

Antifungal effect of phyto-fabricated Silver Nanoparticles

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ABSTRACT

Silver ions have long been known to possess broad spectrum antimicrobial activities. In the present work Silver nanoparticles were fabricated using four different organic wastes. Synthesized particles were characterized by UV-Vis spectroscopy and TEM analysis. Antifungal effects of the synthesized silver nanoparticles were carried out by MIC and Radial growth assay method. The obtained result showed that the MIC values against *F. oxysporum* was 40ppm and against *A.niger* at 50 ppm. *F. oxysporum* was found to be more sensitive to all synthesized AgNPs compared to *A.niger*. Percentage inhibitory activity to radial growth was 100% for both the fungi at 40 and 50ppm of AgNPs.

Keywords: Antifungal, Antimicrobial, Silver nanoparticles

INTRODUCTION

Silver ions and silver salts are well-known antimicrobial agents (Silver and Phung 1996) in various fields due to their growth inhibitory abilities against microorganisms. The antimicrobial nature of silver nanoparticles is the most exploited in the medical field. Many studies have reported that AgNPs can damage cell membrane of microorganisms leading to structural changes, which render them more permeable (Hashimoto *et al.*, 2012 and Lazer, 2011). This effect is highly influenced by the nanoparticles' size, shape and concentration (Lu *et al.*, 2010). In the present work silver nanoparticles (AgNPs) were fabricated from different organic wastes (a green approach). These fabricated particles were tested for their antifungal activity against selected fungi.

MATERIALS AND METHOD

Synthesis and Characterization of silver nanoparticles:

Silver nanoparticles (AgNPs) were fabricated using extracts of Banana stem (BS), cauliflower leaf (CL), pigeon pea seed coat (PP-SC) and saw dust (SD) with 1mM AgNO₃ solution. The change in colour from colourless to yellow indicated the formation of AgNPs. The nanoparticles were characterized using UV-Visible Spectroscopy (Shimadzu UV 1800) and TEM (Transmission electron microscope) analysis. Particles with an

average of less than 30nm size were used for the study. 10-100 ppm of AgNPs synthesized was used for the study.

Fungal cultures:

Aspergillus niger (NFCCI 161) and *Fusarium oxysporum* (NFCCI 245) cultures were obtained from Agarkar Institute, Pune and was maintained on Potato Dextrose Agar (PDA) medium.

Minimum Inhibitory Concentration (MIC) was carried out by broth dilution method (Astuti and Suprpta, 2012 - modified). After 8 days, the concentration at which the fungal growth was inhibited was considered as the minimum inhibitory concentration (MIC) which was determined by visual observation.

Radial growth rate assay (Miyashira *et al.*, 2010):

Sterile Petri dishes were prepared with 15 ml of culture medium and 30, 40, 50 and 70 ppm of AgNPs. After the medium solidified, a mycelial plug (5mm in diameter) of *A.niger* and *F. oxysporum* was placed in the centre of the plate. The cultures were incubated for 7 days in the dark at room temperature. After one week the diameter of fungal colony was measured. The inhibitory activity by the radial growth (IR) was determined according to the formula.

$$IR\% = \frac{dc-dt \times 100}{dt}$$

Where, IR= inhibitory activity to the radial growth

dc = average increase in mycelia growth in control plates.

dt= average increase in mycelia growth in treated plates.

Simultaneously a positive control was run with antifungal bavistin and a negative control plates was run without the AgNPs and bavistin.

Statistical analysis: The data were subjected to Analysis of Variance (ANOVA). Statistical analysis was performed using IRRISTAT software (IRRI,2003). Treatment means were compared using Least Significance Difference (LSD) values at $p \leq 0.05$. Differences among treatments were tested by Duncan's New Multiple Range Test (DMRT). In the tables given in results, mean values followed by same alphabets in superscript (a,b,c,d...) within a column are not significantly different at ≤ 0.05 level.

RESULTS AND DISCUSSION

Synthesized nanoparticles were characterized using UV-Visible Spectroscopy and TEM analysis. The particles showed absorption maxima at 415-445 nm and the average size was found to be in the range of 5 to 30 nm. It was evident that irrespective of source of synthesis of AgNPs, all synthesized AgNPs were very active against the selected fungi. Antifungal activity of the synthesized AgNPs in terms of MIC ranged between 40-60 $\mu\text{g/ml}$ (Table 1). The obtained result showed that MIC of all AgNPs synthesized irrespective of their source, against *F. oxysporum* was 40 $\mu\text{g/ml}$ and against *A. niger* MIC ranged between 50-60 $\mu\text{g/ml}$. Synthesized AgNPs were more active against *F. oxysporum* than *A. niger*.

Radial growth rate assay

The results of determination of Radial growth rate assay of AgNPs fabricated from the various organic wastes against *A.niger* and *F. oxysporum* have been tabulated in table 2& Fig.1. *F. oxysporum* was found to be more sensitive to all synthesized AgNPs compared to *A.niger*. Both fungi showed 100% inhibition in growth with selected concentration of AgNPs.

Table 1: Minimum inhibitory concentration of fabricated AgNPs from organic waste extracts against *A. niger* and *F. oxysporum*.

Source of fabrication of AgNPs	MIC of AgNPs ($\mu\text{g/ml}$)	
	<i>A.niger</i>	<i>F. oxysporum</i> .
BS-AgNPs	60 ^a	40 ^a
CL-AgNPs	50 ^b	40 ^a
PP(SC)-AgNPs	60 ^a	40 ^a
SD-AgNPs	50 ^b	40 ^a
LSD p=0.05	0.987	1.31

Table 2 Radial growth inhibition percentage of AgNPs synthesized from different organic wastes against *A. niger* and *F. oxysporum*. (Values in the column in parenthesis are arcsine transformed values and were used for comparing treatments by ANOVA).

AgNPs	Radial inhibition rate of AgNPs (%)	
	<i>A.niger</i>	<i>F. oxysporum</i>
BS-AgNPs	100 ^a (96.98)	100 ^a (96.98)
CL-AgNPs	100 ^a (96.98)	100 ^a (96.98)
PP(SC)-AgNPs	100 ^a (96.98)	100 ^a (96.98)
SD-AgNPs	100 ^a (96.98)	100 ^a (96.98)
Control	0.00	0.00
LSD	0.00	0.00

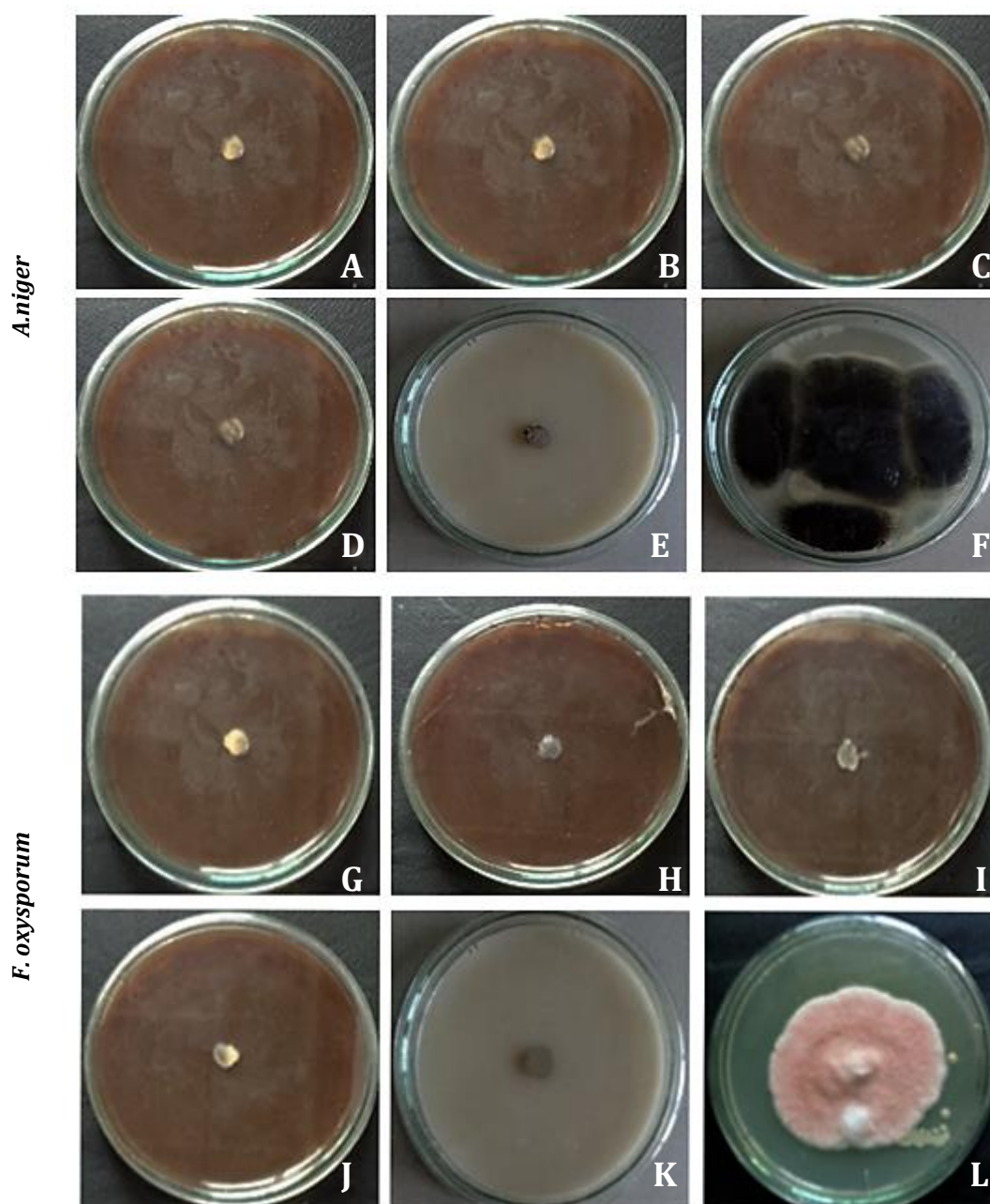


Fig. 1 Radial growth rate assay of AgNPs synthesized from different organic wastes. A&G-BS-AgNPs, & H-CL-AgNPs, C & I-PP (SC)-AgNPs D& J -SD-AgNPs E & K-Positive control, F & L- Negative control against *A.niger* and *F. oxysporum* respectively.

In the present study the size of the synthesized AgNPs ranged from 5-25nm. Strong antimicrobial potency of AgNPs in the range of 10–15 nm has been reported (Siddhartha *et al.*, 2007). Several studies have shown that activity of AgNPs is strongly dependent on the size (Tamayo *et al.*, 2014 and Wu *et al.*, 2014). Smaller nanoparticles seem to have a superior ability to penetrate into the microorganism. Also the interactions with the membranes and any resulting damage, which may lead to cell death, are certainly more evident in the case of nanoparticles with smaller diameter and a positive zeta potential (Gianluigi *et al.*, 2015; Rai *et al.*, 2014; Roe *et al.*, 2008).

Potential antimicrobial activity of Synthesized AgNPs from *Strychnos potatorum* seed extract and banana peel extract have been reported (Suganya *et al.*, 2014; Haytham and Ibrahim, 2015). Kruthika and Somanathan (2014) reported lethal effects of fruit waste mediated AgNPs against *Aspergillus* and *Candida*. AgNPs are effective and fast-acting fungicide against a broad spectrum of common fungi including genera such as *Aspergillus*, *Candida* and *Saccharomyces* (Vikas *et al.*, 2014). Our results indicated the potential application of phytofabricated AgNPs against selected fungi.

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