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PLASTIC DEGRADATION AND ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING *PHYLLANTHUS EMBLICA* LEAVES

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ABSTRACT

Plastic is using as substitute for almost all human utensils. Its dump in everywhere on earth leads pollution in environment, and effects on human health. Now a day's its decomposition is a major issue. In the present study, we used 100 ml aqueous solution of silver nanoparticle for degradation of 0.8 mm plastic material. Silver nanoparticles were synthesized using *Phyllanthus emblica* leaves extract; plant cultivated for fruits. The fruits are used in the preparation of pickle the plant is commercial cultivated for fruits. The fruits and leaves are traditionally used as medicine. Experiments were also conducted to test the efficacy of nanoparticles against pathogenic fungi. The fungal zone of inhibition was determined as compared to the standard drug bavistin. Green Synthesized silver nanoparticles were characterized using UV-VIS Spectroscopy, XRD, and TEM.

Keywords: *Plastic, Decomposition, AgNO₃, plant extract, XRD, and TEM etc.*

INTRODUCTION

Excessive use of plastics in domestic, industrial and agriculture sectors exert pressure on capacities available for plastic waste disposal which cause an additional burden on the environment (Akarsu *et al.*, 2006). Plastics including shopping bags, prepared from polyethylene, Water bottles, after their useful life, find their way to streets, sidewalks, beaches and water bodies ultimately to the block sewerage system which may serve as a suitable habitat for disease causing vectors including mosquitoes (Angulo-Sanchez *et al.*, 1994) and lead to the death of billions of marine animals by ingestion of the plastic debris or entanglement (Asapu *et al.*, 2011). A number of approaches have been proposed for dealing with the plastic waste. These include incineration, landfills, thermal degradation, bio-degradation, and photo-catalysis (Asghar *et al.*, 2011). Many of these are associated, however, with secondary problems. Uncontrolled burning of polyethylene produces vapors which includes many toxic compounds like ketones, acrolein, and methane and pollute the air which causes serious environmental hazards (Briassoulis 2006). Polyethylene wastes buried in soil cause negative effects to soil quality and may affect the drainage patterns leading to declined agricultural yield (Briassoulis *et al.*, 2004). In the plastic degradation studies reported so far Titania has been used in the form of nanoparticles. An option for using a larger surface area material, in the form of nanotubes (Da Silva *et al.*, 2014). Metal nanoparticles exhibit unique chemical and physical properties including large surface/volume ratio, which are useful in different fields such as electronics, photonics, biomedical, catalysis etc. (Ostuni *et al.*, 2001; Joannopoulos *et al.*, 2008; Guo *et al.*, 2013; and Arinstein *et al.*, 2007). Among the various noble metals, silver is the metal of first choice due to their diverse properties especially high antimicrobial and catalytic nature (Mashwani *et al.*, 2015; and Rostami-Vartooni *et al.*, 2016). Thus, researcher attention has been focused on alternative means of degrading the plastic material, in the present study; we used 50 ml aqueous solution of green synthesized silver nanoparticle for degradation of 0.8 mm plastic material. Silver nanoparticles shows positive effect in degradation of plastic material, AgNPs are synthesized using *Phyllanthus emblica* leaves extract, the plant is commercial cultivated for fruits. The fruit is traditionally used as medicine. Pathogenic fungal inhibiting Efficacy of silver nanoparticles is

Research Article

tested by wells method. The fungal zone of inhibition was determined comparing with standard control bavistin. Green Synthesized AgNPs were characterized using UV-VIS Spectroscopy, XRD, and TEM.

MATERIALS AND METHODS

Materials:

Silver nitrate (AgNO_3), *Phyllanthus emblica* leaves, plastic cut pieces of water bottle, *Fusarium udum*, bavistin, Mercuric chloride ect.

Methodology:

Sample collection:

Fresh leaves of *Phyllanthus emblica* from kusnoor village which is just behind Gulbarga University, kalaburgi and used water bottle from foot way of Sedam road kalaburgi were collected in sterilized polythene bag, and brought to Mycology and Plant pathology Laboratory. Plastic bottle was cut into small pieces with surgical blade and stored in laboratory conditions for further studies.

Preparation of leaf extract:

Collected sample *Phyllanthus emblica*, leaves were surface sterilized, and dried under shade. Dried leaves were cut into small pieces and grinded to powder. 10 gram of *Phyllanthus emblica*, leaf powder boiled in 200 ml of distilled water for 10 minutes then filtered it with whatman No. 1 filter paper. The prepared plant extract solution was cooled at 4°C and stored in laboratory condition (Fig. 1) for further experimental work.

Green synthesis of silver nanoparticles:

100 mL of 1 mM aqueous solution of silver nitrate (AgNO_3) was taken in conical flask. Then the prepared leaf extract solution with various concentrations from 5, 10, and 15 mL was added separately and agitated at room temperature. Control treatment (without Silver nitrate, only plant extract and distilled water) was also run along with experimental flask. After 24, 48 and 72 hours of time interval culture filtrate and Silver nitrate solutions turned colourless to dark brown colour due to reduction of Silver nitrate to Silver ions (Fig. 2)

Plastic degradation by Silver nanoparticles:

100 ml Synthesized Silver nanoparticles of *Phyllanthus emblica* was taken in 500 ml beaker. Then 5 Mg small cut pieces of plastic were added into the beaker and kept at room temperature. Along with experimental beaker, Control treatment (without Silver nitrate, only plant extract and distilled water Fig. 3) was also kept along with experimental beaker.

Isolation and inoculation:

Wilted stem of pigeon pea was surface sterilized by running water and broken longitudinal kept in the moist blotter for growth of the fungi, after two days associated fungi were isolated and identified as *Fusarium udum* with the help of published literature. An isolated fungus was further sub cultured on PDA plates (fig. 4) in order to obtain a pure culture.

Antifungal activity:

The antifungal activity of AgNPs was investigated by well diffusion method. Potato dextrose agar plates were prepared, sterilized and solidified, after solidification *Fusarium udum* was inoculated on the plates. 0.20 mg/ml, 0.30 mg/ml AgNPs and 0.20 mg/ ml, 0.30 mg/ ml, Bavitin and distilled water (control) were poured in the wells and kept for incubation at room temperature for five days. (Fig. 5) A zone of inhibition measured and compared with the standard bavistin and silver nitrate solution.

Characterization of synthesized AgNPs

UV-Visible spectroscopy:

The reduction of silver ions was confirmed by testing the supernatant by UV-visible spectrophotometer. (Fig. 6) The UV-visible spectroscopy measurements were performed on Elico spectral photometer as a resolution of 1 nm from 300 to 800 nm.

X-ray diffraction (XRD) study:

Powdered sample was used for XRD; The Coherently diffracting Crystallography domain size of the Silver nano particle was calculated from the width of the XRD peaks (Fig. 7) using scherrer formula.

Research Article

Transmission electron microscopic (TEM) analysis:

The TEM analysis was performed at IIT Mumbai. (Fig. 8) TEM technique was employed to see the size and shape of the synthesized silver nanoparticles.

RESULTS AND DISCUSSION

Plastic degradation:

50 ml Synthesized Silver nanoparticles from *Phyllanthus emblica* was taken in 500 ml beaker. Then 5 Mg small cut pieces of plastic were added into the beaker and kept at room temperature. Along with experimental beaker, Control treatment 5 mg plastic cut pieces with 50 ml plant extract and distilled water was also kept along with experimental beaker. The dependent variable is the amount of decomposition observed after 11 days. This is determined by using the digital weighing scale to measure the weight of the plastic before and after the 11 days. The total weight of the plastic is checked using the digital weight scale. The total weight of the plastic is subtracted by the weight of plastic material decomposed. The values are recorded in the table given below, the result confirms that the synthesized Silver nanoparticles have strong efficacy to decompose plastic materials.

Table 1: AgNPs shows degradation of Plastic.

Solutions	Plastic decomposition after 11 days		
	Start weight (g)	Finish weight (g)	% Decomposition
50 ml AgNPs solution	5 Mg	1 Mg	20%
50 ml <i>Phyllanthus emblica</i> extract (control)	5 Mg	00 Mg	0%
50 ml Distilled water (control)	5 Mg	00 Mg	0%

Antifungal activity:

The AgNPs possess antifungal activity against isolated fungi at the concentrations of 2 mg/ml and 3 mg/ml. The AgNPs were compared favorably with standard bavistin at the concentrations of 0.20 and 0.30 ml. AgNPs exhibited positive effects inhibiting the growth of tested fungi, the MIC of AgNPs was tested against which varied from 0.20 to 0.30 mg/ml whereas bavistin showed 0.20 to 30 mg/ml. The results indicated that biosynthesized AgNPs has a positive antifungal effect.

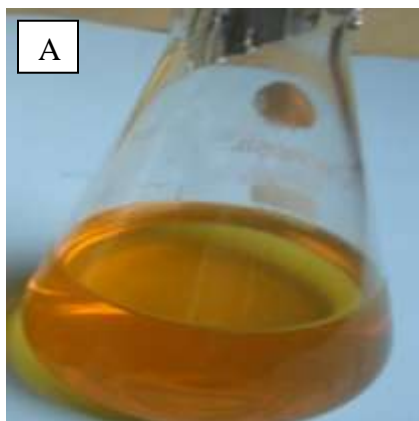
Table 2: AgNPs shows Antifungal Activity against *Fusarium udum*

Fungi	Zone of Inhibition					
	Silver nanoparticles.		Standard bavistin		Distilled water	
	0.20 mg/ml	0.30 mg/ml	0.20 mg/ml	0.30 mg/ml	0.20 ml	0.30 ml
<i>Fusarium udum</i>	42%	56%	41%	54%	-	-

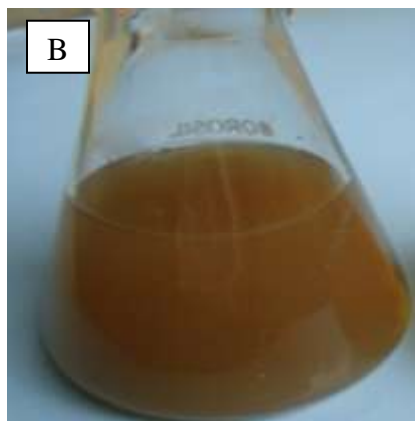


(A) 50ml plantextract

Fig 1: Aqueous plant extract of *Phyllanthus emblica*



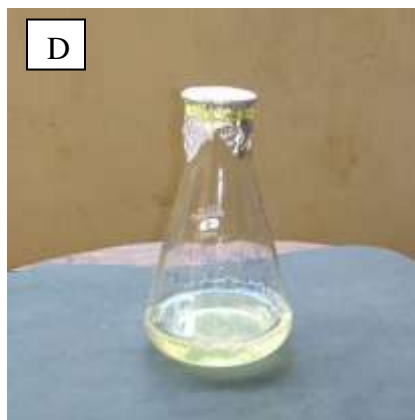
(A) 100 ml AgNO_3 + 5ml plantextract



(B) 1000 ml AgNO_3 + 10ml plantextract



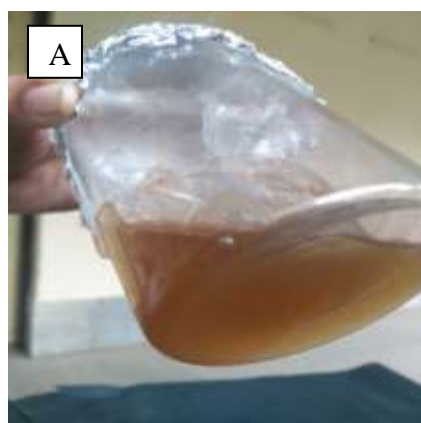
(C) 100 ml AgNO_3 + 15ml plantextract



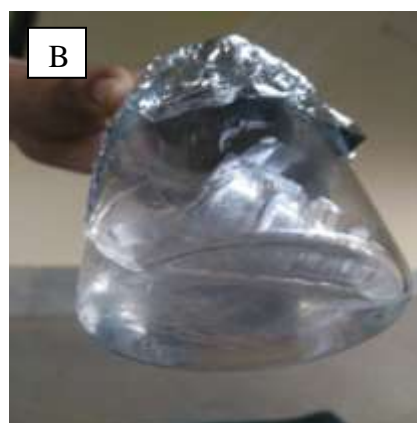
(D) 50 ml DW + 5ml plantextract

Fig 2: Biosynthesis of silver nanoparticles-color change reaction: conical flask containing the aqueous plant extract of *Phyllanthus emblica*

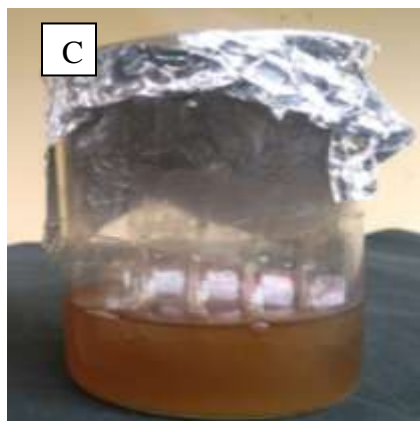
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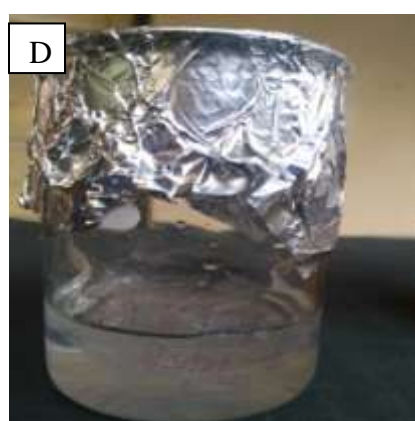
(A) 50 ml AgNO₃ + 5mg plastic



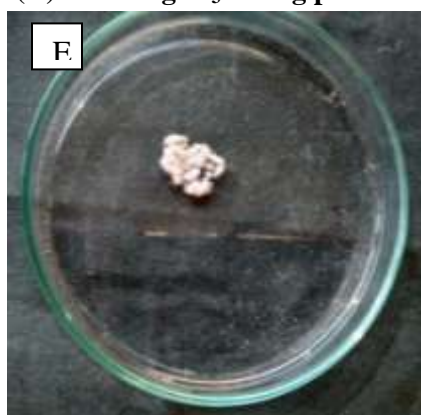
(B) 50 ml D. water + 5mg plastic



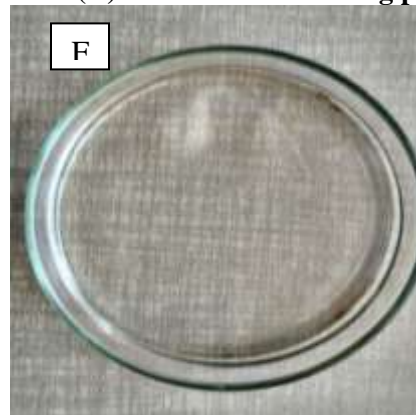
(C) 50 ml AgNO₃ + 5mg plastic



(D) 50 ml D. water + 5mg plastic



(E) 1 mg decomposed plastic filterate



(F) 0 mg Control filterate

Fig 3: Degradation of 0.8 mm water bottle's Plastic material employing Silver nanoparticle

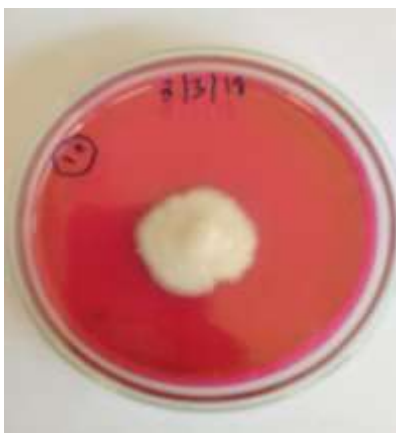


Fig. 4: colony morphology of *Fusarium udum*



CNP: *Phyllanthus emblica*, extract's Silver nanoparticles.

BVST: Bavistin standard control

DW: Distilled water

Fig. 5: Antifungal effect of AgNPs against *Fusarium udum* by well method

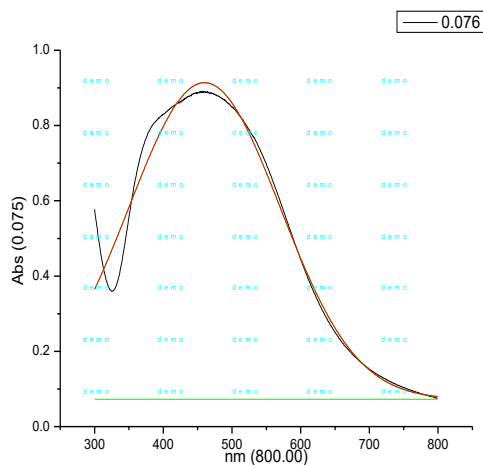


Fig 6: UV-Vis spectrum of silver nanoparticles synthesized using *Phyllanthus emblica* plant extract. UV-Vis spectra recorded as function of time of reaction of an aqueous solution of 1mM silver nitrate solution with the plant filtrate. The time of reaction is indicated next to the respective curves.

Research Article

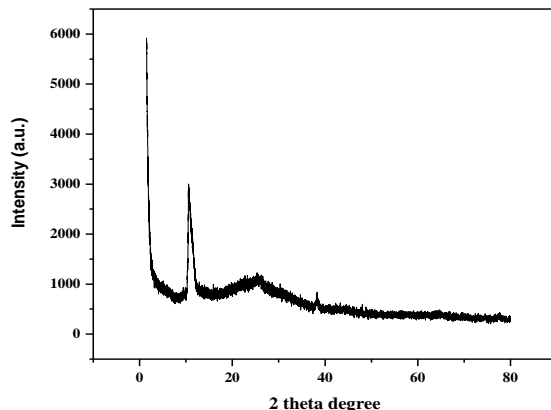


Fig 7: XRD analysis, peaks assigned to the corresponding diffraction signals (111), (200), (220), and (311) facets of Silver.

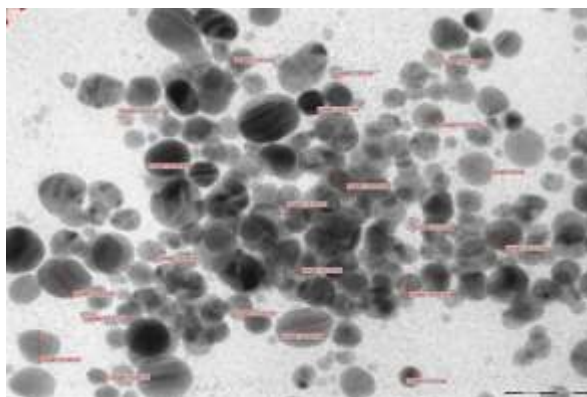


Fig 8: Transmission electron microscopic photographs of synthesized silver nanoparticles from *Phyllanthus emblica*.

XRD study:

Obtained Silver nanoparticles were purified by repeated centrifugation at 3000 rpm for 40 minutes by redispersing silver nanoparticles pellet into 10 ml double distilled water. After drying silver nanoparticles in room temperature structure and composition analysis was carried out by XRD (Fig. 4) The crystallite domain size was calculated by the width of the XRD peaks using Scherer formula $D = 0.96 \lambda / \beta \cos \theta$, where D is crystalline domain size perpendicular to reflecting planes, λ is the x-ray wavelength, β is the full width at half maximum and θ is the diffraction angle.

The average particle size was 30-35 nm. XRD analysis, peaks assigned to the corresponding diffraction signals (111), (200), (220), and (311) facets of Silver. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the (111) plane.

TEM Analysis:

Sample was prepared for Transmission electron microscopic Analysis (IIT Mumbai) TEM Technique was employed to see the size and shape of the synthesized silver nanoparticles; it was observed that there is variation in the particle sizes around 30% of particles in 25 nm range and 25% in 30 nm range and 20% in

Research Article

35 nm ranges. The particles range from 12 nm least to 75 nm high, the TEM image suggests that the particles are polydispersed (Fig. 5) and are rounding spherical in shape.

Conclusion

In the present study Silver nanoparticles were Green synthesized using *Phyllanthus emblica* plant extract. The plant extract in different concentration i.e. 5 ml 10 ml and 15 ml are challenged with 1mM Silver nitrate; change of mixture from color less to dark brown indicates the synthesis of Silver nanoparticles in the reaction mixture. 100 ml of Synthesized Silver nanoparticles applied for the degradation of 0.8 mm plastic material, the dependent variable amount of decomposition observed after 11 days. Antifungal Efficacy of Synthesized AgNPs was tested against *Fusarium udum*. Zone of inhibition was observed. And the crystallite domain size of synthesized Silver nano particles was measured 30-35 nm by XRD analysis, shape and size of the silver nanoparicles was studied by TEM analysis. Results conclude that *Phyllanthus emblica*, plant extract is potential producer of Silver nano particles, and Silver nanoparticle have strong efficacy of plastic decomposition and also fungal inhibition.

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Research Article

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