Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(1): 155–160

P- ISSN: 0976-1675 | **E**- ISSN: 2229-4538

www.rjas.org

Research Paper

Cladosporium sphaerospermum Strain ATCC 11289 Nanoparticles: Green Chemistry Approach

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Received: 25 Oct 2020 | Revised accepted: 31 Dec 2020 | Published online: 06 Jan 2021

A B S T R A C T

A green chemistry approach that incorporates nanotechnology and microbial biotechnology is the microbial synthesis of nanoparticles. Silver nanoparticles biosynthesis using extracellular fungal filtrate of *Cladosporium sphaerospermum* endophytic fungi isolated from the selected tree bark was simple, eco-friendly and robust. The goal of this study was to obtain silver nanoparticles (AgNPs) using aqueous extracts from the strain ATCC 11289 of the filamentous fungus *Cladosporium sphaerospermum* as an alternative to a chemical degradation procedure for harmful dyes. *Cladosporium sphaerospermum's* morphological and molecular characteristics have been established. Sequencing of ITS 18s rRNA, phenotypic characteristics and phylogenetic analysis were carried out. The isolate is recognized and described by the sequencing and phenotypic characterization of ITS 18s rRNA, which demonstrated the strain NF from *Ficus benghalesis* temperate area tree bark as *Cladosporium sphaerospermum* strain ATCC 11289. In addition, UV Vis spectrophotometer, FTIR, SEM and ESI MS tests have characterized the synthesized silver nanoparticles. Changes in parameters such as pH, temperature, silver nitrate solution concentration, optimum absorption parameters in the UV-Visible spectrophotometer have been used to optimize the output of silver nanoparticles Synthesized silver nanoparticles characterized by UV-Visible spectroscopy with a peak of 400-420 nm. For dye degradation of Methylene blue by $NaBH_4$, the obtained nanoparticles with good yield using microbial synthesis can be used. 83 percent of dye decolonization for methylene blue dye has been demonstrated by *Cladosporium*. Future studies on the use of these particles for *in-vitro* biological experiments would be cost-effective and convenient.

***Key words*:** *Cladosporium*, Endophytic fungi, Dye degradation, SEM, ESI-MS, Sodium borohydrate

With its application in science and technology for the synthesis and creation of nano-materials at the nanoscale stage, nanotechnology is an evolving area. Due to its optical, electrical and catalytic properties, the use of metal nanoparticles is gaining traction in the present century to use and optimize the physical properties of nanosized metal particles. Since they are less harmful to humans and the environment, green nanoparticles have been synthesized using biological sources such as plants and microbes [1].

Synthetic dyes in the textile industry are commonly used. The removal from the atmosphere of non-biodegradable harmful chemicals from synthetic dyes is a critical ecological problem. For the decomposition of dye, many techniques such as flocculation, electro coagulation, redox treatment and UV light degradation are commonly used. The present situation, however, needs an improved methodology to resolve the ineffectiveness of the current procedure [2]. Metal nanoparticles have been documented in recent research to be an efficient photo catalyst to degrade chemical complexes of synthetic dyes under visible light illumination [3]. Accordingly, silver nanoparticles could be used as highly economical agents for the rapid removal from the atmosphere of dye-based pollutants and could also be used to monitor other reducible contaminants. Silver nanoparticles have been successfully prepared from isolated endophytic fungus using a green chemistry method. From the leaves of *Ocimum balilicumh*, nine fungal endophytes were isolated [4]. *Aspergillus sp*., *Penicillium sp*., *Cladosporium sp*., and *Alternaria sp*. are the four main isolates [5]. The extracellular synthesis of silver nanoparticles has been verified. Effectively decolorized methylene blue dye by silver nanoparticles up to 96 percent within 72 h of incubation [6].

Green synthesis has now become a vast research field of production. Here we report a new green process for the synthesis of silver and gold nanoparticles using Kashayam, Guggulutiktham, and Ayurvedic medicine for the first time to the best of our knowledge. In the reduction of Methylene Blue (MB) by NaBH44, the size dependent catalytic activity of the synthesized nanoparticles is identified [7].

The use of free, sustainable and eco-friendly reducing agents used for the synthesis of silver nanoparticles has been found to exhibit excellent photocatalytic activity against dye molecules and can be used in water purification and dye effluent treatment systems [8]. In this current study we investigated the isolated endophytic fungi *Cladosporium sphaerospermum* produced by the green synthesis of silver

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nanoparticles. The silver nanoparticles were characterized by UV-Visible Spectroscopy, FTIR, SEM and ESI-MS. Dye reduction was observed in the extracellular biosynthesized silver nanoparticles, and catalytic dye reduction activity by $NaBH_4$ was observed.

MATERIALS AND METHODS

Silver nitrate (Alpha chemika); Sodium borohydride (PubChem); 1% Methylene blue (Science Company):1 gm in 100 ml distilled water. UV-spectrophotometer (Thermo Scientific, Chemito Spectrascan UV 2100 U.V Visible Spectrophotometer), FTIR (Shimatzu).

Production of Silver nanoparticles from endophytic isolates

The biogenesis of silver nanoparticles was carried out with slight modifications. The culture was inoculated in 50 ml PDB and was incubated at 28℃ on for 20 days on rotary shaker at 120 rpm. After incubation the broth with the grown culture was filtered using Whatmann filter paper number and the mycelium was collected and inoculated in 50 ml sterile distilled water. It was then incubated at 28℃ on for 2 days on rotary shaker at 120rpm. After incubation the distilled water with mycelium was filtered using Whatmann No 1 filter paper. The distilled water filtrate was collected in a sterile flask. Silver nitrate was prepared of different concentration (1mM, 2mM, 3mM, 4mM, 5mM) in sterile distilled water. Experimental solution was prepared by adding 10 ml of fungal culture filtrate to 90 ml of each concentration of silver nanoparticles solution and incubated in the dark for 24 hours at room temperature. Characteristics' of silver nanoparticles was done by Spectral analysis for the development of nanoparticles at different reaction conditions were observed using UV–Vis spectrophotometer from 300 to 700 nm [9].

Optimization of various parameters for silver nanoparticles

The environmental conditions exert an influence on growth and development of organism. The enzyme production by fungi is influenced by the condition in which the organisms are cultivated therefore, optimization studies will not only support good growth but also enhance product yield. The production of nanoparticles is also dependent on substrate concentration. The concentration of silver nanoparticles from 0.5 to 5.0 mM was studied. The optimum concentration for the synthesis of nanosilver is confirmed by UV-visible absorption chemical analysis. pH encompasses a sturdy influence on growth and catalyst production that is needed for the biogenesis of silver nanoparticles. Different pH ranging from 6 to 8 was used with the difference of 1.0 to study the influence of pH on silver nanoparticles production from endophytic fungus. Temperature plays a very important role in all reactions. Optimization studies with respect to temperature were carried out with temperature ranging from 25°C to 40°C with difference of 5-10°C for silver nanoparticles production. The sample was analyzed with UV-visible absorption chemical analysis and any result of temperature on nanoparticles was studied [10].

Characterization of synthesized silver nanoparticles

FTIR: FTIR measurements were carried out to identify the potential functional groups of the biomolecules in the fungal extract which are responsible for the reduction of the silver ions into silver nanoparticles.

ESI-MS: Elemental analysis of silver was measured by EDX.

Scanning electron microscope

SEM studies confirm the size and shape of the biosynthesized silver nanoparticles using all three fungal extract. Size of the nanoparticles was observed at different magnifications.

Application of silver nanoparticles for chemical dye reduction / degradation

Nowadays plant and microbes mediate synthesis of nanoparticles has nice interest and accomplishment because of its eco-benign and low time overwhelming properties. In this study silver nanoparticles were successfully synthesized and optimized. The photocatalytic activity of the synthesized silver nanoparticles was examined by degradation of methylene blue under sunlight irradiation. Green synthesized silver nanoparticles have the ability to degrade effectively the dye.

Methylene blue was subjected to reduction using sodium borohydride in the presence of silver nanoparticles in order to assess the efficacy of the catalytic activity of the synthesized silver nanoparticles. Freshly ready 1.0 ml of 10 mM sodium borohydride solution was mixed with 1.5 ml of 1 mM methylene blue, and the mixture was made up to 10 ml using double-distilled water and then stirred for 5 min, the solution mixture was created up to ten metric capacity unit victimization double-distilled water then stirred for five min furthermore. Sufficient quantities of synthesized silver nanoparticles were superimposed to each these solutions and mixed for thirty min with smart agitation, and also the UV-Vis spectrum of the reaction mixture of methylene blue was recorded at 1-min intervals of time for a period of 15 min at 25°C at 665 nm. The test was conducted in a standard quartz cuvette of about 3.0-mL volume. The rate constant of the redox reaction was dependent on the variation in absorption band at 664 nm as a function of time [11]. The dye reduction percentage were calculated by using:

$$\text{Efficiency } \% = C0 - C/C0 \times 100$$

Where, C0= Absorbance of Dye at 0 min.
C = Absorbance of reaction mixture after time interval [11].

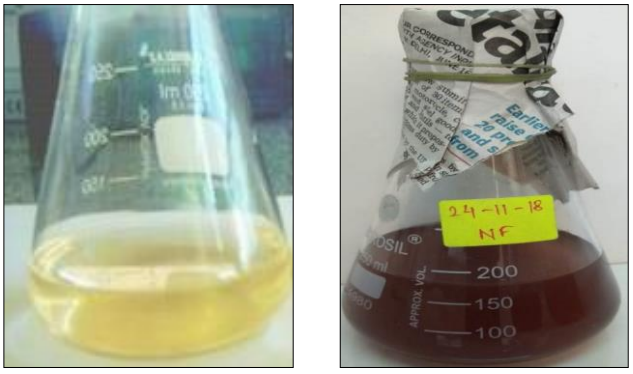


Plate 1 A. Uninoculated broth medium. B. Biogenesis of AgNPs by *Cladosporium sphaerospermum*

RESULTS AND DISCUSSION

Production of Silver nanoparticles from endophytic isolates

After incubation, change in colorless to brown color due to silver nanoparticle synthesis is observed. Biosynthesis of silver nanoparticles using fungal filtrate of fungal isolates. Initially formation of silver nanoparticles was detected by color change from colorless to dark brown [12]. The color change indicated reduction of silver ions (Ag^+) to SNP's

(Ag^o). Later synthesized brown colored solution used for spectrophotometric analysis from 300-700 nm the *Cladosporium sphaerospermum* silver nanoparticles has shown their own maximum absorption in between 400 to 420 nm as the generally silver nanoparticles shows maximum absorption in between 390-440 nm. Then, synthesized nanoparticles further washed with sterile distilled water 3 times by centrifugation to get dry form of it for further FTIR, SEM and EDS studies [13].

Optimization of various parameters for silver nanoparticles

Cladosporium spp from *Ficus benghalesis* tree bark has shown optimized substrate concentration 1mM, pH 7, at 40°C temperature (Plate 2).

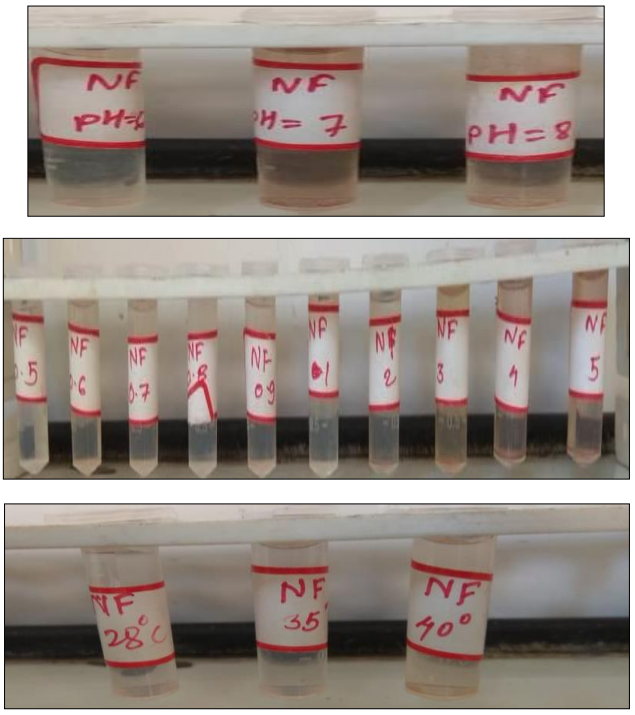


Plate 2 Optimization of Ag nanoparticles by using various parameters such as, substrate concentration, pH, and temperature

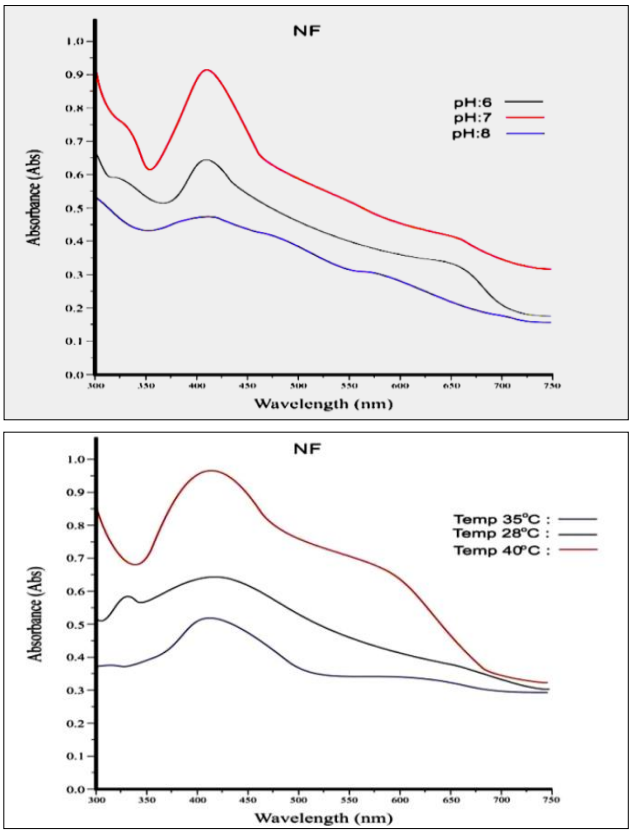
Characterization of synthesized silver nanoparticles

FTIR

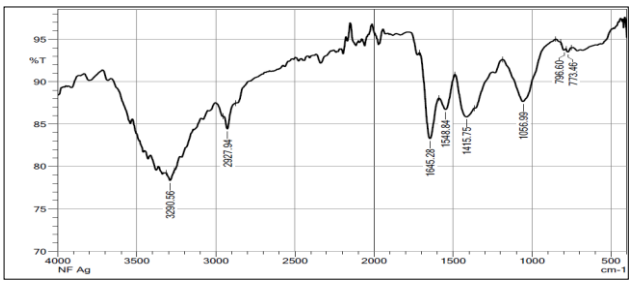
FTIR measurements were carried out to identify the potential functional groups of the biomolecules in the fungal extract which are responsible for the reduction of the silver ions into silver nanoparticles shows a strong absorption peak at 3290 cm^{-1} which indicates presence of carboxylic groups [14]. This useful cluster was changed in synthesized silver nanoparticles. The broad absorption band was observed between 3425 and 2927 cm^{-1} due to the O–H stretching and H-bonded alcohols and phenol groups. A weak band was observed at 1645 cm^{-1} corresponding to N–H bending primary amines [15]. A small peak was formed at 773 cm^{-1} due to the occurrence of alkyl halides. Moreover, the functional bimolecules are hydroxyl, carboxylic, phenol, and amine groups in fungal synthesized silver nanoparticles involved in the reduction of silver ions which was confirmed by FTIR spectrum.

Scanning electron microscope

SEM image shows the size and shape of the biosynthesized silver nanoparticles using all three fungal extract. Size of the nanoparticles was observed at different magnifications. Spherical and rod shape of nanoparticles with high agglomeration was noted with the size range from 200 nm to 500 nm. In this SEM image, some of the nanoparticles show large size due to the aggregation of small size of nanoparticles. Polydispersed nanoparticles were observed in SEM image and revealed the result of UV-Vis spectrophotometer. The surfaces of aggregated nanoparticles were shown to be rough [16].



Graph 1 λ max of synthesized Silver nanoparticles from endophytic fungi



Graph 2 *Cladosporium sphaerospermum* silver nanoparticles FTIR spectrum

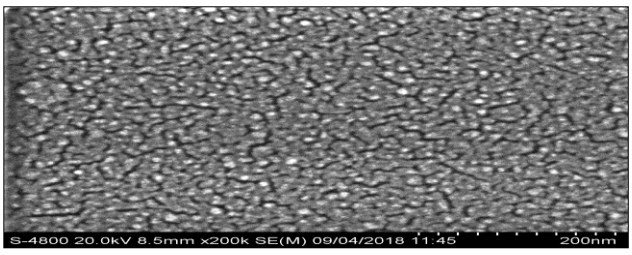
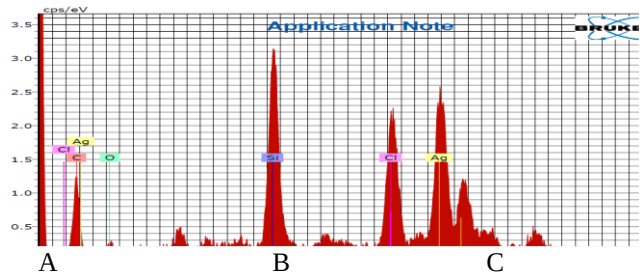


Plate 3 *Cladosporium sphaerospermum* silver nanoparticles

ESI-MS

Elemental analysis of silver was measured by EDX; EDX spectra reveal strong signals in the silver region of 3 keV

and confirm the formation of nanosilver and its elemental nature. This signal was formed due to the excitation of surface Plasmon resonance of silver nanoparticles. Some of the weak signals from Cl were observed. These signals were found due to maybe the presence of impurity from the biological molecules [17].



A B C

Graph 3 *Cladosporium sphaerospermum* silver nanoparticles

Application of silver nanoparticles for chemical dye reduction / degradation

Endophytic fungal isolates, has shown decrease in optical density which indicates that nanoparticles have the ability to reduce chemical dye. Methylene blue is blue in color, as dye started to reduce, the color of dye started to get faint or light blue in color [18]. Photocatalytic degradation of methylene blue was carried out by using green synthesized silver nanoparticles. Thereafter light blue was changed into light green. Finally, the degradation process was completed at 3 h and was identified by the change of reaction mixture color to faint colour. After the absorbance at different time interval has shown color change, blue to light brown then colorless till the 3 hours with decrease in optical density of samples [19].

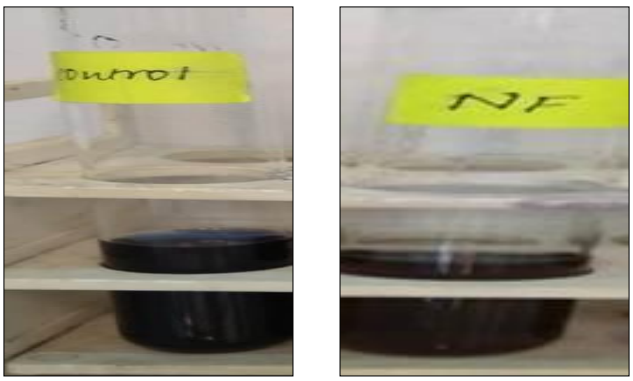
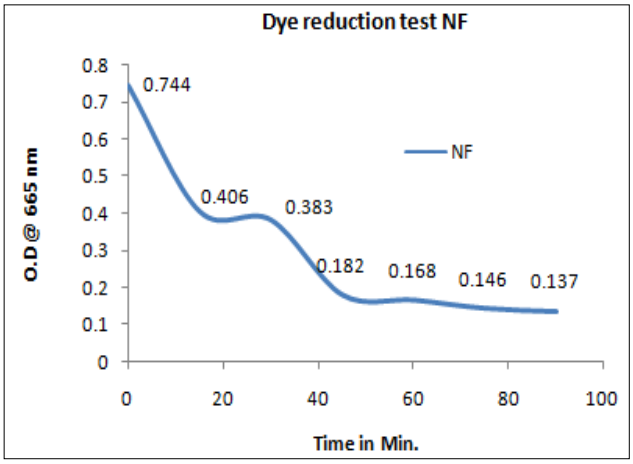


Plate 4 Methylelne blue dye degradation

The extracellular synthesis of silver nanoparticles were confirmed by color change from transparent to yellow-brown after incubation time of 48 hrs. The negative control was used without the fungal filtrate in silver nanoparticles solution. The intensity of brown color increases with respect to concentration of silver nanoparticles according to incubation time. The appearance of brown color was due to excitation of surface Plasmon vibration. To increase the silver nanoparticles synthesis rate, medium optimization processes were carried out under different physicochemical conditions. Environmental conditions profoundly modulate the growth and metabolism fungi [20]. Culture conditions have been the critical components, directly affecting the productivity and also the process economics. Optimization of physical parameters will not only support good growth also enhance the product yield. The growth conditions such as, substrate concentration, pH, and temperature etc. [21].



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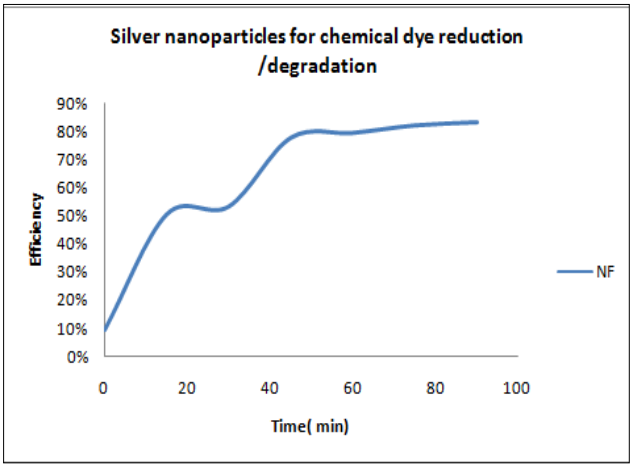

B

Graph 4 A. Methylene blue dye reduction by silver nanoparticles, B. % efficiency of dye reduction from NF*: Cladosporium sphaerospermum*

Table 1 Silver nanoparticles for chemical dye reduction

Time	Methylene blue dye reduction % efficiency
Min	NF
	9%
15	50%
30	53%
45	78%
60	80%
75	82%
90	83%

The optimization of extracellular synthesis of silver nanoparticles was performed for different concentrations ranging from 0.5 to 5mM. The optimum concentration was found to discussed below, was used further for characterization and optimization of various parameters such as pH and temperature [22]. When the silver nanoparticles concentration increased to 2 mM the particle size may increase due to the aggregation of large silver nanoparticles. pH has a strong influence on growth and enzyme production which is required for biosynthesis of silver nanoparticles [23]. Different pH ranging from 6 to 8 with a difference of 1 was used to study effect of pH on silver nanoparticles production from endophytic fungal isolates. Temperature plays an important role in all reactions. Optimization studies with respect to temperature were carried out with temperature of 28°C, 35°C and 40°C respectively [24]. The sample was analyzed with UV-Visible spectroscopy and further effect of temperature on nanoparticles was studied. It has shown

optimized conditions such as for *Cladosporium sphaerospermum* from *Ficus benghalesis* tree bark has shown optimized substrate concentration 1mM, pH 7, at 40ºC temperature. This finding is quite interesting as it contradicts the report of [25]. They found decrease in optical density after 1 mM substrate concentration and accordingly they proposed 1mM substrate concentration as the optimal silver nanoparticles production condition [26]. In acidic conditions we cannot observe any characteristic absorbance band for silver nanoparticles formation. These results revealed that, pH value started from 6 to 8 supported the maximum synthesis of silver nanoparticles. On the other side at high temperature of 40°C was observed optimum reaction temperature in case of *Cladosporium sphaerospermum*. The maximum SPR peak intensity was detected at 70°C results revealed also that by increasing in the reaction temperature, a sharp narrow UV spectra peak at lower wavelength region (412 nm at 70°C) are developed, which indicate the formation of smaller nanoparticles, whereas, at lower reaction temperature, the peaks observed at higher wavelength regions (440 nm at 30°C) which clearly indicates increase in silver nanoparticles size. Those findings are in agreement with the fact that when the temperature is increased, the reactants are consumed rapidly leading to the formation of smaller nanoparticles [27].

The characterization of synthesized silver nanoparticles was studied by UV-Visible Spectroscopy and characterized spectrum range was between 400 to 420 nm and absorbance peak was obtained at 411nm. For FTIR measurement, the dried and powdered sample of silver nanoparticles is used [28]. It is carried out for identification of effective biomolecules which are responsible for production and stabilization of silver nanoparticles from silver metal ions of silver nanoparticles solution. The protein molecules from fungal mycelia not only act as a reducing agent but also as a stabilizing agent by providing site for silver nanoparticles binding by negatively charged free amino acid residue [29].

The peaks observed in the FTIR spectrum shows the presence of proteins, which may act as a ligand for silver nanoparticles. This evidence suggests that the release of extracellular protein molecules could possibly influence the reduction and stabilization of the silver nanoparticles [30]. The presence of nitrate reductase was observed in the extracellular proteins secreted by the fungus [31]. This might be responsible for the bioreduction (of Ag^+ to Ag^- and the subsequent formation of silver nanoparticles), formation of stable nanoparticle Various studies have indicated that NADH, NADH- dependent nitrate reductase are involved in the biosynthesis of metal nanoparticles have reported that certain NADH-dependent reductase was involved in the reduction of silver ions by *F. oxysporum* [32]. Size and shape of synthesized silver nanoparticles was characterized through scanning electron microscope, it shows spherical particles with size less than 50 nm, confirms that reduction of silver ions to silver nanoparticles [33]. The reduction of Ag^+ ions to elemental silver by endophytic fungus is further confirmed by EDX analysis, which shows an optical absorption peak in the range of 3kev. This is typical for the absorption of metallic silver nanocrystallites [34].

For the reduction of methylene blue, the bond dissociation energy (BDE) plays an important role in formation or breaking of new bond. In reaction between Methylene Blue dye and $NaBH_4$, in which $NaBH_4$ acted as a donor and the dye as acceptor [35]. The addition of silver nanoparticle in reaction mixture performed as potential intermediate between Methylene blue dye and $BH4^-$ ions. At first, it lowered the BDE and made the electron transfer between them more efficient [36]. Thus, the rate of reduction of Methylene blue by $NaBH_4$ was increased in the presence of silver nanoparticles. Silver nanoparticles synthesized from endophytic fungal extract of decolorized methylene blue dye, which is basic dye, quite fast [37]. These results suggested that silver nanoparticles can be used for the treatment of textile effluents as well chemical dye production industries. In future studies, various application such as antimicrobial activity for multidrug resistant microorganisms as well as reduction of harmful industrial dyes, artificial food coloring, toxic food dyes, etc. [38]. In this study, SNPs were synthesized by biological means using endophytic cell free extract. The SNPs synthesis process at laboratory scale is quite inexpensive and non-toxic, eco-friendly as compared to the chemical methods. The synthesized silver nanoparticles also showed efficient degradation of methylene blue dye thus have potential in industrial application other than possible pharmaceutical application as antibacterial agents.

CONCLUSIONS

The aqueous fungal extract was added to silver nitrate solution, the color of the silver nitrate reaction medium was changed from transparent to brown and that indicates reduction of silver ions to silver nanoparticles. Thus, synthesized silver nanoparticles characterized by UV-Visible spectroscopy, which shows characterized peak between 400-420 nm. FTIR spectrum was examined to identify the effective functional molecules. SEM and ESI-MS analysis also done to study the shape and chemical structure of synthesized nanoparticles. *Cladosporium sphaerospermum* from *Ficus benghalesis* tree bark has shown optimized substrate concentration 3mM, pH 7, at 40ºC temperature.

Declaration of interest

The authors report no conflicts of interest.

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