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PHYTOGENIC SYNTHESIS OF SILVER NANOPARTICLES USING *IPOMOEA OBSCURA* (L) LEAVES EXTRACT AND ITS FUNGICIDAL ACTIVITY AGAINST PHYTOPATHOGENS

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ABSTRACT

The current study was carried out to synthesize the silver nanoparticles using leaf extract of *Ipomoea obscura* through green chemistry approach. Synthesis of silver nanoparticles were confirmed by the change in colour of reaction mixture from pale yellow to dark brown and absorption band obtained at 430 nm by UV-visible spectroscopic studies. Characterization of synthesized nanoparticles was carried out by XRD, DLS, FTIR, SEM and EDAX for their structure, size, possible functional groups, shape and elemental composition respectively. The XRD spectrum of the synthesized silver nanoparticles showed three distinct diffraction peaks at 2θ values 37.89°, 43.96° and 64.32° corresponding to lattice planes (111),(200) and (220) respectively confirming that the particles are face centred cubic in nature. The dynamic light scattering spectroscopic (DLS) analysis showed that the particles are 46nm in size. SEM images of nanoparticles revealed the cubic shape of particles and the Energy Dispersive X-ray analysis (EDAX) confirmed the significant presence of elemental silver. Synthesized silver nanoparticle showed good fungicidal activity against *Didymella bryoniae*, *Fusarium oxysporum*, *Fusarium moniliforme* and *Aspergillus flavus*.

Keywords: *Ipomoea obscura,* Phytogenic synthesis, Silver nanoparticles, Fungicidal activity, Phytopathogens.

1. INTRODUCTION

Nanoscience and nanotechnology is the rapidly growing field of science which has created the interest among the researchers since the early 90s of the last century. This branch of science is concerned with the synthesis, manipulation and application of particles with nanoscale (1-100nm). Nanoscience has become an integral part of present day technology and said to be a key technology of the 21st century due to its interdisciplinary nature of research [1, 2].

Nanoparticles can be synthesized by various approaches of physical and chemical methods which involve the use of chemicals as reducing and stabilizing agent, require high pressure and high temperature and sometimes releases toxic by-product to the environment. These conventional methods used for the nanoparticles synthesis are costly, toxic, and not eco-friendly. In this regard, green chemistry approach is considered as an easy, cost effective, efficient and novel strategy for synthesis of metal nanoparticles. In green synthesis, chemical reducing agents and the stabilizers are replaced by the extracts obtained by the biological sources which act as reducing and capping agents [1, 3]. Among the biological sources, plant extracts are used widely because of their ease of extraction, abundant availability and presence of proteins, carbohydrates and several types of secondary metabolites which acts as reducing agents without producing any toxic by-product [4-6].

Silver and its salts have been known for its antimicrobial properties from ancient times and used for treatment of skin ulcer, bone fracture, supporting wound healing, water and air purification, etc. If ionic silver is converted to nanoparticles, it might exhibit additional antimicrobial properties because of its small size and large surface to volume ratio leading to the differences in both chemical and physical properties with its bulk counterparts [7, 8]. Due to the desirable electrical, chemical, optical, thermal, biological and catalytic properties exhibited by the silver nanoparticles [4, 9], their synthesis and applications acquired more importance in the field of electronics, chemistry, textiles industries, cosmetics, drug delivery systems, as biosensors, therapeutics, diagnostics, antimicrobial and environmental remediation [10-15]. The invention of active nano-silver material enabled technologies in the field of agriculture to enhance the quality of agricultural products as well as in the

management of agricultural and horticultural crop diseases is gaining momentum [16].

The present study was carried out to synthesize the silver nanoparticles by green chemistry approach using leaf extract of *I. obscura, a* commonly available climber with medicinal importance and to evaluate its efficacy as antifungal agents against agriculturally important phyto-pathogenic fungi.

2. MATERIAL AND METHODS

2.1. Plants and chemicals

Fresh leaves of *I. obscura* were collected from Botanical garden, Department of studies in Botany, University of Mysore, Manasagangotri campus, Mysuru, Karnataka State, India. Silver nitrate ($AgNO_3$) was purchased from HIMEDIA and double-distilled water was used in the entire experiment.

2.2. Test pathogens

Agriculturally important phytopathogens namely, *Didymella bryoniae, Fusarium oxysporum, Fusarium monili-forme* and *Aspergillus flavus,* which were available in the Department of Studies in Botany, University of Mysore, Mysuru were used in the present study to evaluate the efficacy of synthesized silver nanoparticles against them.

2.3. Preparation of Leaf extract

Fresh leaves of *I. obscura* were washed with tap water for two-three times to remove the surface dust particles adhere to leaves and then with double distilled water. Excess water on the leaf surface was drained out and ten grams of leaves were cut into small pieces of 0.5 to 1cm and boiled with 100ml of double distilled water for about 5-10 minutes, cooled and filtered through Whatman No. 1 filter paper. The extract obtained was stored at 4°C for further use.

2.4. Synthesis of Silver nanoparticles

Leaf extract of *I. obscura* was mixed slowly with 1 mM Silver nitrate solution with varying proportion *viz.* 9:1, 3:1, 1:1 and 1:9 ratio to standardize the metal precursor to leaf extract ratio [17, 18] and the reaction mixture was kept in dark condition at room temperature. Change of color from pale yellow to brown indicated the formation of silver nanoparticles which was further confirmed by UV-visible spectroscopic study. After the completion of reaction, the solution was centrifuged at 12000 rpm for 20 minutes and the obtained silver nanoparticles were dried and stored for further characterization.

2.5. Characterization of synthesized silver nano-particles

2.5.1. UV-visible spectroscopy

The small amount of bio-reduced sample was taken in a quartz cuvette and optical density was observed at the wavelengths ranged between 300-700nm by using UV-Vis spectrophotometer Beckman Coulter DU730 to study the formation of silver nanoparticles and results were tabulated.

2.5.2. XRD analysis

X-Ray Diffraction (XRD) study was carried out to determine the crystalline nature of synthesized silver nanoparticles between 2θ angle 10 to 80° using Rigaku Destop Miniflex II X-Ray Diffractometer.

Further, the size of nanoparticles was calculated using Debye-Scherrer formula [19].

$$D=K\lambda/\beta\cos\theta$$

Where; D: Particles size (diameter), K: Scherrer constant (K= 0.89), λ: Wavelength of X-ray (0.15406 nm), β: Full width at half maximum (FWHM) in radians, θ: diffraction angle (Bragg's angle (2θ))

2.5.3. Dynamic Light Scattering (DLS) Analysis and Zeta potential

The average particle size, the distribution and zeta potential were analyzed by Microtrac (USA) particle size analyzer.

2.5.4. Fourier Transform Infra-red Spectroscopy (FTIR)

The possible functional groups of phtyochemicals in the leaf extract which acted as reducing and capping agent in the nanoparticle synthesis was analyzed by Fourier transform infrared (FTIR) spectroscopy in the range from 600 to 4000 cm^{-1} using the instrument PerkinElmer Spectrum Two Spectrophotometer.

2.5.5. Scanning Electron Microscopy (SEM) and EDAX analysis

The morphological characteristics of phytosynthesized silver nanoparticles using *I. obscura* leaf extract were studied using Hitachi S-3400N Scanning Electron Microscope. Images were captured by placing minute amount of nanoparticles on the carbon tape coated over copper grid and sputter coated with gold. The presence of elemental silver and composition of the particles were studied by Energy Dispersive X-ray Spectroscopy (EDAX).

2.5.6. *Fungicidal efficacy of synthesized silver nanoparticles*

The fungicidal efficacy of the *I. obscura* leaf extract mediated synthesized silver nanoparticles were tested against the *D. bryoniae, F. oxysporum, F. moniliforme* and *A. flavus* by agar well diffusion method. 100µl inoculum prepared from seven days old fungal cultures, was uniformly spread over petriplate containing PDA media and 8mm wells were punched on the petriplates using sterile cork-borer to load the test samples. 100µl of Silver nanoparticles (1mg/ml), plant extract, Silver nitrate, DMSO and standard fungicide (1mg/ml) with commercial name Sectin (Fenamidone 10% + Mancozeb 50%**)** were loaded to respective wells. Sectin and DMSO were used as positive and negative control respectively. The plates were incubated at room temperature for 3-7 days and zone of inhibition was measured in millimeter. All tests were carried out in triplicate.

2.6. Statistical analysis

All the data was subjected to one-way analysis of variance (ANOVA) using graph pad prism 5 software and represented at $P < 0.05$ level of significance.

3. RESULTS AND DISSCUSSION

3.1. Synthesis of silver nanoparticles

When 1mM silver nitrate solution is added with leaf extract of *I. obscura* in varying proportions (9:1, 3:1, 1:1 and 1:9), the silver ions were reduced to silver nanoparticles, which was confirmed by the change in colour from pale yellow to dark brown [20, 21] due to excitation of surface Plasmon vibration of metal electrons in the reaction mixture [22, 23] with 9:1 ratio. Whereas, change in colour was not found in other reaction mixtures with different ratio (fig.1A) indicating that, only 9:1 ratio of metal precursor to leaf extract could be the optimum proportion for synthesis of silver nanoparticles (fig.1B). Biomolecules present in the leaf extract acted as reducing agent during the silver nanoparticles synthesis [24, 25].

3.2. Characterization of synthesized silvernanoparticles

3.2.1. *UV- spectroscopic analysis*

The synthesis of silver nanoparticles was confirmed from intense peak obtained at 430nm due to surface Plasmon resonance of conductive electrons on metal nanoparticles in reaction mixture with 9:1 proportion of metal precursor to leaf extract, whereas no peaks were found at particular wavelength in reaction mixture with other proportions (fig. 2A & B). The results are in agreement with previous results in which Surface Plasmon resonance band ranged between 420nm to 460nm [26-29].

3.2.2. *X-Ray Diffraction Studies*

The three distinct diffraction peaks obtained by XRD studies (fig.3) of synthesized silver nanoparticles at 2θ angles 37.89°, 43.96° and 64.32° corresponds to lattice planes (111), (200) and (220) respectively confirmed the face centred cubic nature of synthesized nanoparticles. The obtained results are in accordance with other earlier reports [30, 31]. Further, the synthesized nanoparticles are in the range of 19nm to 34nm in size.

Fig. 1A: Showing color change in reaction mixtures with different proportions viz. 9:1, 3:1, 1:1 and 1:9 respectively from left to right

Fig. 1B: Leaves extract, Silver nitrate (1mM) and Reaction mixture (9:1) respectively from left to right

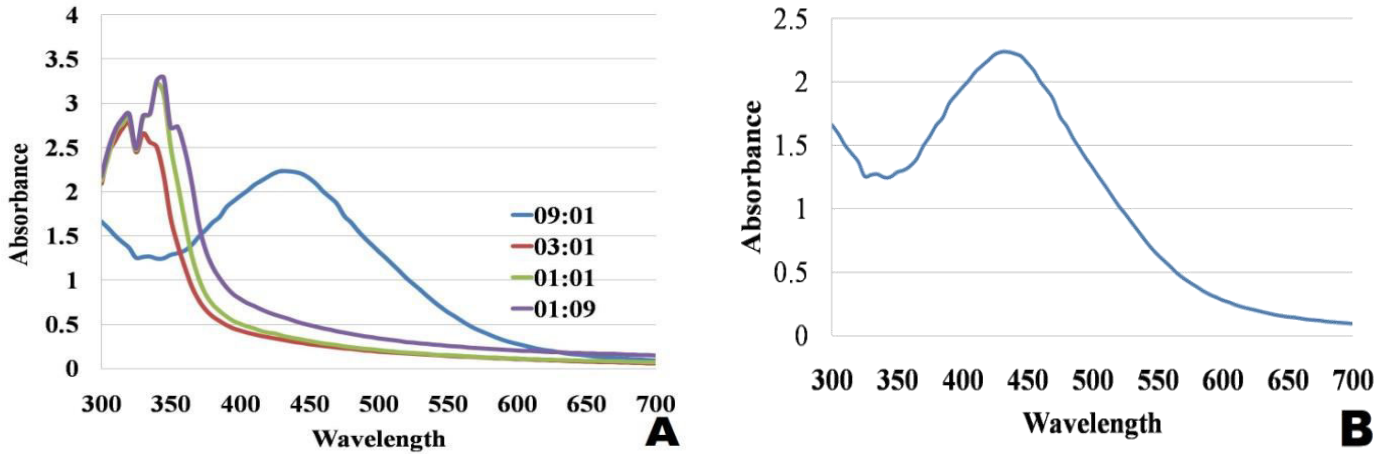

Fig. 2: (A) UV- Visible spectra of reaction mixtures with different ratio, (B) UV- Visible spectra of reaction mixture (9:1) showing intense peak at 430nm

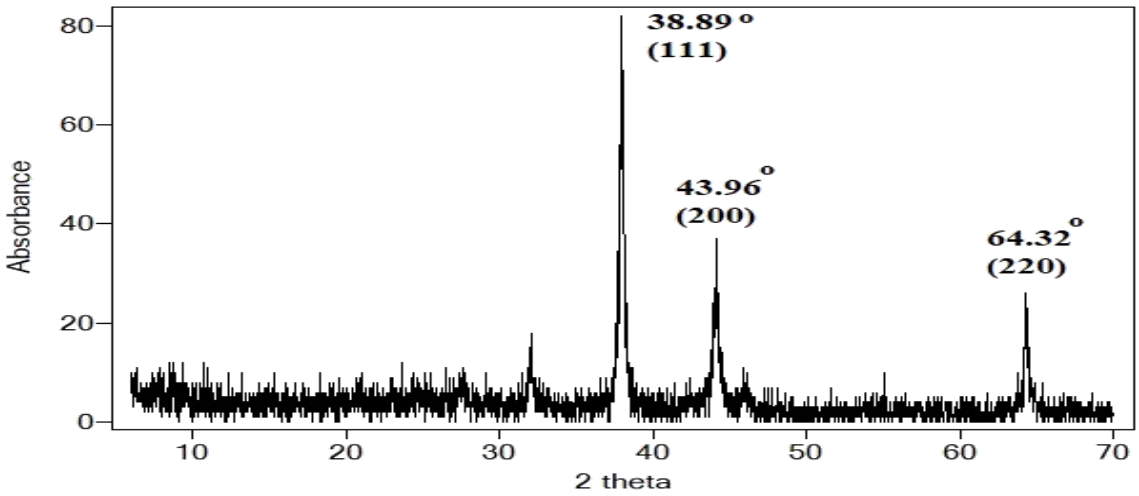

Fig. 3: X-ray diffraction spectra of *I. obscura* leaves extract mediated silver nanoparticles

3.2.3. Dynamic Light Scattering (DLS) Analysis and Zeta potential

Dynamic Light Scattering (DLS), often referred to Photon correlation spectroscopy (PCS) is a common reliable technique to determine the particle size in colloidal suspensions [32]. The histogram obtained by the DLS analysis (fig.4) showed the average particle size of 46.5nm of the synthesized silver nanoparticles in colloidal solution. The difference in the diameter volume obtained in the XRD and DLS is mainly due to sample preparation. In XRD studies, the analysis was carried out in dry state, whereas in DLS the size of the particle was obtained at hydrate state. Therefore, nanoparticles will have a larger hydrodynamic volume due to solvent in hydrate state [33, 34]. The negative zeta potential of -9.4 mv of silver nanoparticles indicates the repulsive nature and stability of the particles [35, 36].

3.2.4. Fourier Transform Infra-Red Spectroscopy (FTIR)

The spectrum (fig.5) obtained by the Fourier Transform Infra-Red Spectroscopic analysis (FTIR) showed the peaks at different wave numbers which corresponds to the possible biomolecules in leaf extract which acted as reducing and capping agent [37,38]. The peak at 2156.07cm^{-1} corresponds to the C≡C stretch of alkynes, peak at 2109.36cm^{-1} corresponds to the C≡C terminal alkyne group (monosubstituted) and the peaks at 666.75cm^{-1}, 656.23cm^{-1} and 631.80cm^{-1} corresponds to the C-H bend of alkyne or OH out of plane bend of alcohol [39, 40].

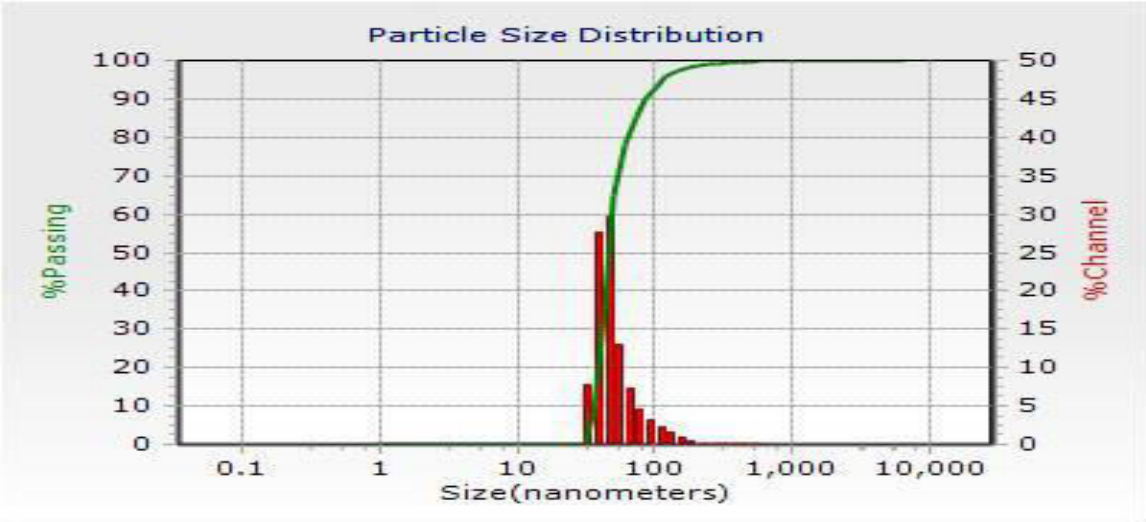

Fig. 4: Histogram of Dynamic light scattering spectroscopy

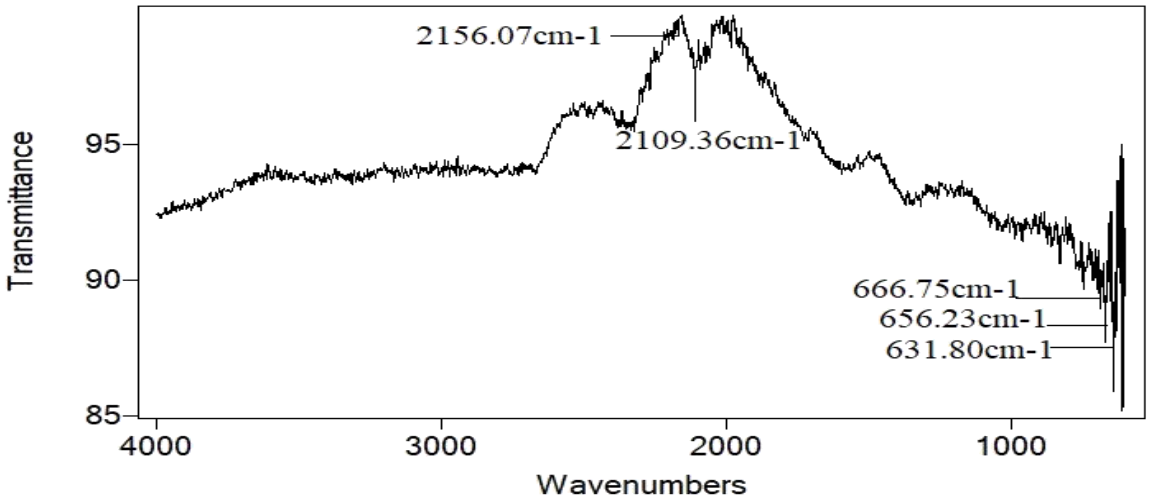

Fig. 5: FTIR spectrum of Phyto-synthesized Silver nanoparticles

3.2.5. Scanning Electron Microscopy and EDX analysis

Scanning Electron Microscopic images (fig. 6A & B) clearly showed that the particles are cubic in shape with varying sizes. The presence of elemental silver (85.36%) was confirmed by the strong signals of Silver in the EDAX spectrum (fig. 7) along with traces of oxygen (14.64%) which may be due to the biomolecules and capping agents attached to the silver nanoparticles which corroborates with earlier reports [40, 41].

3.3. Antifungal activity

The values of the zone of inhibition by phytosynthesized silver nanoparticles against the fungal pathogens *Didymella bryoniae, Fusarium oxysporum, Fusarium monili-formae* and *Aspergillus flavus* are given in table 1. The silver nanoparticles were proved to be promising agents in inhibiting the growth of all the tested pathogens. *Didymella bryoniae* found to be more susceptible to the synthesized silver nanoparticles with inhibition zone of 19.66mm which was considerably higher than the standard (Sectin) fungicide which showed 18.33mm zone of inhibition (fig.8). *F. oxysporum, F. moniliforme* and *A. flavus* were also found to be susceptible to synthesized silver nanoparticles with inhibition ozone of 18.33mm, 18.66mm and 18.66mm respectively which was considerably less compared to the standard fungicide (Sectin) which showed 22.66mm, 22.33mm and 22.66mm zone of inhibition against the *F. oxysporum, F. moniliforme* and *A. flavus* respectively.

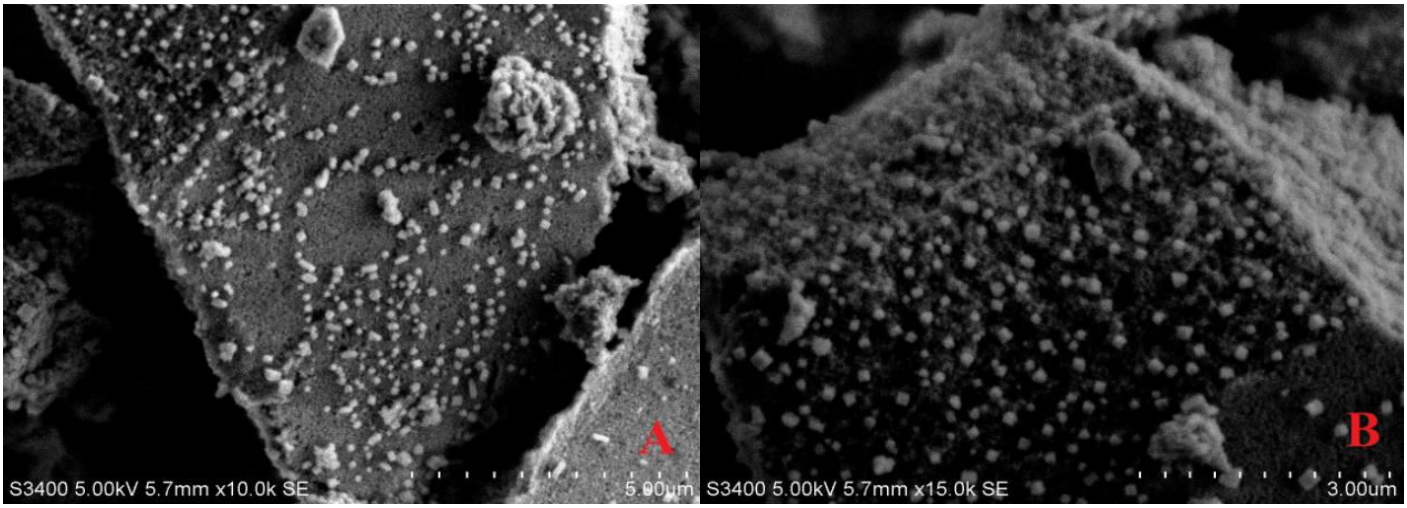

Fig. 6: (A) & (B): Scanning electron microscopic image of silver nanoparticles

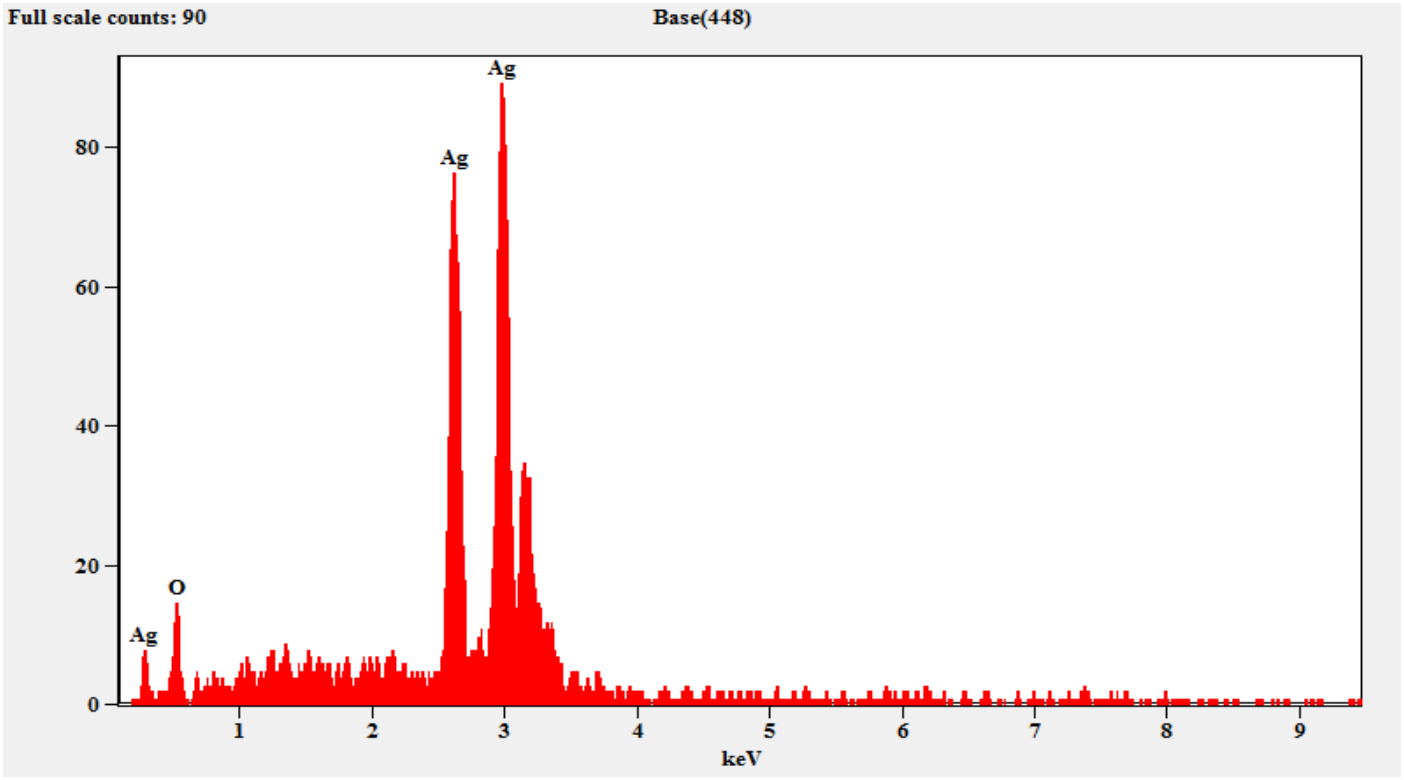

Fig. 7: EDAX spectrum of synthesized silver nanoparticles and elemental composition

Table 1: Antifungal activity of synthesized AgNPs using leaves extract of *I. obscura* against fungal phyto-pathogens

Fungal Pathogens	Zone of inhibition in mm				
	Plant extract	DMSO	$AgNO_3$	SNP	Sectin
D. bryoniae	0	0	0	19.66±0.33	18.33±0.33
F. oxysporum	0	0	0	18.33±0.33	22.66±0.33
F. moniliforme	0	0	0	18.66±0.33	22.33±0.33
A. flavus	0	0	0	18.66±0.33	20.66±0.33

Each value represents the mean of three replicates per treatment. ± SE = Standard error

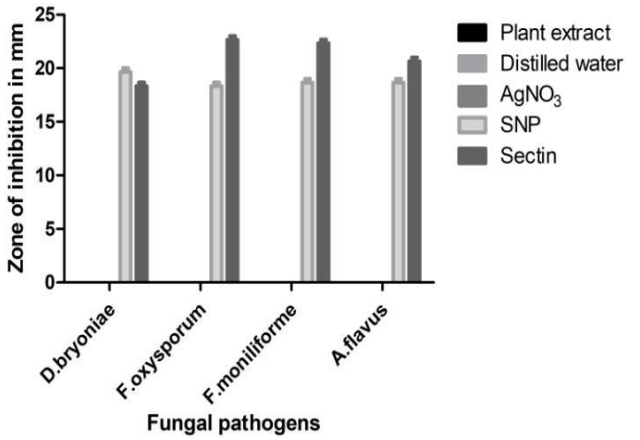

Fig. 8: Antifungal activity of synthesized AgNPs

4. CONCLUSION

Application of nanotechnology in the field of agriculture is gaining importance in recent years, particularly in managing phytopathogens, as there are several reports that, most of the chemical fungicides are becoming insensitive to several important fungal species including *D. bryoniae, F. oxysporum, F.moniliforme* and *A. flavus*. In the present study cubic shaped silver nanoparticles with average particles size of 46.5nm were synthesized through green chemistry method using leaf extract of *I. obscura* which is acost effective and an eco-friendly method. Antifungal activity of biosynthesized nanoparticles showed that they have potential to use in the management of plant diseases caused by agriculturally important fungi. However, further field experiments are to be conducted to validate the results as these experiments are carried out in the laboratory condition.

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Conflict of Interest

Authors declare that there are no conflicts of interest

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