Mycopath (2015) 13(1): 1-6 **Research Article**

Environmentally friendly synthesis of silver nanoparticles using *Moringa oleifera* (Lam) leaf extract and their antibacterial activity against some important pathogenic bacteria

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Abstract

Conventionally physical and chemical methods are used for the biosynthesis of silver nanoparticles. Biological methods of nanoparticles synthesis are cost effective, easily scaled up and environmental friendly. A green synthesis of silver nanoparticles has been achieved using environmentally acceptable plant extract. It was observed that *Moringa oleifera Lam* leaf extract can reduce silver ions into silver nanoparticles at room temperature. The synthesized nanoparticles have been characterized by the UV–visible spectroscopy, high resolution transmission electron microscopy. Further, the antibacterial activity of silver nanoparticles was evaluated by well diffusion method and it was found that the biogenic silver nanoparticles have antibacterial activity against *Pantoea agglomerans* (27mm) followed by *Ralstonia solanacearum*, *Erwinia amylovora* and *Pseudomonas lachrymans* (19.66 mm, 16.66 mm and 13 mm, respectively) and the lowest for *Agrobacterium tumefaciens* and no effect on *Erwinia carotovora* compared to control. Investigation on the antibacterial activity of synthesized silver nanoparticles against some phytopathogenic bacteria reveals high potential as antibacterial agent in controlling various plant diseases caused by bacteria.

Keywords: Antibacterial, silver nanoparticles, phythopathogenic bacteria, *Moringa oleifera.*

Introduction

In recent years, scientists have focused on the increase of food production needed for the fast expansion of world population. Unfortunately, substantial yield losses occur due to insects and plant diseases caused by fungi, bacteria and viruses (Fletcher *et al.,* 2006). Bacteria have also unfavorable effects on quality, safety and preservation of food (Kotan *et al.*, 2010, 2013). Synthetic chemicals are widely used in the control of plant diseases. However, these chemicals may cause toxic residues in treated products (Isman, 2000). Synthetic pesticides can also cause environmental pollution owing to their slow biodegradation (Barnard *et al.,* 1997). In addition, the risk of developing the resistance by microorganisms and the high cost-benefit ratio are other disadvantages of synthetic pesticides usage (Brent and Hollomon, 1998). The growing awareness about the environment has forced the researchers to develop green methods to synthesize their desired product. The major requirements for any green synthesis are non-toxic chemicals, environmentally benign solvents and renewable materials. Nanostructures of noble metals particularly silver nanostructures with 1–100 nm size has attracted significant interest over the years because of the unique size dependent optical, electrical and magnetic properties (Kumar *et al.,* 2014; Nagarajan and Ramasamy, 2014).

Moringa oleifera Lam. (Family: Moringaceae) has gained much importance in the recent days due to its multiple used and benefits to agriculture and industry (Ashfag *et al.,* 2012). Regarded as a miracle plant, all the parts of moringa plant are used for medicinal and other purposes. Roots, flowers, bark, stem, leaves and seeds of moringa possess antimicrobial properties (Anjorin *et al.,* 2010; Dwivedi and Enespa, 2012). Moringa leaves were reported to possess anti-atherosclerosis and anti-oxidant effects (Chumark *et al.,* 2008; Verma *et al.,* 2009). The major phytochemical constituents in the leaves are phenolic compounds and flavonoids such as cryptochlorogenic acid, isoquercetin and astragalin (Vongsak *et al.,* 2012). These compounds are famous for their wide-ranged activities including anti-oxidation, anti-hypertension and anti-

inflammation (Gasparotto *et al.,* 2011; Soromou *et al.,* 2012). This study was carried out to synthesize AgNPs using aqueous extract of *M. oleifera*, which acted as reducing and capping agent for the reduction of silver ions. The synthesized silver nanoparticles were examined through UV–visible spectroscopy, High resolution transmission electron microscopy (HRTEM) analysis, followed by the study of antimicrobial activity against phythopathogenic bacteria.

Material and Methods

Silver nitrate was purchased from Windsor Laboratories Limited, United Kingdom, Sodium chloride were purchased from Sigma-Aldrich Chemie GmbH, Riedstr, Steinheim, Germany. Nutrient Agar, peptone, meat extract and yeast extract were purchased from Loba Chemie. PVT. LDT. India and *Moringa oleifera Lam* leaves were purchased from the local market, the fine powder was obtained from the dried leaves by using Mill (Glen Creston Stammore, England). Finally, the leaf powder was sterilized in an autoclave at a pressure of 15 Ib/sq inch and the temperature 121°C for 5 min. All the aqueous solutions were prepared using de-ionized water obtained from Water distiller LABCONCO water PROT.M PS LABCONCO Corporation, Kansas City, Missouri 64132-USA. The bacterial strains tested were obtained from Bacterial Disease Research Department. Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt. These were originally isolated from different hosts. These strains were *Agrobacterium tumefaciens* (peach), *Erwinia amylovora* (pear), *Erwinia carotovora* (potato), *Pantoea agglomerans* (mango), *Pseudomonas lachrymans* (cucumber) and *Ralstonia solanacearum* (potato).

Preparation of aqueous extract

Aqueous extract of *M. oleifera* leaves was prepared using 10 g of leaf powder was added to 100 mL milliliter of deionized water at 60 °C for 5 min. This extract was filtered through nylon mesh, followed by Millipore filter (0.45 µm).The filtered extract was stored in refrigerator at 4 °C for further studies.

Synthesis of silver nanoparticles (AgNPs)

For synthesis of silver nanoparticles, the Erlenmeyer flask containing 50 milliliter of $AgNO_3$ (2 mM) was reacted with 10 milliliter of the aqueous extract of *M. oleifera* leaf at room temperature.

UV-Visible spectroscopy of AgNPs

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into deionized water. UV-Vis spectral analysis was done by using UV-Visible spectrophotometer double beam.

High resolution transmission electron microscopy (HR-TEM) of AgNPs

The morphology and size of the biosynthesized silver nanoparticles were investigated by HRTEM using Tecnai G 20, FEI, The Netherland at an accelerating voltage of 200 kV. The sample for TEM analysis was prepared by placing a drop of silver nanoparticles solution onto a carbon-coated copper grid, followed by water evaporation in air at room temperature Sathishkumar *et al.* (2009).

Antibacterial activity of AgNPs

Antibacterial activity of the synthesized silver nanoparticles were determined, using the well diffusion method against six phytopathogenic bacteria, *A. tumefaciens*, *E. amylovora*, *E. carotovora, P. agglomerans*, *P. lachrymans* and *R. solanacearum*. All glassware and media used were sterilized in an autoclave at 121 °C for 20 min. The bacterial suspension (10^8 CFU mL^{-1}) was spread on the surface of nutrient agar (NA) which contained different ingredients, peptone (5.0 g), Beef extract (1.0 g), yeast extract (2.0 g) and agar (15.0 g) in distilled water. Atlas (1995). Petri plates were prepared by pouring 20 mL of nutrient agar for all the bacteria. The inoculums were spread on the surface of the solidified media. Once the agar was solidified, agar wells of 6-mm diameter were prepared with the help of a sterilized stainless steel cork borer, and then wells were filled with 20 µL of silver nanoparticles. The plates were kept 30 min for diffusion and then incubated at 28 ºC for 48 h. (Distilled water used as a control). The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each bacterium was recorded and expressed in millimeter. The inhibition zones were compared with that of control. The experiment was performed in three replicates.

Results and Discussion

The green synthesis of silver nanoparticles using of *M. oleifera* leaves extract was

successfully carried out, as the change in the color of the solution from yellowish brown to dark brown color (Fig. 1) exhibits the reduction of the silver nitrate in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Shankar *et al.,* 2004).

The formation of silver nanoparticles was confirmed through measurement of UV-Visible spectrum of the reaction mixture. The UV-Visible spectrophotometric analysis of colloidal reaction mixture of silver nanoparticles synthesized using *M. oleifera* leaves extract showed sharp peak at 434 nm in the spectrum (Fig. 2).

HRTEM has been employed to characterize the size, shape and morphologies of formed silver nanoparticles. HRTEM image is the evidence that the morphology of silver nanoparticles is nearly spherical in shape and few nanoparticles were agglomerated. The nanoparticles were not in direct contact even within the aggregates and were surrounded by thin layer of organic material, indicating stabilization of the nanoparticles by a capping agent. The particle size measured from the HRTEM images was observed to be 5 to 50 nm (Fig. 3).

The AgNPs synthesized from *M. oleifera* leaves extract exhibited potent antibacterial activity against some of the phytopathogenic bacteria used in this study. After 48 h of incubation at 28 ºC, the zone of inhibition was observed in plates loaded with AgNPs. All the plates showed clear zones around the wells are depicted and the measured diameter of zones is in (Fig. 4). The zone formation indicates that the AgNPs suppressed the growth of microorganisms. Aruna *et al.* (2012). The result of antibacterial test (diameters of zone of inhibition) is tabulated in Table (1). The highest zone of inhibition was observed for *P.agglomerans* (27 mm) followed by *R. solanacearum*, *E. amylovora* and *P. lachrymans* (19.66, 16.66 and 13 mm, respectively) and the lowest for *A. tumefaciens* and no effect on *E. carotovora.* From these results, it is evident that the nanoparticles synthesized may have important applications in controlling various plant diseases caused by bacteria.

The exact mechanism that silver nanoparticles employ as an antimicrobial is not clearly known and is a debated topic (Prabhu and Poulose, 2012). It is well established that Ag^{+} and Ag^{-} ions founded compounds have strong antimicrobial activity; several researchers are keen in using other inorganic nanoparticles as antibacterial agents. Jeeva *et al.* (2014). Also, AgNPs may attach to the cell wall thus leading to cell disruption by varying membrane permeability and cell respiration. AgNPs can penetrate inside the cell also since, they have a greater affinity to react with sulfur containing proteins in the cell wall and inside the cell and also phosphorous containing elements such as DNA (Hajipour *et al.,* 2012).The action of AgNps on the bacteria was also due to the interaction with thiol group compounds found in the respiratory enzymes of bacterial cells thus inhibiting the respiration process in bacteria (Li *et al.,* 2011).

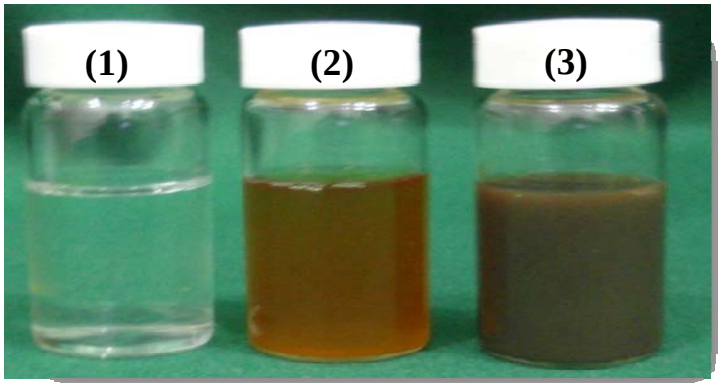

Fig. 1: Synthesis of AgNPs by *M. oleifera* leaves extract. The figure shows vials containing samples of the culture filtrate after exposure to silver nitrate alone (1), *M. oleifera* leaves extract (2) and *M. oleifera* leaf extract with $AgNO_3$ (3). It is observed that the color of the solution turned from colorless to brown, indicating the formation of AgNPs.

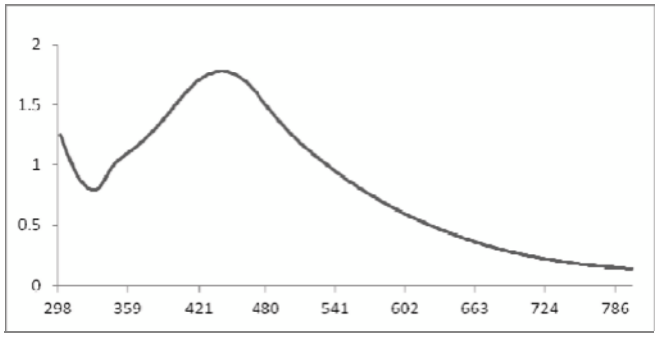

Fig. 2: UV-vis spectrum of green synthesized silver nanoparticles.

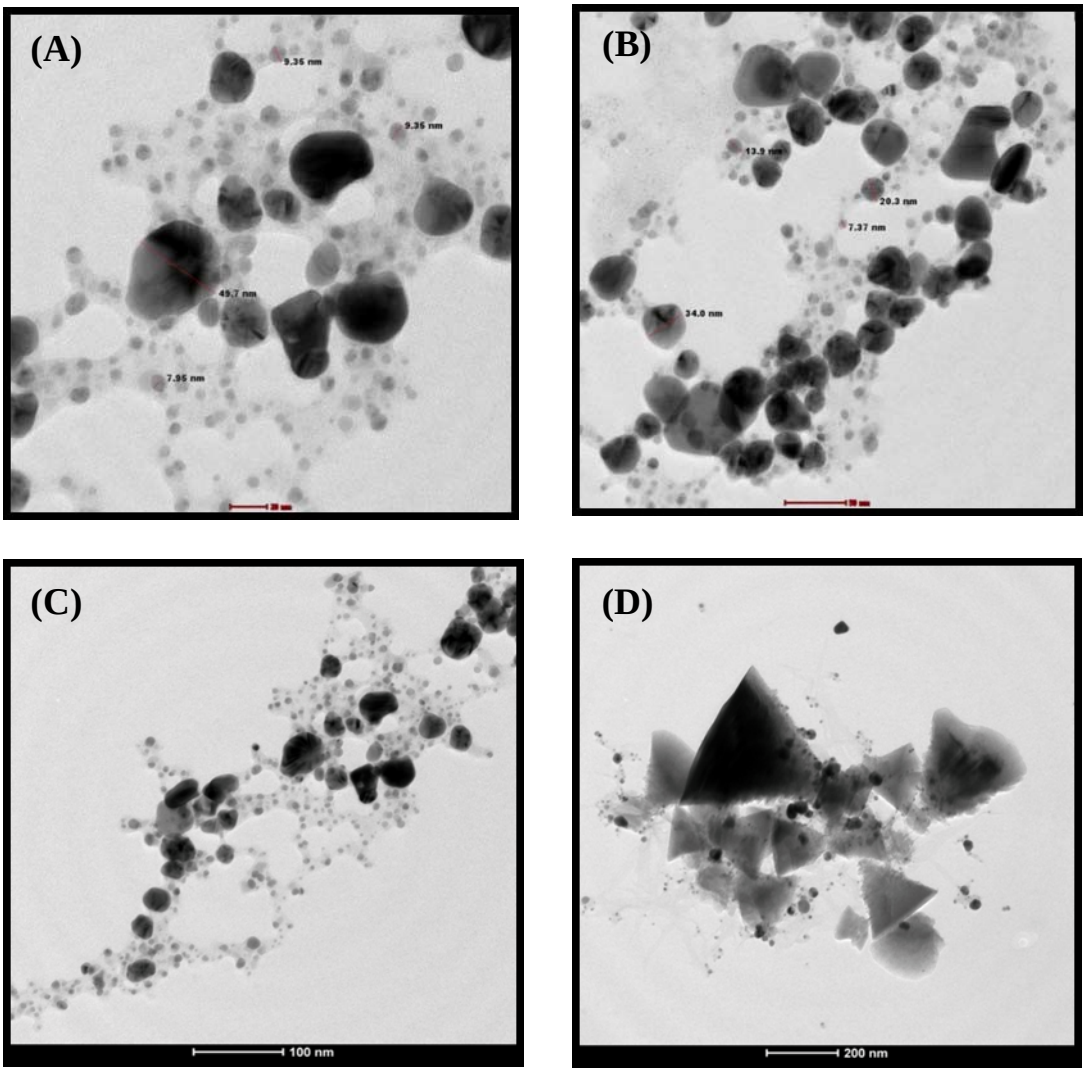

Fig. 3: HRTEM micrograph of AgNPs synthesized by *Moringa oleifera* leaf extract fluorence scale bar: (A) 20 nm (B) 50 nm (C) 100 nm (D) 200 nm.

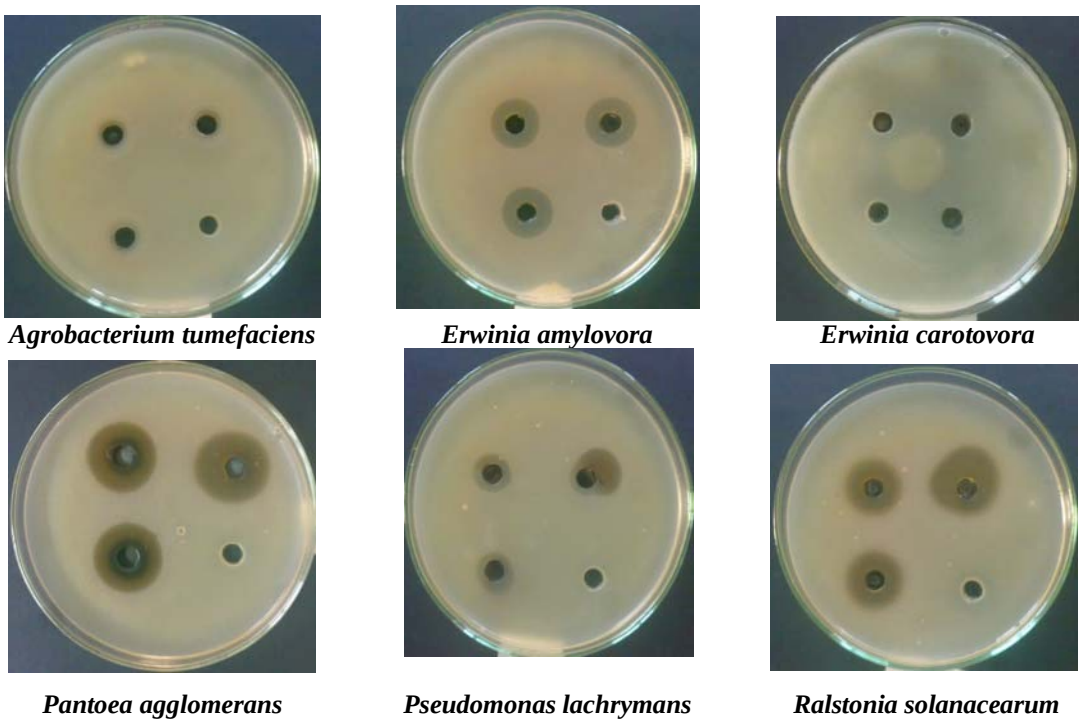

Fig .4: Antibacterial activity of synthesized silver nanoparticles against phytopathogenic bacteria.

Table 1: inhibition zone (mm) of silver nanoparticle synthesized from *M. oleifera* leaf extract against phytopathogenic bacteria. .

S.No.	Bacterial Strains Used	Inhibition of zone (mm)
1	*Agrobacterium tumefaciens*	9.00 ± 0.00
2	*Erwinia amylovora*	16.66 ±0.57
3	*Erwinia carotovora*	0.0 ± 0.00
4	*Pantoea agglomerans*	27.00 ± 2.00
5	*Pseudomonas lachrymans*	13.00 ± 1.00
6	*Ralstonia solanacearum*	19.66 ± 2.08
	Control	0.0 ± 0.00

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Conclusion

The biological synthesis of silver nanoparticles is rapid, simple, safe, one-step, cost effective, eco-friendly and novel synthesis route for preparing silver nanoparticles was carried out using *M. oleifera* leaf extract as a reducing and capping agent at room temperature. The synthesized silver nanoparticles were characterized by UV-visible spectrometer and HR-TEM analysis. The size of the nanoparticles ranges from 5 to 50 nm with spherical shape. These nanoparticles showed excellent inhibitory activity against *Pantoea agglomerans, Ralstonia solanacearum and Erwinia amylovora* compared to control. The presented results are initial data and evaluation of the activity should be continued in green house and field to determine the efficacy and activity of the prepared silver nanoparticles.

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