



## RESEARCH ARTICLE

### GREEN SYNTHESIS OF SILVER NANOPARTICLES FOR PLANT DISEASE DIAGNOSIS

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#### ABSTRACT

As the total population is expanding, interest for nourishment is additionally expanding. But food losses occur due to crop diseases caused by pathogens such as bacteria, viruses and fungi. So it is important to utilize the advanced technologies, for example, bio and nanotechnologies in agricultural sciences. Metallic nanoparticles are being utilized in every phase of science along with engineering. Among the all noble metal nanoparticles, silver nanoparticle are an arch product from the field of nanotechnology which has gained boundless interests because of their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal in addition to anti-inflammatory activities. The introduction of nano silver offers an alternative where all detection and identification of plant pathogens. Green synthesis of silver nanoparticles can be conceivably utilized to overcome the biological risk generated by nanoparticles derived from chemical methods, especially in the plant. This paper provides an overview of green synthesis and application of silver nanoparticles for the disease diagnosis and detection methods in plants.

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## INTRODUCTION

Nanotechnology has a wide scope of application in medicine, industry and agriculture and can revolutionize the entire society. In agriculture, nanotechnology has potential scope for use in the natural resource exploitation and conservation, and production and protection of the crops and livestock. In agriculture, Nanotechnology has started significant work to make new functional materials for food industry development, product development and instrumentation for food safety and Bio-security (Joseph and Morrison, 2006). Nanotechnology gave new tools for the molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients etc. The new technology based smart delivery systems will help the agricultural industry combat viruses and other crop pathogens. Use of nanoparticles in plant disease management is a novel and fancy approach that may prove very effective in future with the progress of application aspect of nanotechnology (Mujeebur and Tanveer 2014).

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Plant diseases are major limiting factor in sustainable crop production. It is estimated that about 20–30 % of the field crops are annually lost due to infection of diseases (Nezhad 2014; Sankaran et al. 2010; Mann et al. 2008). Although, combined infestation of pests and diseases in plants could result up to 82 % losses in attainable yield in case of cotton and over 50 % losses for other major crops (Pan et al. 2010; Thind 2012). Further, if we combine these losses with post-harvest spoilage and deterioration in quality, these losses become more critical particularly for resource poor countries like India. Usually, the bacterial, fungal, and viral infections, spread over larger area in crops, groves and plantations through accidental introduction of vectors or through infected seed or plant materials. Another route for the spread of pathogens is through ornamental plants that act as hosts. These plants are frequently sold through mass distribution before the infections are known. In this context, early detection of diseases is of key importance to prevent disease spread with minimal loss to crop production (Sankaran et al. 2010; Martinelli et al. 2014). Traditional methods for identifying plant pathogens rely on the interpretation of visual symptoms and/or the isolation, culturing and laboratory identification of the pathogen.

Table 1. Silver Nanoparticles synthesis from different plant sources

Plant name	Plant tissue	Size (Nm)
Camellia Sinensis (Loo <i>et al.</i> , 2012)	Leaves	2-10
Lantana Camara (Thirumurugan <i>et al.</i> , 2011)	Leaves	40
Ovalifoliolata Boswellia (Ankanna <i>et al.</i> , 2010)	Bark	30-40
Euphorbia Milli (De Matosa <i>et al.</i> , 2011)	Latex	10-50
Glycyrrhiza Glabra (De Matosa <i>et al.</i> , 2011)	Roots	20-30
Terminalia catappa (Bindhani and Panigrahi., 2014)	Leaves	20-40
Aloevera (Chandran <i>et al.</i> , 2006)	Leaves	50-350
MorindaCoreia Buk. Ham (Kannan <i>et al.</i> , 2014)	Leaves	--
Pin (Song and Kim, 2009)	Leaves	15-500
Persimmon (Song and Kim, 2009)	Leaves	15-500
Medicago Sativa (Gardea et al., 1999; 2003)	Leaves	20-40
Mirabilis Jalapa (Vankar and Bajpai., 2010)	Flower	60-70
Geranium Leaf (Shankar <i>et al.</i> , 2003)	Leaves	16-40
Capsicum Annuuml (Agarwal <i>et al.</i> , 2014)	Callus	15
Cinnamomum Caphora (Huang <i>et al.</i> , 2007)	Leaves	55-80
Eucalyptus Hybrida (Dubey <i>et al.</i> , 2009)	Leaves	--
Alternanthera dentate (Nakkala <i>et al.</i> , 2014)	Leaves	50-100
Acorus calamus (Nakkala JR <i>et al.</i> , 2014)	Rhizome	31
Boerhaavia diffusa (Suna <i>et al.</i> , 2014)	Whole plant	25
Tea extract (Nabikhan <i>et al.</i> , 2010)	Leaves	20-90
Tribulus terrestris (Mariselvam <i>et al.</i> , 2014)	Fruit	16-28
Cocous nucifera (Mariselvam <i>et al.</i> , 2014)	Inflorescence	22
Abutilon indicum (Sadeghi and Gholamhoseinpoor; 2015)	Leaves	7-17
Pistacia atlantica (Sadeghi <i>et al.</i> , 2015)	Seeds	10-50
Ziziphora tenuior (Ulug <i>et al.</i> , 2015)	Leaves	8-40
Ficus carica (Geetha <i>et al.</i> , 2014)	Leaves	13
Cymbopogan citratus (Masurkar <i>et al.</i> , 2011)	Leaves	32
Acalypha indica (Kumarasamyraja and jeganathan; 2013)	Leaves	0.5
Premna herbacea (Kumar <i>et al.</i> , 2013)	Leaves	10-30
Calotropis procera (Gondwal and Pant; 2013)	Plant	19-45
Centella asiatica (Rout <i>et al.</i> , 2013)	Leaves	30-50
Argyrea nervosa (Thombre <i>et al.</i> , 2014)	Seeds	20-50
Psoralea corylifolia (Sunita <i>et al.</i> , 2014)	Seeds	100-110
Brassica rapa (Narayanan and Park; 2014)	Leaves	16
Coccinia indica (Kumar <i>et al.</i> , 2013)	Leaves	10-20
Vitex negundo (Zargar <i>et al.</i> , 2011)	Leaves	5-30
Melia dubia (Kathiravan <i>et al.</i> , 2014)	Leaves	35
Portulaca oleracea (Firdhouse and Lalitha; 2012)	Leaves	<60
Thevetia peruviana (Rupiasih <i>et al.</i> , 2013)	Latex	10-30
Pogostemon benghalensis (Gogoi 2013)	Leaves	>80
Trachyspermum ammi (Vijayaraghavan <i>et al.</i> , 2012)	Seeds	87-100
Swietenia mahogany (Mondal <i>et al.</i> , 2011)	Leaves	50
Musa paradisiacal (Bankar <i>et al.</i> , 2010)	Peel	20
Moringa oleifera (Prasad and Elumalai; 2011)	Leaves	57
Garcinia mangostana (Veerasamy <i>et al.</i> , 2010)	Leaves	35
Eclipta prostrate (Rajakumar and Abdul; 2011)	Leaves	35-60
Nelumbo nucifera (Santhoshkumar <i>et al.</i> , 2011)	Leaves	25-80
Acalypha indica (Krishnaraj <i>et al.</i> , 2010)	Leaves	20-30
Allium sativum (Ahamed <i>et al.</i> , 2011)	Leaves	4-22
Aloe vera (Chandran <i>et al.</i> , 2006)	Leaves	50-350
Citrus sinensis (Kaviya <i>et al.</i> , 2011)	Peel	10-35
Eucalyptus hybrid (Dubey <i>et al.</i> , 2009)	Peel	50-150
Memecylon edule (Elavazhagan <i>et al.</i> , 2011)	Leaves	20-50
Nelumbo nucifera (Santhoshkumar <i>et al.</i> , 2011)	Leaves	25-80
Datura metel (Kesharwani <i>et al.</i> , 2009)	Leaves	16-40
Carica papaya (Jain <i>et al.</i> , 2009)	Leaves	25-50
Vitis vinifera (Gnanajobitha <i>et al.</i> , 2013)	Fruit	30-40

Table 2. Silver Nanoparticles activity against microorganism

Plant	Microorganism	References
Tea	E. coli	Nabikhan <i>et al.</i> , 2010
Cocous nucifera	Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa and Salmonella paratyphi	Sadeghi <i>et al.</i> , 2015
Solanus torvum	P. aeruginosa, S. aureus, A. flavus and Aspergillus niger	Govindaraju <i>et al.</i> , 2010
Alternanthera dentate	Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and, Enterococcus faecalis	Nakkala <i>et al.</i> , 2014
Tribulus terrestris	Streptococcus pyogens, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureus	Mariselvam <i>et al.</i> , 2014
Aloe vera	E. coli	Zhang <i>et al.</i> , 2010
Boerhaavia diffusa	Aeromonas hydrophila, Pseudomonas fluorescens and Flavobacterium branchiophilum	Vijay <i>et al.</i> , 2014
Trianthema decandra	E. coli and P. aeruginosa	Geethalakshmi and Sarada, 2010
Argimone mexicana	Escherichia coli; Pseudomonas aeruginosa; Aspergillus flavus	Khandelwal <i>et al.</i> , 2010
Svensonia hyderabadensis	A. niger, Fusarium oxysporum, Curvularia lunata and Rhizopus arrhizus	Sun <i>et al.</i> , 2004
Cymbopogan citratus	P. aeruginosa, P. mirabilis, E. coli, Shigella flexaneri, S. someni and Klebsiella pneumonia	Kumarasamyraja and jeganathan, 2013
Abutilon indicum	S. typhi, E. coli, S. aureus and B. subtilus	Sadeghi and Gholamhoseinpoor, 2015

These techniques suffered from some major drawbacks such as lack of sensitivity, time-consuming and costly etc. Additionally, the accuracy and reliability of these assays depend largely on the experience and skill of the person making the diagnosis (Sankaran *et al.* 2010; Kashyap *et al.* 2011; Alvarez 2004). One of the most promising nanomaterials is quantum dots (QD), which have been widely used in a broad range of bio-related applications including rapid detection of a particular biological marker with extreme accuracy (Kashyap *et al.* 2015). Biosensor, quantum dots, nanostructured platforms, nano-imaging and nanopore DNA sequencing tools have the potential to raise sensitivity, specificity and speed of the pathogen detection, facilitate high-throughput analysis, and can be used for high-quality monitoring and crop protection (Khiyami *et al.* 2014). Furthermore, nanodiagnostic kit equipments can easily and quickly detect potential plant pathogens, allowing experts to help farmers in the prevention of epidemic diseases. Currently, a vast library of nanostructures has been synthesized and documented, with different properties and applications (Savaliya *et al.* 2015; Khiyami *et al.* 2014). Metal nanoparticles have been applied in biosensors as marker tags to replace enzymes as the label. Stripping voltammetry as an electrochemical technique can be applied to detect the metal nanoparticles directly making the assay simple to perform. Gold (AuNP) and silver nanoparticles (AgNP) can be used in these methods including different inorganic nanocrystals (ZnS, PbS and CdS) for analyte detection (Upadhyayula 2012). The unique physical and chemical properties of nanoparticles such as colloidal gold can provide excellent application in a wide range of biosensing techniques (Rosi and Mirkin 2005; Khan and Rizvi 2014). Nanoparticles can also be exploited in conductivity based sensors where they can induce a change in the signal upon the attachment of the nanoparticles tagged antibody with the antigen captured on the sensor surface (Servin *et al.* 2015). Various strategies such as antibody-antigen, adhesion receptor, antibiotic and complementary DNA sequence recognitions have been developed for a specific detection between target phytopathogenic cells and bio-functionalized nanomaterials (Conde *et al.* 2014). Gold nanoparticles are excellent markers to be used in biosensors due to ease in alternation of their optical or electrochemical procedures to identify pathogens. A number of nanoparticle-based experiments have been performed to develop biomolecular detection with DNA- or protein functionalized gold nanoparticles, which are used as the target-specific probes (Thaxton *et al.* 2006). These detection methods include conductive polymer nanowires (Pal *et al.* 2008), carbon nanotubes (Poonam and Deo 2008), nanoporous silicon (Yang *et al.* 2008) and gold nanoparticles (Wang *et al.* 2010). Singh *et al.* (2010) used nano-gold based immunosensors that could detect Karnal bunt disease in wheat (*Tilletia indica*) using surface plasmon resonance (SPR). Generally there are two approaches which are involved in the syntheses of silver nanoparticles, either from “top to bottom” approach or a “bottom to up” approach. In bottom to top approach, nanoparticles can be synthesized using chemical and biological methods by self-assemble of atoms to new nuclei which grow into a particle of nanoscale as shown in a while in top to bottom approach, suitable bulk material break down into fine particles by size reduction with various lithographic techniques e.g. grinding, milling, sputtering and thermal/laser ablation. The green synthesis of nanoparticles is a part of ‘bottom to up’ approach. The small particles (atoms) self-assemble and makes network like structure followed by nuclei formation that convert into nanoparticles.

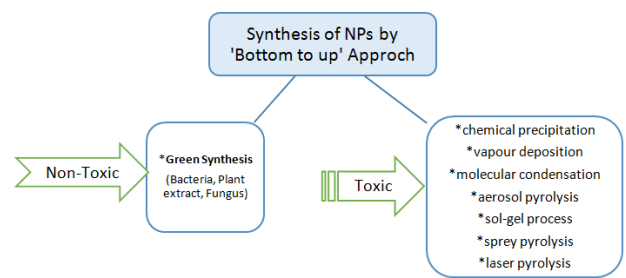


Figure 1. Nanoparticles synthesis by ‘Bottom to up’ process

Due to their medicinal and antimicrobial properties, silver nanoparticles have been incorporated into more than 200 consumer products, including clothing, medicines and cosmetics. The advancement of green syntheses over chemical and physical methods is: environment friendly, cost effective and easily scaled up for large scale syntheses of nanoparticles, furthermore there is no need to use high temperature, pressure, energy and toxic chemicals (Dhuper *et al.*, 2012). Although; among the various biological methods of silver nanoparticle synthesis, microbe mediated synthesis is not of industrial feasibility due to the requirements of highly aseptic conditions and their maintenance. Therefore; the use of plant extracts for this purpose is potentially advantageous over microorganisms due to the ease of improvement, the less biohazard and elaborate process of maintaining cell cultures (Kalishwaralal *et al.*, 2010). This review deals with the utilization of the application of Nanotechnology in controlling plant diseases.

#### Application of Silver Nan particles in Plant Diseases Diagnosis

The antifungal effectiveness of colloidal nano silver (1.5 nm average diameter) solution, against rose powdery mildew caused by *Sphaerotheca pannosa Var rosae*. Rose powdery mildew disease causes leaf distortion, leaf curling, early defoliation and reduced flowering. Nano silver colloid is a well dispersed and stabilized silver nano particle solution and is more adhesive on bacteria and fungus, hence are better fungicide. Anderson classified the nano silver as pesticide. Silver is now an accepted agrochemical replacement (Anderson 2009). Nanoparticles because of ultra-small size, even smaller than a virus particle and high reactivity, may affect the activity of microorganisms. The silver has been generally found non injurious to microorganisms. However, silver NPs inhibited the colonization of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. The highest antimicrobial activity of silver nanoparticles (30 nm) synthesized by *Solanum tuberosum* and *Ocimum tenuiflorum* leaf extracts was found against *S. aureus* and *E. coli*, respectively. The antibacterial activity of the synthesized Ag NPs/PVP (hybrid materials based on polyvinylpyrrolidone with silver nanoparticles) against three different groups of bacteria *Staphylococcus aureus* (gram positive bacteria), *E. coli* (gram-negative bacteria), *P. aeruginosa* (nonferment gram-negative bacteria), as well as against spores of *Bacillus subtilis* has been studied (Bryaskova *et al.*, 2011). The Ag NPs/PVP were tested for fungicidal activity against different yeasts and molds such as *Candida albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata* and *Aspergillus brasiliensis*. The hybrid materials showed strong antifungal effects against the tested microbes (Bryaskova *et al.*, 2011). The nanopesticide formulations also offer large specific surface area and hence, increased affinity to the target (Yan *et al.*, 2005).

Nanoemulsions, nanoencapsulates, nanocontainers and nanocages are some of the nanopesticide delivery techniques that may prove effective in plant protection programmes (Lyons and Scrinis, 2009). Corradini *et al.* (2010) explored the possibility of utilizing chitosan nanoparticles, a highly degradable antibacterial material for slow release of NPK fertilizer. Liu *et al.* (2006) developed kaolin clay based nano layers to be used as cementing and coating material for controlled release of fertilizers.

## Conclusion

Production of silver nanoparticles is nontoxic and eco-friendly approach. There are various microbes, fungus and plants parts were used for the synthesis of silver nanoparticles but plants extract was less biohazard, requirements of low aseptic conditions and elaborate process of maintaining cell cultures is easy as compare of microbes. The silver nanoparticles used for various diseases identification, management and treatment. The plant based extracts provides synthesis of a controlled size and morphology of silver nanoparticles. In medicine, silver nanoparticles are being used as antimicrobial agents in bandages, toothpastes for example. Applications are in targeted drug delivery and clinical diagnostics are in developing stage. Use of nanoparticles in plant disease management is a novel and fancy approach that may prove very effective in future with the progress of application aspect of nanotechnology.

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