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Green synthesis and characterization of silver (Ag) nanoparticles using neem leaf extract and its antifungal activity against seed borne pathogens in chilli

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ABSTRACT : The silver nanoparticles (AgNPs) synthesized using hot water neen leaf extract (NLF) as reducing and stabilizing agent are reported and evaluated for antibacterial activity against chilli seed borne pathogens. The effect of extract concentration, contact time, pH and temperature on the reaction rate and the shape of the Ag nanoparticles were investigated. The data revealed that the rate of formation of the nanosilver size decreased significantly in the basic medium with different concentration (1 mM, 5 mM and 10 mM). Synthesized was characterized by UV–vis spectroscopy, X-ray diffraction, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The nanoparticle synthesis using different concentration of silver nitrate and 5 mM was getting nano sized silver particles AgNPs. The silver nanoparticles were with an average size of 55 to 350 nm and mostly rod shape. The antifungal activity of synthesized AgNPs was observed in seed health test and different concentration of silver NPs (750 mg, 1000 mg and 1250 mg) were used for seed health test. The AgNPs at 750 mg concentration significantly inhibited the seed borne pothogens. Thus AgNPs showed broad spectrum antifungal activity at lower concentration and may be a good alternative therapeutic approach in future.

KEY WORDS : Green synthesis, Silver nanoparticles, Neem leaf extract, Antifungal activity

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Chilli (*Capsicum annuum* L.) is an important vegetable cum spice crop which is grown for both domestic and export market. It is a rich source of Vitamin C, A and B. India is the largest producer of chillies in the world (8.5 lakh tonnes) followed by China (4 lakh tonnes), Pakistan (3 lakh tonnes) and Mexico (3 lakh tonnes). Andhra Pradesh ranks first in India both in area and production with 2.04 lakh hectares producing 323 thousand tones. Chilli crop suffers with many fungal, bacterial and viral diseases resulting in enormous yield losses. Among the fungal diseases, in recent years dry root rot of chilli caused by *Sclerotium rolfsii* is of major concern and causing the high economic losses in chilli (Kalmesh and Gurjar, 2001). It affects the yield severely whenever it occurs at any stage of the crop. Presently, greater emphasis should be placed on nanofungicides to control the soil borne pathogens and to avoid the development of resistant strains effectively. Hence, a holistic approach is formulated for the effective management and to examine the antifungal activity against seed borne pathogens in chilli.

Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis (Bhattacharya and Gupta, 2005). A great deal of effort has been put into the biosynthesis of inorganic material, especially metal nanoparticle using micro-organisms and plants (Mohanpuria *et al.*, 2008 and Farooqui *et al.*, 2010).

RESEARCH METHODS

Synthesis of silver nanostructures :

Materials :

Silver nitrate $AgNO_3$ was obtained from Sigma–Aldrich chemicals and used as received. Deionized water was used throughout the reactions. All glass wares were washed with dilute nitric acid HNO_3 and distilled water, then dried in hot air oven. Neem leaf was boiled for 15 min, filtrated and completed to 100 ml to get the extract. The filtrate used as reducing agent was kept in the dark at 10° C to be used within one week. A stock solution of $AgNO_3$ 2×10^{-2} M was prepared by dissolving 0.34 g/ 100 ml de-ionized water.

Characterization :

The prepared silver nanoparticles were subjected to various characterized techniques like X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscope with EDS (SEM/EDS) and particle size analyzer.

Scanning electron microscope (SEM) :

SEM FEI QUANTA 250 was used to characterize the size and morphology of the nanoparticles. Sample of test naoparticles (0.5 to 1.0 mg) was dusted on one side of the double sided adhesive carbon conducting tape, and then mounted on the 8 mm diameter aluminum stub. Sample surface were observed at different magnification and the images were recorded.

Transmission electron microscope (TEM) :

TEM FEI TECHNAI SPRIT, make or source was used to analyze the sample. Dilute suspensions of nanoparticles (0.50 mg) in pure ethanol (15 ml) were prepared by ultrasonication. A drop of the suspension placed on 300-mesh lacy carbon coated copper grid, dried and the images were recorded at different magnification.

X-ray diffraction :

The X-ray diffractograms have been recorded on Powder XRD (Bruker D8 Advance Powder X-ray Diffractometer, Germany). The machine exploits Cu-Kα radiation (0.154 nm) for measuring the crystalline nature of atoms in the material (Toraya, 1986). The diffractograms were recorded in the range of 2θ = 10-80 degrees at a scanning speed of 0.080 and step times 1s at room temperature 25°C.

Seed health – Pathogen infection :

Seed health testing for fungal infection was carried out using blotter technique. Twenty five seeds in four replicates were placed equidistantly on three layered sterile blotter paper moistened with 0.2 per cent 2, 4-D solution in sterile Petri plates under aseptic condition and incubated at 20±2°C for seven days with alternate cycles of 12 h in near ultraviolet light (NUV) range and for the remaining 12h in dark. On the eighth day, the seeds were examined for the presence of fungal infection. The number of infected seeds was counted and the mean value was expressed in percentage (ISTA, 2010).

RESEARCH FINDINGS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

SEM and TEM analysis :

The formation of silver nanostructures was initially confirmed with settlement of nanoparticles at different concentration (Fig. 1) due to surface plasmon resonance phenomenon provides a convenient signature to indicate the formation of Ag NPs in the reaction mixture. The surface morphology of the nanoparticles synthesized and examined under SEM revealed that flower-like silver nanostructures (Fig. 2) measuring 300 to 350 nm

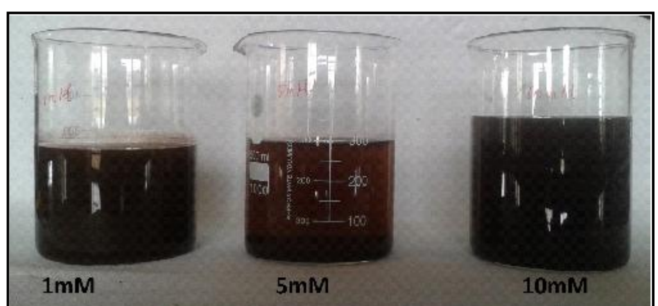

Fig. 1 : Colour change during the phytoreduction of $AgNO_3$ into Ag NPs at the beginning and after 24 h of reaction

Table 1 : Effect of concentration of silver nitrate on size of silver particles

Concentration of SLS added (M)	Effective concentration of silver nitrate in the reaction mixture (mM)	Particle size (nm)
1.0 mM	0	1.2 µm
5 mM	5	300-350
10 mM	25	641 nm

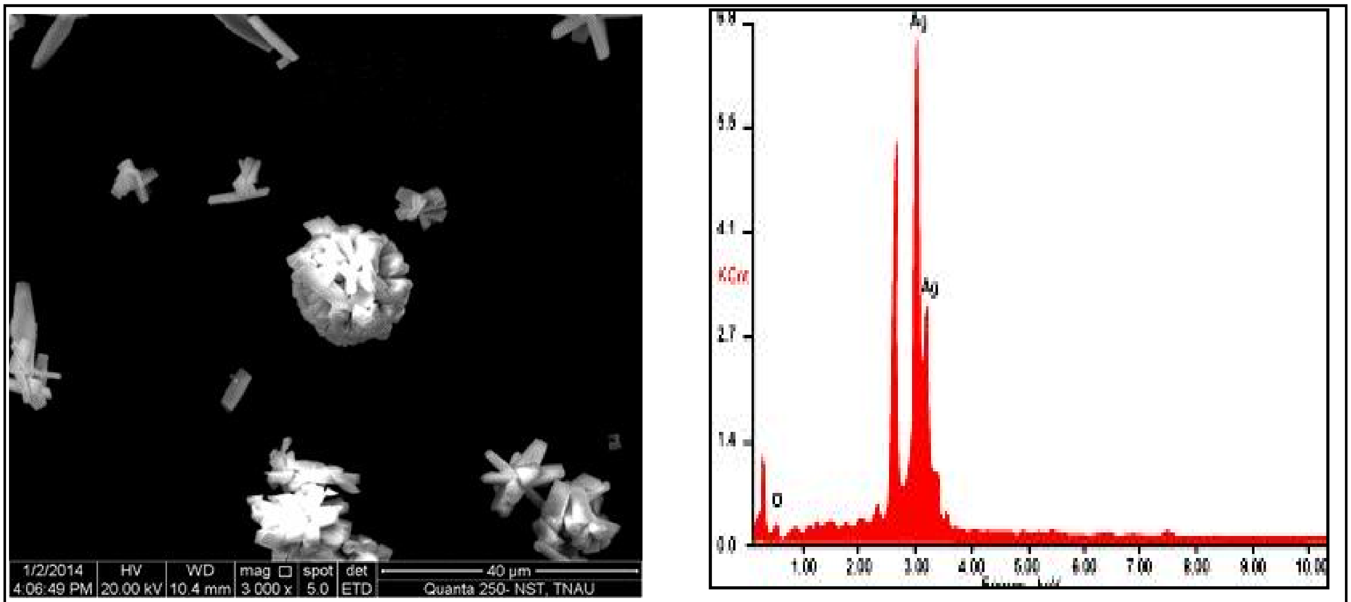

Fig. 2 : SEM / EDS image of Ag nanoparticles

diameter. In this study, $AgNO_3$ was used to prepare rod shaped nano Ag at mild reaction temperature. The same morphology was obtained when using $AgNO_3$ as reported by Lee and Meisel (2005).

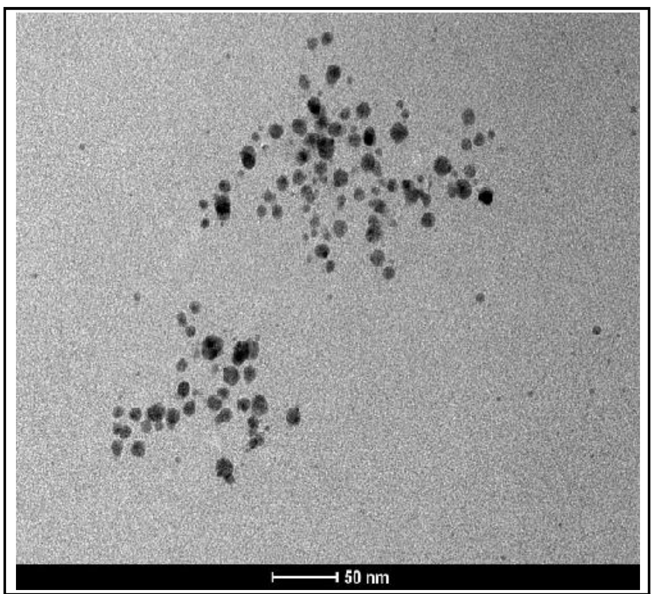

Fig. 3 : TEM image of Ag nanoparticles

To confirm the results of SEM, the same nanoparticles were characterized under TEM. Ag nanoparticles were diagnosed primarily to be cylindrical in shape as against spherical under SEM but measured 100 nm in conformity (Fig. 3).

Power XRD analysis :

The phase structure and the purity of the Ag were

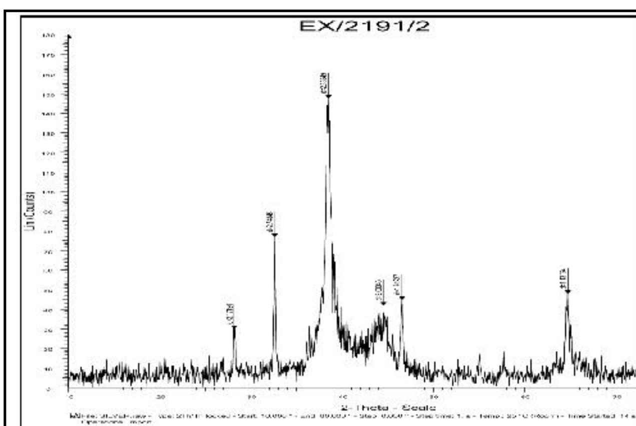

Fig. 4 : Powder XRD patterns of Ag nanoparticles

Table 2 : Effect of inorganic nanoparticles on pathogen infection (%) of stored (Accelerated aging) seeds of chilli cv. PKM 1

Treatments (mg /kg^{-1})	TiO_2	ZnO	Ag	Cu	Mean
500	13	12	7	9	10
750	10	12	6	8	9
1000	8	10	4	9	8
1250	12	9	4	6	8
Mean	11	11	5	8	9
Control	16				
	Treatments		Dosage		Treatment x Dosage
S.E.$\pm$	0.17		0.15		0.35
C.D. (P=0.05)	0.35**		0.31**		0.70**

examined by Power X-ray diffraction studies. The powder XRD pattern of the as obtained silver nanoparticles is shown in (Fig 4). The peak positions of the sample exhibited monoclinic structure of Ag which was confirmed from the (JCPDS) Card No-087-0720. Further, no other impurity peak was observed in the XRD pattern, showing the single phase sample formation. Lattice parameters of unit cell of Ag was found to be a = 2.33 Å. The d-spacing value is in good agreement with the standard values reported by the (JCPDS) Card No-087-0720.

Silver nanoparticles treated seeds can control the seed borne diseases. In this experiment were observed for the occurrence of pathogens in the seeds due to nano seed treatment (Table 2). The treatments, seeds treated with Ag @ 1250 mg kg^{-1} which was protected the seeds with minimum seed pathogen infection (5.8 %) followed by Ag @ 1000 mg kg^{-1} (6.4 %) while maximum seed pathogen infection (18.2 %) was observed in control seeds.

Maintenance of seed quality during storage/ carryover of seed is very much essential. In this context, pathogens play a major role in determining the storage life of seed. Experiment results suggest that seeds treated with Ag NP @ 1250 mg had minimum seed pathogen infection in accelerated aged chilli seeds as compared to control (Table 2). The antimicrobial effect of silver is mainly dependent on superficial contact wherein silver can inhibit enzymatic systems of the respiratory chain and alter DNA synthesis which helps to protect the seeds from any fungal pathogens (Brett, 2006). Naturally, silver nanoparticles possess superior antimicrobial activity and it exhibits multiple modes of inhibitory action against microorganisms (Park *et al.*, 2006) and may be used with relative safety for control of various plant pathogens compared to synthetic fungicides (Min, 2009).

Conclusion :

Rod shaped silver nanoparticles were synthesized using neem leaf extract as reductant and stabilizer. Silver nanoparticles were synthesized by a well-controlled one-step solution phase glucose reduction method. It is confirmed by XRD analysis. The spherical Morphology is confirmed by both SEM / EDS and TEM. The method is found to be reliable, simple in approach and cost effective. The silver nanoparticles thus synthesized are potential enough to kill pathogenic fungi.

REFERENCES

Bhattacharya, D. and Gupta, R.K. (2005). Nanotechnology and potential of microorganisms. *Crit. Rev. Biotechnol.*, **25** : 199-204

Brett, D.W. (2006). A discussion of silver as an antimicrobial agent: alleviating the confusion. *Ostomy Wound Manage*, **52**: 34-41.

Farooqui M.A, Chauhan, P.S., Krishnamoorthy, P. and Shaik, J. (2010). Digest. *J. Nanomater Biostruct*., **5**: 43.

ISTA (2010). International rules of seed testing. *Seed Sci. & Technol.*, **11** : 20-35.

Kalmesh, M. and Gurjar, R.B.S. (2001). *Sclerotium rolfsii* – A new threat to chilli in Rajasthan. *Mycol. & Plant Pathol.*, **31**(2) : 261

Lee, P.C. and Meisel, D. (2005). Adsorption and surface-enhanced raman of dyes on silver and gold sols. *J. Physics Chem.*, **86** : 3391-3395

Min, J.S. (2009). Effects of colloidal silver nanoparticles on sclerotium-forming phytopathogenic fungi. *Plant Pathol.*, **25**: 376–380.

Mohanpuria, P., Rana, N.K. and Yadav, S.K. (2008). Biosynthesis of nanoparticles: technological concepts and future applications. *J. Nanopart Res.*, **10** : 507-517

Park, H.J., Kim, S.H., Kim, H.J. and Choi, S.H. (2006). A new composition of nano sized silica-silver for control of various plant diseases. *Plant Pathol. J.*, **22** : 295–302.

Toraya, H. (1986). Whole- powder- pattern fitting without reference to structural model: application to x-ray powder diffraction data. *J. Appl. Cryst.*, **19**: 440-447.