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National Academy of Agricultural Science (NAAS) Rating : 3.03

Antifungal effect of plant mediated silver nanoparticles against *Fusarium oxysporum* f sp *lycopersici*

R. Surega*, S. Ramakrishnan*, B. Anita*, K. Gunasekaran* and S. Nakkeeran*

ABSTRACT: *The plant mediated silver nanoparticles synthesized using 0.01M of $AgNO_3$ solution and plant extracts of Euphorbia hirta as reducing and capping agentwere characterized using UV-Vis absorption spectroscopy andX-Ray Refractive Diffraction and found to possess all the desirable physical and chemical traits of metal nanoparticles. In vitro assay showed that the synthesized plant mediated silver nanoparticles is inhibitory to Fusarium oxysporumf splycopersici.*

Keywords: *Antifungal agents, UV- Vis, XRD analysis, Nanoparticlesand Fusarium oxysporumf splycopersici*

INTRODUCTION

The silver has been known for its natural antibacterial and antifungal properties over 100 years (Morones *et al.,* 2005). Silver nanoparticles are very reactive and inhibit microbes respiration and their metabolism; suppresses electron transfer systems and transport of substrates in the microbial cell membrane as they cause physical damage according to Bragg and Rannie (1974) and Thurman *et al.* (1989). However attention is currently focused on plant mediated nanoparticles due to harmful effects of nanoparticles in plant protection. The pathogen *Fusarium oxysporum* causes wilt diseases in many economically important crops and reduced the yield by 60 per cent (Tripathi *et al.,* 2009). Therefore in the present study it is programmed to synthesize plant mediated nanoparticles and to study their *in vitro* effect on *Fusarium oxysporum* f sp *lycopersici.*

MATERIALS AND METHODS

Synthesis of silver nanoparticles using plant extracts

The fresh leaves of *Euphorbia hirta,* were used for the synthesis of plant mediated silver nanoparticles. The plant extracts of

E. hirta were obtained by boiling 50 g of fresh chopped leaves in 500 ml of distilled water at 100 °C for 30 min and filtered through Whatman No. 1 filter paper and the total volume was made as 400 ml. The filtered plant extract was finally used for the synthesis of plant mediated silver nanoparticles as follows.

Twenty five ml of leaf extract was added to 100 ml of 0.01 M of $AgNo_3$ in a 250 ml conical flask and heated for 10 min at 90 °C. The content of the flasks were stirred at 150 rpm at 30 °C using the magnetic stirrer. The process was continued till the change of color from green to dark brown indicating the synthesis of silver nanoparticles. The extract was centrifuged at 6000 rpm for 20 min and the pellet was kept in hot air oven overnight at 70 °C to make it as a fine powder of nanoparticles.

Characterization of silver nanoparticles

The synthesis of plant mediated silver nanoparticles was confirmed by taking 0.5 mg nanoparticles to disperse in 20 ml distilled water followed by sonification for 32 min at regular intervals for measuring the absorbance through scanning with UV–vis spectra at the wavelength of 200–700 nm in Beckman–DU20 spectrophotometer. Then the XRD (Philipse PW 1830) analysis was performed to measure the size and nature of the dried synthesized plant mediated nanoparticles by coating on XRD gridat the voltage of 40 kV.

Efficacy of silver nanoparticles against *Fusarium oxysporum* f sp *lycopersici*

The antifungal activity of plant mediatedsilver nanoparticlesat different concentrations *viz.*, 100, 250,

* Department of Nematology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, *E-mail: sureka.supa@gmail.com*

500, 750, 100, 125 and 1500 ppm was evaluated against *Fusarium oxysporum* f sp *lycopersici* using poisoned food technique *in vitro* using potato dextrose agar (PDA) medium treated The PDA medium without nanoparticles served as untreated control. The media containing plant mediated silver nanoparticles was incubated at room temperature (30 ± 2 °C). After 48 hr of incubation, an agar plug of 8 mm dia containing the fungus was inoculated simultaneously at the center of each petri dish and incubated at 28 ± 2 °C. After a weak of incubation, inhibition zones were measured. The assay was run twice with three replications for each concentrations. The inhibition rate observed at 7 days after inoculation (DAI) was calculated using the following formula:

$$\text{Inhibition rate (\%)} = \frac{R - r}{R} \times 100$$

Where 'R' is radial growth of fungi in control plate and 'r' is the radial growth of fungi in plant mediated silver nanoparticle plates treated with plates.

The data generated through Completely Randomized Design (CRD) adopted in the present study were scrutinized for statistical significance.

RESULTS AND DISCUSSION

Characterization of silver nanoparticles UV-Vis Spectra

The characterization of synthesized plant mediated silver nanoparticles through UV-Visshowed the brownish colour of reactive solution due to excitation of surface plasma resonance. Hence, there was reduction of pure $Ag^{+}$to Ag^{0} ions with plant extracts of *Euphorbia hirta* as measured by the UV-Vis spectrum. When the plant extract got exposed to $AgNO_3$ solution the maximum absorbance was noticed at 434 nm indicating the formation of silver nanoparticles in the reaction mixture. Further the broadening of peak in the Figure. 1 indicated that the resultant synthesized plant mediated nanoparticles are poly dispersal. The finding of Elumalai *et al.* (2010) who observed the similar absorbance peak at 430 nm in an attempt of synthesizing plant mediated silver nanoparticles using *Tridax procumbans* support the present finding.

X-Ray Refractive Diffraction

The XRD pattern for diffraction analysis of plant mediated silver nanoparticles showed the strong diffraction peaks at 2θ values as 28.6°, 32.5°, 38.38°, 44.56°, 46.42°, 55.57°, 64.78° and 77.02° corresponding

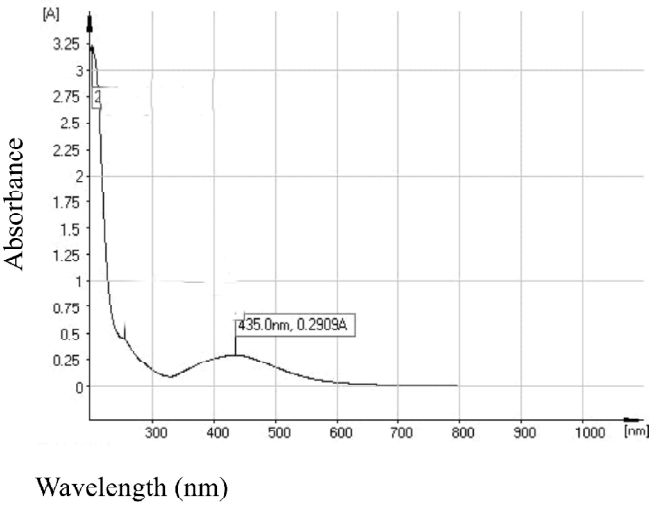

Figure 1: Characterization of plant mediated silver nanoparticles through UV-Vis Spectra

to the crystal plane values of 101, 004, 111, 200, 200, 211, 220 and 311 respectively indicated the formation of silver nanoparticles on library search as per JCPDS Card No. 040783. The computed values of different diffraction peaks of XRD as face centred cubic (fcc) structure showing regular arrangement of atoms with the synthesized nanoparticles as indicated generally by high intense peak reflection value of 111. Further the intensity of peaks reflected in the present study indicated the high degree of crystalline nature of silver nanoparticles. However, the broad diffraction peaks indicated that the crystalline nature of nanoparticle size is very small and it was calculated as 23 nm using the Debye–Scherrer formula (Fig. 3). The results coincides with the report of Bhati *et al.* (2014) synthesized the plant mediated silver nanoparticles using *Tridax procumbans*.

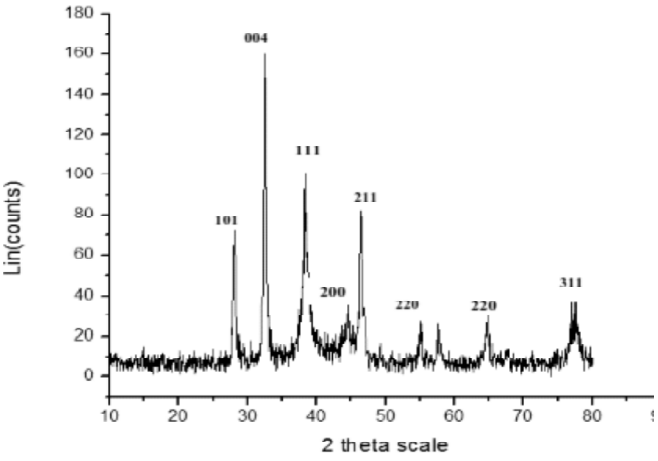

Figure 2: Characterization of plant mediated silver nanoparticles through XRD

Effect of silver nanoparticles against *Fusarium oxysporum* f sp *lycopersici*

The effect ofplant mediated silver nanoparticles showed various level of inhibition on mycelial growth of *Fusariumoxysporum* f sp *lycopersici* (Table 1). The plant mediated silver nanoparticles caused *Fusarium oxysporum* f sp *lycopersici*mycelial deformity with crinkled hyphal surface. Whereas untreatedfungal mycelia was normal and intact with profuse branching and under SEM at various resolutions (Fig. 3). All the concentrations of plant mediated nanoparticles resulted with significant inhibition in the mycelial growth of *Fusarium oxysporum* f sp *lycopersici*. However it was highest with 1250 ppm (86.06%) of plant mediated silver nanoparticles in the present study. The findings of the present study was supported by Krishnaraj *et al.* (2012),Gopinath and Velusamy (2013) and Yehia and Ahmed (2013) who reported that the green synthesized silver nanoparticles possessing strong antifungal activity against the various phytopathogenic fungi like *Fusarium oxysporum* and *Peniciliumex pansum etc.*,

Table 1
Effect of green synthesized silver nanoparticles on mycelial growth of *Fusarium oxysporum* f sp *lycopersici*

Nanoparticles conc. (ppm)	*Radial mycelial growth*	*Per cent inhibition*
100	17.5 (24.4)	65.98
250	15.3 (22.8)	68.16
500	13.1 (21.1)	70.47
750	10.0 (18.4)	74.24
1000	7.5 (15.9)	77.79
1250	7.5 (15.9)	77.79
1500	3.6 (10.0)	86.06
Untreated control	90.0 (71.6)	-
CD(P= 0.05)	2.44	

Figures in parentheses are arcsine transformed values

CONCLUSION

The plant mediated silver nanoparticles using *E. hirta* possessing all desirable physical and chemical characters of nanoparticles were effective to inhibit the growth and development of *Fusarium oxysporum* f sp *lycopersici*.

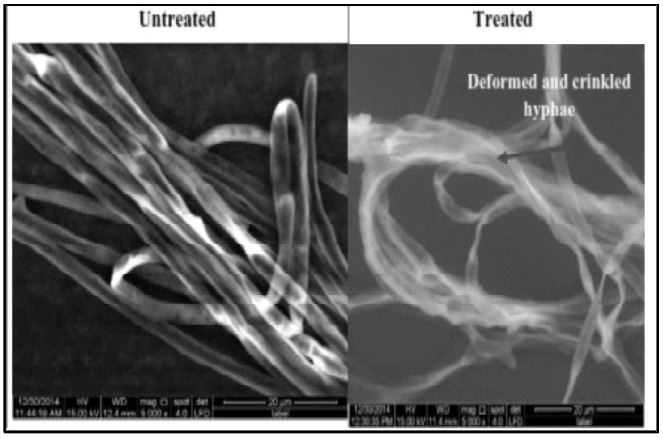

Figure 3: Comparison of silver nanoparticles treated and untreated *Fusarium oxysporum* f sp *lycopersici*

ACKNOWLEDGMENT

We gratefully acknowledge the Nano Mission, Department of Science and Technology, New Delhi for financial support to undertake the present study.

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