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Silver Nanoparticle: Synthesized and Antimicrobial Activity on Target Plant Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Author Shantamma wrote the protocol and wrote the first draft of the manuscript. Authors KTR, NBP and RA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The growth rate of agricultural production is reducing worldwide every year due to mainly biotic and abiotic stresses including plant diseases. Various organic and inorganic methods are being used to protect plants from disease causing pathogens. Among them, use of pesticides is the most prevalent one incurring millions of dollars on pesticides globally for control of plant diseases. In recent years, environmental hazards and ill effects caused by indiscriminate use of pesticides have been widely discussed. Therefore, agriculture scientists are finding an alternative antimicrobial compounds such as nanoparticles for the management of diseases with least adverse effect on nature and ecosystem. Herein we reviewed the synthesis, antimicrobial efficacy and compatibility of silver nanoparticles which could help to develop the novel technology for crop protection.

Keywords: Plant pathogens; antimicrobial activity; plant diseases; agrochemicals, silver nanoparticle.

1. INTRODUCTION

Nanotechnology is the application of science and technology to control the matter at molecular level. The term nanotechnology was first defined by Norio Taniguchi of Tokyo Science University in 1974. Nanotechnology emerged from the physical, chemical, biological and engineering sciences, where novel techniques are being developed to probe and influence atoms and/or molecules. The development of nanotechnology conjunction with biotechnology significantly expanded the application domain of nanomaterials in various fields. A variety of carbon-based, metal and metal oxide based dendrimers (nano-sized polymers) and biocomposite nanomaterials [1] are developed. Major types include single-walled and multi walled carbon nanotubes, magnetized iron (Fe) nanoparticles, aluminum (AI), copper (Cu), gold (Au), silver (Ag), silica (Si) zinc (Zn) nanoparticles and zinc oxide (ZnO), titanium dioxide (TiO₂) and cerium oxide (Ce₂O₃).

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. Nanoparticles (NPs) are commonly defined as solid colloidal particles with sizes typically in the range of 1.0 nm to 1000 nm in one or more dimensions [2.3]. In material science and colloidal chemistry, the term colloidal particle is about a small amount of matter having size typical for colloids and with a clear phase boundary. The dispersed-phase particles have a diameter ranging between 1 and 1000 nanometer. The antimicrobial property of silver nanoparticles is exclusively governed by the size of the particles in turn total surface area. Hence, the most effective nanoparticles with potential antimicrobial property is in the range of 10-100 nm in diameter.

Though there are many reports on inhibitory effect of silver particles on many microbial pathogens under in vitro, but very few reports are there on field performance of these silver particles against plant pathogens. knowledge about compatibility of agrochemicals with silver nanoparticles will be very vital in selecting the compatible combinations for effective management of insect pests, diseases and weeds. Combined application of pesticides is even though a labour saving shortcut method, but an understanding and knowledge of pesticide compatibility is essential in order to avoid problems which may arise from combinations of incompatible agrochemicals. Pesticides combinations may show physical, chemical or

phytotoxic incompatibility causing undesirable results [4]. Information on compatibility of different agrochemicals with silver particles is essential for adopting the technology for field use.

The age old plant diseases of great significance worldwide with respect to there devastating nature, many causative plant pathogenic fungi and bacteria have its own bearing on farming community and industry at global level. During favorable conditions crop gets destroyed within no time due to such devastating pathogens. Hitherto control of such plant disease traditionally was relied mainly on foliar applications of chemical fungicides, resulting in very high use of pesticides in the crop production. Besides the high costs, the risk of resistance development problem of residues in food chain is high due to use of potentially high risk pesticides. In order to find alternative molecules to the chemical fungicides and bactericides, new molecules such as silver nanoparticles at low concentrations of ionic silver with size measuring between 10-1000 nm in diameter have been reported to have inhibitory effect on many fungal and bacterial plant pathogens besides safe to human beings and environment were synthesized and tested under both in vitro and field conditions.

2. SYNTHESIS OF SILVER NANO PARTICLES

2.1 Chemical Methods

The commercial synthesis of nanoparticles is largely done by chemical methods. There are different chemical methods to synthesize the nanoparticles, however, choice of the method may vary with the material available and the expected product. Some important chemical methods are reduction method, colloidal method, sonochemical method *etc.*

Among the chemical method, the chemical reduction method has been employed by various workers. The method involving the reduction of ${\rm Ag}^{\dagger}$ to ${\rm Ag}^{0}$ based on drop wise addition of 0.001 M ${\rm AgNO_3}$ to 0.002 M sodium borohydride (NaBH₄) with continuous stirring for about 20 min on an ice bath was also employed wherein formation of silver nanoparticles was observed by a change in colour of the mixture from colorless to yellow [5]. Similarly, the silver nanoparticles synthesized by chemical method measured 60 nm and found highly effective against gram positive bacteria such as

Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus [6], whereas nanoparticles measuring 20 nm size employed as metal ion contaminant removal media for the removal of Fe⁺ and Mn⁻ metal ions from the ground water [7].

2.2 Biological Methods

Biological methods of synthesizing nanoparticles using microorganism, enzyme and plant or plant extract have been suggested as possible ecofriendly alternatives to chemical or physical methods of synthesizing the nanoparticles.

2.3 Synthesis of Nanoparticles by using Bacteria and Fungi

The magnetotactic bacteria are able to synthesize magnetic nanoparticles. Magnetotactic bacteria are motile which move along geometric field lines, they produce magnetosomes; unique intracellular structures containing magnetic particle in narrow range of very low oxygen concentration. Magnetotactic bacteria usually mineralize either oxide magnetite (Fe_3O_4) or iron sulfide (Fe_3S_4) [8].

The biosynthesis of nanoparticles takes place extra-cellular, intra-cellular and on the surface of the cell wall. Extra cellular synthesis of nanoparticles was observed in *Fusarium* oxysporum with silver and gold-silver nanoparticles. The reduction of silver ions by F. oxysporum strains have been attributed to nitrate-dependent reductase and ashuttle quinine extracellular process [9]. Similarly, extracellular synthesis of silver nanoparticles by the fungi Aspergillus fumigatus and Fusarium semitectum measured 2 and 5 nm, respectively [10], whereas silver nanoparticles produced extracellularly by photoautotrophic Cyanobacterium and Plectonema boryanum measured 1 to 40 nm [11]. Apart from the efficacy on their own, the silver nanoparticles, synthesized extracellularly using a common fungus, Alternaria alternata were evaluated for their part in increasing the antifungal activity of fluconazole against Phoma Phoma herbarum, glomerata. Fusarium semitectum, Trichoderma sp. and Candida albicans [12].

Silver nanoparticles were also synthesized biologically by treating the aqueous silver nitrate solution with culture supernatants of different strains of *Enterobacteria* such as *Klebsiella pneumonia* [13]. Later, the novel method of

biosynthesis of silver nanoparticles using a combination of culture supernatant of *Bacillus subtilis* and microwave irradiation was tried. This method was extremely rapid and produced smaller silver nanoparticles with size ranging 5-50 nm [14].

2.4 Synthesis of Nanoparticles Using Plant Extracts

An important branch of nanotechnology is use of plant extract for biosynthesis of nanoparticles. Recently, the extract of *Aloe vera* plant has been successfully used to synthesize single crystalline triangular gold nanoparticles (50-350 nm in size) and spherical silver nanoparticles (~15 nm in size) with high yield by the reaction of chloroaurate ions for Au and silver ions for Ag with the Aloe vera plant extract [15]. Cinnamon zeylanicum bark has been reported to have remarkable pharmacological effects and it was found useful to synthesize AgNP's with size of 30-40 nm [16]. Extract of Cycas (Cycadaceos), a common gymnospermic plant having flavonoids broadly belonging to the class of phenolic compounds has been used to synthesize AqNPs with size ranging from 2-6 nm with an average of 3.29 ± 0.22 nm [17].

Shankar et al. [18] reported that silver nanoparticles could be synthesized by using aqueous extract of *Azadiracta indica* leaves. Similarly, Philip [19] reported rapid biosynthesis of well dispersed silver nanoparticles by aqueous *Mangifera indica* leaf extract. The colloid consists of well dispersed triangular, hexagonal and nearly spherical nanoparticles having size ~20 nm. Silver nanoparticles were rapidly synthesized at room temperature by treating silver ions with the *Citrus limon* extract [20].

3. EVALUATION OF SILVER NANO PARTICLES UNDER *IN-VITRO* CON DITIONS

3.1 *In vitro* Evaluation of Silver Nano particles Against Fungi

The three different forms of silver nanoparticles at 10 ppm concentration found exhibiting detrimental effects on both fungal hyphae and conidial germination of *Raffaelea* sp., a wilt causing pathogen of Oak tree [16,21-23]. *In vitro* assays of silver ions and nanoparticles at 50 ppm concentration had a significant effect on the colony formation of *Bipolaris sorokiniana* and *Magnaporthe grisea* [21]. Three different types of

nanosilver liquids *viz.*, WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R were inhibited fungal growth by > 90 per cent when tested at 7 ppm concentration against *Sclerotium cepivorum* causing lethal disease in onion under *in vitro* condition [24]. Silver nanoparticles also used as an agent for antifungal treatment of various seed borne plant pathogens- *Rhizoctonia solani*, *Aspergillus flavus* and *Alternaria alternata* by inhibiting the mycelial growth when used at 100 µg/ml concentration [25]. Similar efficacy has been reported against soil borne pathogens *viz.*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor* [23].

The silver nanoparticles of 20-30 nm size found effective at 25, 50, 100 and 200 ppm concentrations in a dose-dependent manner against rampant rice leaf blast fungus Magnaporthae grisea by inhibition of both hyphal growth and number of colonies under laboratory studies [26]. Similarly, nanosilver suspension at 50 ppm concentration inhibited the growth of Cladosporium cladosporoides and Aspergillus niger by 90 per cent and 70 per cent, respectively. The AgNPs even at 15 ppm concentration inhibited the mycelial growth of Alternaria alternata and Botrytis cinerea by 59.30 and 52.90 per cent, respectively [27].

Silver nanoparticles at concentrations of 6 and 8 ppm were found significantly inhibited the growth of Rhizoctonia solani AG1, R. solani AG4, Macrophomina phaseolina. Sclerotinia sclerotiorum and Pythium aphanidermatum by 75 to 80 per cent, whereas at 10, 12 and 14 ppm concentration maximum inhibition of fungal growth up to 90-100 per cent was observed [28]. In an effort to evaluate the plant mediated silver nanoparticles against Fusarium oxysporum f. sp. lycopersici by poisoned food technique, it was observed that at 1250 ppm concentration the maximum mycelial growth inhibition of 86.06 per cent was observed [29]. AqNP when used for treatment of rice seeds infested with Gibberella fujikuroi at 150 μg ml⁻¹ for 10 min to 24 h significantly decreased G. fujikuroi on the seed surface, but equally reduced viability of G. fujikuroi conidia by 50 per cent when directly exposed at 0.015 to 1.5 µg ml concentration for 1 to 20 min [30].

Maximum inhibition of *Phytophthora infestans* growth was recorded at 500 ppm concentration (86.13%) followed by 250 ppm (74.81%) concentration of colloidal silver, whereas lowest inhibition was recorded at 10 ppm (3.31%) [31].

3.2 *In vitro* Evaluation of Silver Nano particles Against Bacteria

Antibacterial properties of AgNPs were related to total surface area of the nanoparticles. Smaller particles with larger surface to volume ratio had greater antibacterial activity [32] and the shape of the particle was also known to influence antibacterial activity of Ag-NPs against the gram negative Escherichia coli [33]. It was found that, Ag-NPs exhibited significant antibacterial activity against Escherichia coli and multidrug resistant Staphylococcus aureus [34]. On E. coli strains the antibacterial activity mainly dependent on particle size, as the smaller particles were highly toxic than the bigger sized particles [35]. Silver nano particles were recorded combined inhibitory effect when impregnated at 10 mg L⁻¹ with four broad spectrum antibiotics namely, amoxicillin, chloramphenicol, erythromycin and rifamycin against four major bacterial pathogens viz., subtillus, B. cereus, Klebsiella Bacillus pneumoniiea and Vibrio cholera [36], whereas against Aeromonas hydrophila causing a variety of diseases in both fish and humans at different concentrations ranging from 15.3 µg/ml to 153.6 µg/ml [37]. The silver nanoparticles reported effective against causative of zoonosis disease in animal and humans, Brucella under an intramacrophage condition. Under cell culture studies, the silver nanoparticles at 4-6 ppm concentrations acting inside the macrophage cells and the findings highlights the potential way for utilising silver nanoparticles as a new nano drug against intramachrophage Brucella melitensis [38].

Similarly, silver nanoparticles and AgGO nanocomposites at 100 mg/ml exhibited stronger antibacterial properties against gram-negative bacteria (*Salmonella typhi* and *Escherichia coli*) than gram-positive bacteria (*Staphyloccocus aureus* and *S. epidermidis*) [39].

Antimicrobial activity of silver nanoparticles was investigated against plant pathogenic bacteria (Erwina caratovora, E. amylovora, Dickya chransanthemi, D. dianthicale, Pectobacterium wasaibiae, P. atrosepticum chransanthem and P. wasabiae) and pathogenic fungi (Fusarium oxysporum, Alternaria alternata and Aspergillus flavus). The results revealed that the silver nanoparticles have higher antimicrobial activity against the examined bacteria and fungi as compared to antibiotics [40]. The commercial nanosilver product, NanoCidR L2000 reported to have strong antibacterial effect against four important foodborne pathogens Escherichia coli

O157:H7, Salmonella typhimurium and Vibrio parahaemolyticuswith MIC (minimum inhibitory concentration) values of of 3.12 µg/ml, whereas 6.25 µg/ml against Listeria monocytogenes [41].

Complete inhibition of *Xanthomonas axonopodis* pv. *punicae* was recorded at 25, 50 and 100 ppm of colloidal silver and even effective inhibition of bacterial colonies were recorded at lower concentrations *viz.*, 2.5, 5 and 10 ppm [31].

4. COMPATIBILITY OF SILVER NANO PARTICLES WITH OTHER FUNGICIDES AND ANTIBIOTICS

Increased bactericidal efficacy of silver nanoparticles (40 μ g/ml) was observed when combined with amoxicillin antibiotic (0.525 mg/ml) against Escherichia coli on Luria-Bertani (LB) medium than when they were applied Similarly, nanosilver and separately [42]. amoxicillin (4000 µg/ml) recorded enhanced antibacterial activity against Streptococcus Staphylococcus mutans. aureus Pseudomonas aeruginosa at lower concentrations [43], whereas enhanced antibiosis was observed against Bacillus cereus, Staphylococcus aureus, Proteus vulgaris and Escherichia coli [44].

AgNPs when combined with gentamycin, vancomycin, trimethoprim and ciprofloxacin against multiple drug resistant bacteria exhibited the synergistic effect and resulted in 0.2–7.0 (average, 2.8) fold increase in antibacterial activity, which clearly revealed that nanoparticles can be effectively used in combination with antibiotics in order to improve their efficacy against various pathogenic microbes [45].

Enhanced antifungal activity of silver nanoparticles (10 µI) was observed when combined with fluconazole (10 mg) against fungal pathogens. This synergistic activity potentiated the effect of fluconazole by 0.37 fold against *Alternaria alternata* and *Fusarium oxysporum* while it showed 0.35 fold increase against *Cladosporium herbarum* [46].

Physically incompatible reaction was observed when two concentrations of colloidal silver 100 and 250 ppm combined with two fungicides namely cymoxanil 8% + mancozeb 64% WP and dimethomorph 50% WP at 0.2 percent, due to change in the pH (8) of mixed solution [31].

Physically compatible reaction was noticed when colloidal silver particles solution were mixed with

streptomycin sulphate 90% + tetracycline hydroxide 10%. There was no formation of unstable mixtures, crystal flakes or sludge and pH of the solution was turned acidic (5.6) [31].

Enhanced antifungal activity of cymoxanil 8% + mancozeb 64% WP and dimethomoph 50% WP at 0.2 percent was observed against *Phytophthora infestans* in the presence of colloidal silver particles under *in vitro* condition [31].

Colloidal silver at two concentrations of 100 and 250 ppm when combined with streptomycin sulphate 90% + tetracycline hydroxide 10% showed additive effect on inhibition of *Xanthomonas axonopodis* pv.punicae than as when they were applied alone [31].

5. EVALUATION OF SILVER NANO PARTICLES UNDER FIELD CONDITIONS

The nanosilver liquid found effective against white rot of green onion disease caused by *Sclerotium cepivorum* and reduced disease severity and increased biomass of plants under greenhouse condition [24]. Silver nanoparticles at 100 ppm concentration reported to have both preventive and curative efficacy in reducing the severity of powdery mildew disease of cucumber and pumpkin under field condition [47].

The titanium dioxide (TiO₂) reported to have antibacterial activity against *Xanthomonas* bacterial blight on geranium and leaf spot on poinsettia at 25 and 75 mM and recorded 85 and 93 per cent reduction in lesions number/size, respectively in the first trial, whereas 87 and 92 per cent reduction in lesions number/size, respectively in the second trial [48].

Silver nanoparticles were sprayed at different concentrations *viz.*, 25, 50, 100 and 200 ppm on rice seedlings under greenhouse condition to evaluated the effectiveness against rice blast pathogen. Silver nanoparticles at 100 ppm when sprayed three times *i.e.* 3 hours before inoculation, 1 and 5 days after artificial inoculation with spore suspension. It was found that silver nanoparticles were highly efficient when applied both as preventive and after inoculation with 26.7, 15.3 and 20 per cent incidence, respectively as compared to the untreated plants which showed 80 per cent disease incidence [26].

Table 1. Efficacy of silver nanoparticles against plant pathogenic fungi and bacteria at different concentrations under in vitro conditions

SI.No	Nanoparticles studied	Concentration	Against fungi/bacteria	Efficacy	Author
1	Silver	10 ppm	Raffaelea sp.	Inhibitory effect on germination of fungal hyphae and conidia	Kim et al. [16,21-23]
2	Silver	50 ppm	Bipolaris sorokiniana and Magnaporthe grisea	Inhibitory effect on formation of fungal colonies	Jo et al. [21]
3	Silver	7 ppm	Sclerotium cepivorum	Inhibited the growth of fungi by 90%	Jung et al.[24]
4	Silver	100 μg/ml	Rhizoctonia solani, Aspergillus flavus and Alternaria alternata	Inhibition of mycelial growth	Kaur et al.[25]
5	Silver	100 μg/ml	Rhizoctonia solani, Sclerotinia sclerotiorum and S. minor	Inhibition of mycelial growth	Min et al. [23]
6	Silver	25, 50, 100 and 200 ppm	Magnaporthae grisea	Inhibition of both hyphal growth and number of colonies	Elamawi et al. [26]
7	Silver	50 ppm	Cladosporium cladosporoides and Aspergillus niger	Inhibited the fungal growth by 70-90%	Elamawi et al. [26]
8	Silver	-	Fusarium oxysporum, Alternaria alternata and Aspergillus flavus.	Exhibited antifungal activity	Askar et al. [40].
9	AgNPs	15 ppm	Alternaria alternata and Botrytis cinerea	Inhibited the growth of mycelia	Ouda [27]
10	Silver	10, 12 and 14 ppm	Rhizoctonia solani AG1, R.solani AG4, Macrophomina phaseolina, Sclerotinia sclerotiorum and Pythium aphanidermatum	90 to 100% of fungal growth inhibition	Mahdizadeh et al.[28]
11	Silver	1250 ppm	Fusarium oxysporum f. sp. Lycopersici	Inhibited the growth of mycelia by 86.06%	Surega et al. [29]
12	AgNP	150 μg ml ⁻¹	Gibberella fujikuroi	Reduced viability of <i>G. fujikuroi</i> conidia by 50%	Jo, et al. [30].
13	Colloidal silver particles	500 ppm	Phytophthora infestans	Inhibited the mycelial growth by 86.13%	Shantamma [31]
14	Ag-NPs	-	Escherichia coli	Antibacterial activity	Pal et al. [33]

15	Ag-NPs	-	Escherichia coli and Staphylococcus aureus	Antibacterial activity	Ingle et al. [34]
16	Ag-NPs	-	Escherichia coli	Antibacterial activity	Panacek et al. [35]
17	Silver nano particles with broad spectrum antibiotics	10 mg L ⁻¹	Bacillus subtillus, B. cereus, Klebsiella pneumoniiea and Vibrio cholera	Antibacterial activity	Geoprincy [36]
18	Ag-NPs	15.3 μg/ml to 153.6 μg/ml	Aeromonas hydrophila (causes disease in both fish and humans)	Antibacterial activity	(Sarkar et al. [37]
19	Silver	4–6 ppm	Intramachrophage Brucella melitensis	Antibacterial activity	Alizadeh et al. [38]
20	Silver particles and AgGO nanocomposites	100 mg/ml	Gram-negative bacteria (Salmonella typhi and Escherichia coli) Gram-positive bacteria (Staphyloccocus aureus and S. epidermidis)	Exhibited stronger antibacterial properties	Chook et al. [39]
21	Silver	-	Erwina caratovora, E. amylovora, Dickya chransanthemi, D. dianthicale, Pectobacterium wasaibiae, P. atrosepticum chransanthem and P. wasabiae)	Antimicrobial activity	Askar et al. [40]
22	Nano silver- NanoCidR L2000	3.12 μg/ml, and 6.25 μg/ml	Escherichia coli O157:H7, Salmonella typhimurium, Vibrio parahaemolyticus and Listeria monocytogenes	Antibacterial effect	Zarei et al. [41]
23	Colloidal silver particles	2.5, 5, 10, 25,50 and 100ppm	Xanthomonas axonopodis pv. punicae	Complete inhibition of bacterial growth	Shantamma [31]

The AgNPs were recorded efficient against nematode pathogen *Meloidogyne graminis* infesting Bermuda grass (*Cynadon dactylon* and *C. transvalensis*) when applied at biweekly intervals at 90.4 mg/m² by reducing the fall formation and improving the quality of turf grass without phytotoxicity effect [49].

Treatment of *Gibberella fujikuroi* infested seeds with AgNP at 150 μg ml⁻¹ for 12 or 24 h significantly improved seedling emergence and height. Adverse effects on germination rate and seedling growth were not observed with any of the AgNP treatments (150 μg ml⁻¹ for up to 48h exposure). The antifungal effect of AgNP against *G. fujikuroi* suggested that, AgNP is a new antifungal compound that can be used for managing important seedborne fungal pathogens of rice [30].

Among the different combinations of colloidal silver particles and chemical fungicides tested against late blight of potato under field conditions, 500 ppm of colloidal silver in combination with 0.2 per cent of cymoxanil 8% + mancozeb 64% recorded maximum disease reduction as compared to colloidal silver alone and its combination with dimethomorph 50% WP [31].

No significant enhancement of inhibitory activity was found against pomegranate bacterial blight between streptomycin sulphate 90% + tetracycline hydroxide 10% alone and in combination with colloidal silver particles [31].

6. CONCLUSION

Management of fungal and bacterial diseases of food crops is economically important. Recently, a greater effort has been given to development of safe management methods that cause less harm specially to humans, animals and environment at large with a focus on overcoming deficiencies of synthetic fungicides. Different types of nano has significant antifungal particles antibacterial activity. Thus, it can be effectively used against plant pathogenic fungi and bacteria to protect the crop plants and their products, in place of using the commercially available synthetic fungicides, which are less ecofriendly at large. Moreover, this report opens up for further research on the areas of understanding the mode of action of nano particles on the phytopathogenic fungi and bacteria, and impact on plant metabolism could help us using nanoparticles more wisely in agriculture.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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