

Original Research Article

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Sunlight Induced Mediated Silver Nanoparticles from Seeds of *Thevetia peruviana* L., Characterization and their Antifungal Efficacy against *Curvularia lunata* (Wakker) Boedijn

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ABSTRACT

Green synthesis of nanoparticles is one of the most effective processes at a very affordable cost. Green synthesized nanoparticles have wide range of antimicrobial property. *Thevetia peruviana* is commonly called as yellow oleander has chemicals (thevetin A and thevetin B) which help in bio-reduction of metals and antimicrobial property. Ripen yellow oleander seeds were used to prepare extract. Silver nanoparticles were synthesized by exposing AgNO₃ (1mM) with *T. peruviana* seeds extract (10 %) to the bright sunlight, autoclaved method and combination of both methods. Colour change was noted from light milky white to dark orange in all the three methods tried. Synthesized silver nanoparticles were characterized by UV-Visible spectrophotometer and Atomic Force Microscope. Absorption spectra of sunlight exposed AgNPs was 425 nm, autoclaved method 415-420 nm and combination of both recorded 350 nm. Size of AgNPs varied from 50 to 200 nm and spherical to irregular in shape. Antifungal efficacy of AgNPs after 24 h of incubation showed more than 95.00 per cent inhibition of *Curvularia lunata* (Leaf spot of maize) in sunlight and autoclaved method while combination of both methods recorded 58.68 per cent and control (no inhibition). Inhibition of spore germination was on par with fungicide (propiconazole @ 0.1 %). Silver nanoparticles synthesized from sunlight and autoclaved method were more effectively inhibited spore germination compared to combination of both methods and control.

Keywords

Silver nanoparticles,
Thevetia peruviana,
Curvularia lunata,
Spore germination.

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Introduction

Nanoscience has become one of the leading sciences within the last decade with fundamental and applied research prospects in all basic cognitive sciences such as physical, life and earth sciences (physics, chemistry, biology, medicine, engineering and agriculture). The application of nanotechnology in industrial production will create a new path of revolution in few years and the common man will get maximum

benefit when it is commercialized. In the days to come, application of new tools of nanotechnology will definitely revolutionize both agriculture and food industry. Green nanotechnology in agriculture has been theoretical and begun. Green nanotechnology can be applied in various aspects of agriculture viz., manufacture of nano fertilizers, nano pesticides, growth regulators, textiles and food processing. Bio-synthesis of

plant based nanoparticles is economically feasible compared to nanoparticles developed through chemical synthesis. Plants are often attacked by various pathogens such as fungi, bacteria and viruses which results in great loss to farmers. Application of nanotechnology in plant pathology helps in detection, management of plant pathogens and to study the host-pathogen interactions.

Many research workers tried the extracts of different plant species for green synthesis of nanoparticles which showed antimicrobial activity. In the present study we used *Thevetia peruviana* (Yellow oleander) seed extract for the synthesis of nanoparticles from silver nitrate. *T. peruviana* is a small ornamental shrub, which grows up to a height of about 3 to 4 meters. This plant is native to Central and South America but now frequently grown throughout the tropical and sub-tropical region of the world. It is an evergreen small shrub that bears yellow, trumpet like flowers and its fruit is black in colour encasing a large seed. It has a milky sap containing a compound- thevetin (A and B) that is used as a heart stimulant but in its natural form, it is extremely poisonous, especially the seeds. It has immense medicinal properties. This plant has also got very good antimicrobial activity (Alhashimi *et al.*, 2013). Silver nitrate itself has got antimicrobial activity (Kim *et al.*, 1998) but if it is converted into nano size then its property and activity will be changed with respect to inhibiting or enhancing the microbial growth (Jo *et al.*, 2009). In this context an attempt was made where *T. peruviana* seeds were used for the green synthesis of silver nanoparticles (AgNPs). Further, antifungal activities of such synthesized nanoparticles were tested against *Curvularia lunata*, which causes leaf spot in maize crop. *Curvularia* leaf spot of maize was reported for the first time in India from Varanasi region of Uttar Pradesh (Mandokhot and Chaudhary (1972). Grain yield loss varied from 8.3 to 22.6 per cent and fodder yield 9.4

to 24.8 per cent in different commercial hybrids.

Materials and Methods

Preparation of seed extract

Ripen seeds of *T. peruviana* were collected within the premises of campus of Agricultural College, University of Agricultural Sciences, Dharwad. Twenty five grams of seeds was crushed in to paste by using grinder. Volume was made up to 250 ml by adding Millipore filtered water. Further, such extract was boiled for 5 min and filtrated through Whatman No. 1 filter paper and centrifuged at 15,000 rpm for 25 min for removal of seed debris. Finally, supernatant collected was transferred to clean screw cap glass bottles covered with aluminum foil and stored at 4 °C for further use.

Green Synthesis of silver nanoparticles

For synthesis of silver nanoparticles, silver nitrate (HiMedia Laboratories, Mumbai) @ 1mM is used as precursor and *T. peruviana* seed extract @ 10 per cent as reducing agent. Twenty ml of silver nitrate was mixed with 5 ml of seed extract and subjected to different methods of green synthesis. Silver nanoparticles were synthesized by following different methods such as exposing this mixture to bright sunlight for 1 h, autoclaving at 121°C, 15 lb pressure for 15 minutes and combination of both methods. Control was maintained with 5 ml of *T. peruviana* seed extract + 20 ml of deionized water.

Characterization of silver nanoparticles

Detection of λ_{max} of AgNPs was done by using UV-Visible spectrophotometer (Spectrum, SP-UV500DB/VDB). Reaction mixture of 10 μ l was diluted with de-ionized water to make a final volume of 3 ml and absorbance was measured between range of

300-700 nm wave length. Size and shape of the nanoparticles were determined by using AFM (NanosurfAG, Swiss, FlexAFM, with 24bit C3000) and analysis was done by Nanosurf Reporter software.

Antifungal studies against *Curularia lunata*

The pathogen was isolated from infected leaves of maize showing typical symptoms. Culture was isolated on potato dextrose agar (PDA) by following standard tissue isolation method and further purification of culture was done by single spore isolation method. Spore germination method was carried out to know the efficacy of AgNPs against *C. lunata*. Conidia collected from seven days old culture was transferred into cavity slides with eight different treatments, the slides were incubated at room temperature (27±1°C) for germination. Observations were taken at 6 h and 24 h after incubation. Phytotoxicity of green synthesized AgNPs was tested on tomato seedlings of 30 days old by spraying under glasshouse condition.

Results and Discussion

The formation of AgNPs in the reaction mixture of yellow oleander seed extract and silver nitrate was preliminarily indicated by colour change in reaction mixture. The colour

change in the reaction mixture was noticed immediately after exposing to bright sunlight and drastic colour change was observed within 10 minutes of exposure and final observation was recorded after one hour (Fig. 1). Sunlight exposed and autoclaved treatments showed change in colour from milky white to dark orange, whereas in combination of both treatments the final colour of the reaction mixture was darker than other two treatments (Fig. 2).

The change in colour of reaction mixture may due to presence of theveridoside (thevetin A and thevetin B) digitoxigenin, cerberin, peruvoside and theveside in the plant (Sangodare *et al.*, 2012). Similarly, Rupiasih *et al.*, (2013) synthesized AgNPs from *T. peruviana* latex extract and reported that biomolecules present in the latex responsible for the reduction of AgNPs. The UV-Visible spectrum of sunlight exposed AgNPs was 425 nm, autoclaved method 415-420 nm and whereas combination of both the method recorded 350 nm (Fig. 3). Similar result was observed by Brahmachari *et al.*, 2014) when AgNPs were synthesized from *Ocimum sanctum* leaves extract but absorption maxima differed from Rupiasih *et al.*, 2013) who used *T. peruviana* latex extract for the synthesis of AgNPs, where absorption maxima was 570 nm.

Table.1 Efficacy of antifungal activity silver nanoparticles against *Curularia lunata*

Sl. No.	Treatment	Spore germination at	
		6 h	24 h
1	Sterile distilled water	23.44 (4.94)*	80.13 (9.01)
2	Sucrose (2 %)	93.50 (9.72)	100.00 (10.05)
3	<i>Thivetia peruviana</i> seed extract (10 %)	100.00 (10.05)	100.00 (10.05)
4	Silver nitrate (1 mM)	00.00 (1.00)	01.81 (1.61)
5	Silver nitrate (1 mM) + Seed extract (10 %) → Sunlight	00.00 (1.00)	00.48 (1.19)
6	Silver nitrate (1 mM) + Seed extract (10 %) → Autoclaving	00.00 (1.00)	03.86 (2.07)
7	Combination of sunlight and autoclaving method	56.28 (7.56)	58.68 (7.72)
8	Propiconazole (0.1%)	00.00 (1.00)	00.00 (1.00)
S.Em.±		0.13	0.24
CD @ 1%		0.55	1.00

* $\sqrt{x+1}$



Fig.1 Gradual color change in bright sunlight exposure treatment during the synthesis of AgNPs by 10 % of *T. peruviana* seed extract at different time intervals

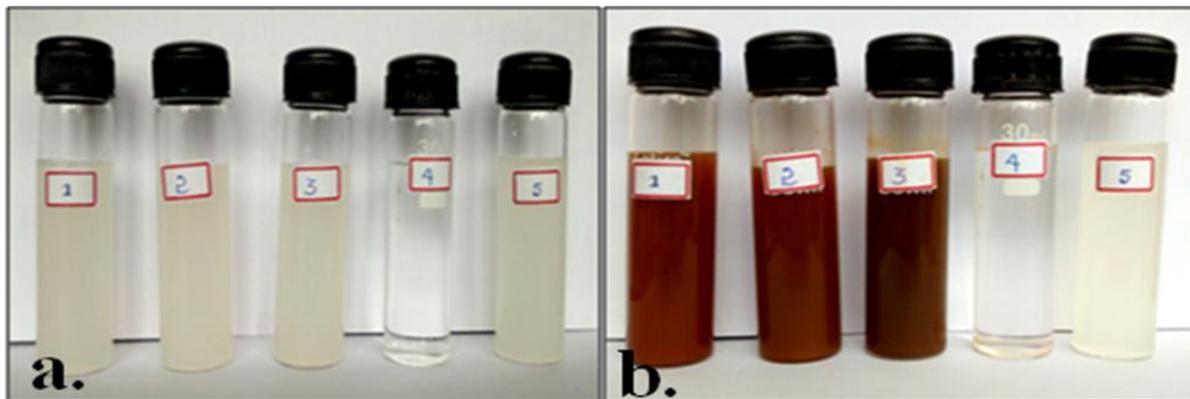


Fig.2 Synthesis of AgNPs from *T. peruviana* seed extract through different methods of green synthesis. Reaction mixture before (a.) and after (b.) subjecting to treatments [1. Exposure to sunlight, 2. Autoclaving, 3. Combination of exposure to sunlight and autoclaving, 4. AgNO₃ (1 mM) alone, 5. *T. peruviana* seed extract (10 %)]

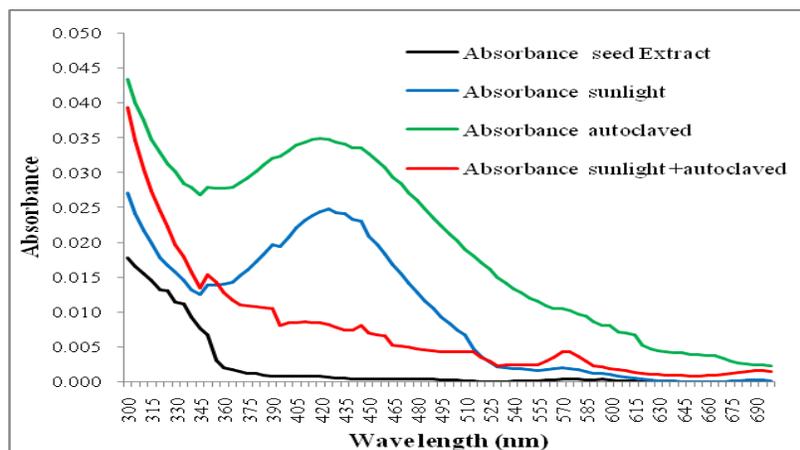


Fig.3 UV-Vis absorbance for AgNPs synthesized by different methods from *T. peruviana*

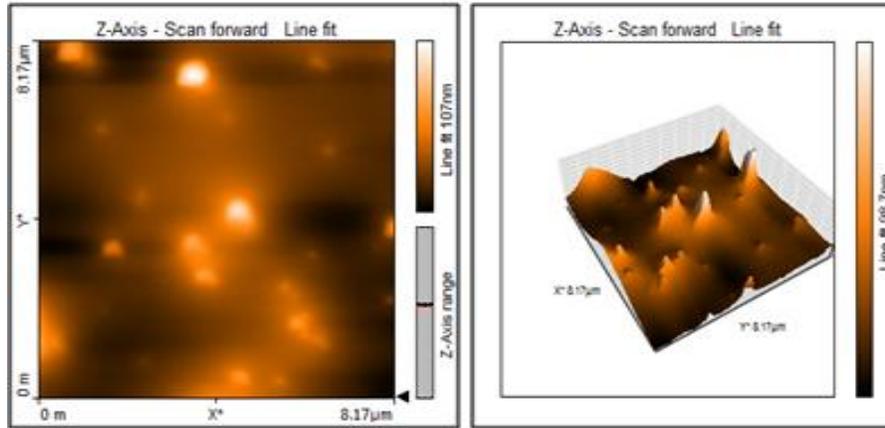


Fig.4 AFM images of green synthesized AgNPs using *T. peruviana* seed extract

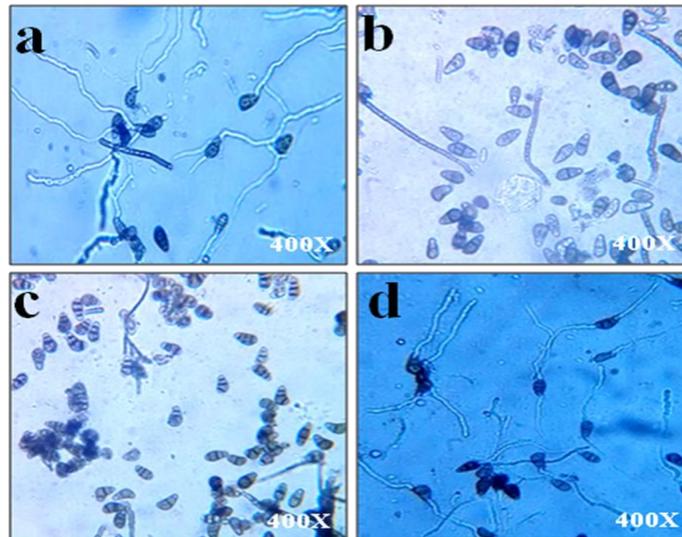


Fig.5 Antifungal efficacy of AgNPs against spore germination of *Curvularia lunata*. a. Control (water) b. AgNPs -Sunlight exposed c. AgNPs -autoclaved method d. *Thivetia peruviana* seed extract

The AFM image analysis revealed the topography, size of AgNPs. In general, it was observed that size of the AgNPs was ranged between 50-200 nm (Fig. 4), whereas shape of AgNPs was spherical to irregular.

Antifungal studies of green synthesized AgNPs against *C. lunata* was carried out. Observation regarding conidial germination was recorded at 6 h and 24 h after incubation for all treatments and the results are presented in the table 1. The conidia were failed to germinate after 6 h which were treated with AgNPs synthesized through

exposing to bright sunlight, autoclaving method and popiconazole @ 0.1 per cent. But, in case of combination (sunlight exposure and autoclaving method) treatment 56.28 per cent conidial germination was observed. The observation recorded 24 after incubation revealed that more than 95.00 per cent inhibition of conidia of *C. lunata* in sunlight and autoclaved method while combination of both methods recorded 58.68 per cent and control (no inhibition), it may due to the subjecting of reaction mixture to excess heat might have changed the properties of AgNPs

compared to other treatments. *T. peruviana* seed extract itself has anti-microbial property but in this study 10 per cent of seed extract has induced the 100 per cent conidial germination (Fig. 5), while AgNPs synthesized from these seed extract showed zero per cent germination, it may be due to photolytic activity of reaction mixture. The antimicrobial activity of *T. peruviana* against *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* has been reported (Alhashimi *et al.*, 2013) and showed effective inhibition but very less research literature is available on antimicrobial activity against plant pathogens compared to other microorganisms.

Green nanotechnology is very interesting research area, synthesis of AgNPs from plant extract is eco-friendly and cost effective method compared to other methods. Present study revealed the rapid synthesis of silver nanoparticles from *T. peruviana* seed extract under bright sunlight, autoclaved method and combination of both methods. Silver nanoparticles synthesized from sunlight and autoclaved methods were more effectively inhibited the spore germination compared to combination of both methods and control. AFM observation confirmed that the size of the green synthesized AgNPs were in the range of 50-200 nm and they were having spherical to irregular shape. Bio-organic components which are present in plant might have acted as a stabilizer for the synthesized silver nanoparticles and which were effective against plant pathogens.

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