increased number of leaves			
Enhanced plant height			
Improved dry weight of plant			
Increased dry weight of inflorescences			
Increased enzymatic activity	Soaking of seeds	Silver nanoparticles (100, 200, 500, 1000, 2000, 4000 mg L^{-1})	Castor (*Ricinus communis*)
Enhanced content of parahydroxy benzoic acid			

(continued)

Table 2.1 (continued)

Effects	Method of application	Nano-silver type and concentration	Crop
Increased plant height	Sprayed every 7 days for 14 weeks	Silver nanoparticles (average size of 50 nm, bulk density 0.92 g ml^{-1}, specific surface area 10.1 m^2 g^{-1}) at 500, 1000, 1500, 2000, 2500, and 3000 ppm)	Cucumber (*Cucumis sativus*)
Improved number of fruits			
Enhanced weight of fruit			
Increased length of fruit			
Increased germination percentage	Adding to medium in vitro	Silver nanoparticles (0, 20, 40, 60, 80, 100 mg kg^{-1} and 0, 30, 60, 90, 120, 150, 180 mmol L^{-1})	Fennel (*Foeniculum vulgare*)
Improved root fresh weight			
Enhanced root length			
Increased root length	Seed soaking (15 ml by 12 days)	Nano-silver (0, 10, 20, 30, 40 μg ml L^{-1})	Fenugreek (*Trigonella foenumgraecum*)
Improved root fresh weight			
Enhanced root dry weight			
Increased seed germination			
Increased diameter of canopy area	Soaking of seeds	Nano-silver (0, 20, 40, 60, 80, and 100 ppm)	Ferula rigidula (*Thymus kotschyanus*)
Shortened flowering time			
Improved essential oil			
Enhanced herb yield			
Increased α-terpinyl acetate content			
Enhanced root length	Adding to medium and soil	Silver nanoparticles (0.5, 15 mg L^{-1} or 0.5, 15 mg/kg)	Fodder beet (*Beta vulgaris*)
Increased stem length			
Improved carotenoid content			
Inhibited seedling growth	Seed soaking for 1 h	Silver nanoparticles (1, 5, 10, 20, 40 mg L^{-1})	Ryegrass (*Lolium multiflorum*)
Enhanced root length	Adding to IAA and BA		Hibiscus (*Hibiscus rosa-sinensis*)
Increased number of roots			

(continued)

Table 2.1 (continued)

Effects	Method of application	Nano-silver type and concentration	Crop
Increased root length	Adding to seeds (15 ml by 14 days)	Silver nanoparticles (10, 20, 30, and 40 μg m L^{-1})	Lentil (*Lens culinaris*)
Improved shoot length			
Increased dry mass			
Enhanced seed germination			
Inhibited seedling growth	Adding to agar medium and soil	Silver nanoparticles (5, 10, 20, 40 mg L^{-1} and 500, 1000, 2000 mg kg^{-1})	Mung bean (*Phaseolus radiatus*)
Increased root length	Adding to medium	Silver nanoparticles (25, 50, 100, 200, and 400 ppm)	Mustard (*Brassica juncea*)
Enhanced chlorophyll content			
Improved photosynthetic quantum efficiency			
Increased seed germination	Seed soaking for 2 h	Nano-silver (20 and 50 ml L^{-1})	Pearl millet (*Pennisetum glaucum*)
Improved shoot length			
Enhanced root length			
Increased antioxidative enzyme activities	Sprayed of 50 mL	Nano-silver (0, 20, 40, 60, and 80 mg L^{-1})	*Pelargonium* ‘Flowerfairy’ and ‘Foxi’ (*Pelargonium zonale*)
Reduced lipid peroxidation			
Improved petal longevity			
Decreased petal abscission			
Increased stem length	Adding to medium	Nano-silver (0, 1.0, 1.5, and 2.0 ppm)	Potato (*Solanum tuberosum*)
Improved root length			
Decreased of number of isolated protoplasts			
Decreased in the viability of isolated protoplasts			

(continued)

Table 2.1 (continued)

Effects	Method of application	Nano-silver type and concentration	Crop
Increased flavonoid content	Adding to medium	Silver nanoparticles (average size 20 nm, spherical in shape, and specific surface area of 18–22 $m^2\ g^{-1}$) at 0, 2, 10, 20 mg L^{-1}	Potato 'White Desiree' (*Solanum tuberosum*)
Enhanced total phenolics			
Improved growth and development of explants under in vitro culture condition			
Diminished water content	Adding to medium in vitro	Nano-silver (125, 250, and 500 mg L^{-1})	Radish (*Raphanus sativus*)
Decreased root length			
Reduced bacterial contamination	Adding to medium in vitro	Nano-silver (0, 50, 100, and 150 ppm)	Rose (*Rosa hybrida*)
Reduced phenolic exudation rate			
Increased number of seed	Soaking of seeds	Silver nanoparticles (20, 40, 60 ppm)	Safflower (*Carthamus tinctorius*)
Improved number of inflorescences			
Increased number of roots	Soaking corms for 90 min	Nano-silver (0, 40, 80, or 120 ppm)	Saffron (*Crocus sativus*)
Improved root length			
Enhanced leaves dry weight			
Increased germination index in early stage	Soaking of seeds (5 s three times of 1 h)	Silver nanoparticles (0, 25, 50, 75, and 100 mg L^{-1})	Tomato 'Peto Early CH' 'Primo Early' 'Cal.J.n3' 'Early Urbanay VF' 'King Stone' 'Super Stone' 'Super Strain B' (*Lycopersicon esculentum*)
Decreased root length			
Decreased shoots length			
Increased germination percentage	Seed soaking for 2 h	Silver nanoparticles (0.05, 0.5, 1.5, 2, 2.5 mg L^{-1})	Tomato (*Solanumly copersicum*)
Improved germination rate			
Enhanced root length			
Increased seedling fresh and dry weight			
Improved shoot dry weight	Sprayed on foliar	Silver nanoparticles (50 and 75 ppm)	Brassica 'Pusa Jai Kisan' (*Brassica juncea*)
Increased shoot fresh weight			
Enhanced shoot length			

2.9 Conclusions

Biogenic silver nanoparticles are better than chemical and physical preparation and have the ability to destroy crop insect and fungal and bacterial infection that attack plant crop. Resistance problem for many bacteria and fungi was overcome.

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