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Original Research

Compatibility of *Beauveria bassiana* strains on the biosynthesis of silver nanoparticles

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ABSTRACT:

Nowadays synthesis of nanomaterials by using bio-root is limelight of modern nanotechnology. In the present investigation, we have isolated four strains viz: KFRI 330 (A), KFRI 332 (B), KFRI 351 (C) and KFRI 352 (D) of *Beauveria bassiana* from the forest soils in Kerala. Spore count was tested for all the strains of *B. bassiana* stored in the laboratory. Silver nanoparticles were synthesized from the four strains of *B. bassiana* and the formation of nanoparticles was observed within 48 hours. The synthesized silver nanoparticle has been characterized by UV-Vis spectroscopy, FT-IR and TEM analysis. The appearance of UV-Vis Peak (SPR 440 nm) revealed the reduction of silver metal ions to silver nanoparticles by using the fungal strains. The possible bio-molecules involved in nanoparticles synthesis was identified by HPLC analysis. The functional groups involved in the silver nanoparticles synthesis were identified. The amide group is responsible for the synthesis of silver nanoparticles. From the TEM analysis, the size of the AGNPs has been measured as 4-70 nm (mean 10.7±0.04 nm). It was evident from the HPLC result that primary amines act on capping as a well as a stabilizing agent.

Keywords:
B. bassiana, Silver nanoparticles, Characterization, HPLC.

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INTRODUCTION

Improvement of naturally invigorated test forms for the union of nanoparticles is a significant branch of nanotechnology. The biosynthesis of nanoparticles has built up expanding consideration because of the developing need to extend safe, practical and earth well-disposed advancements for the nano-materials union. Organic strategies for nanoparticles union utilizing microorganism (Klaus *et al*., 1999; Nair and Pradeep, 2002; Konishi *et al*., 2007; Sangappa and Thiagarajan, 2012), and their protein (Wilner *et al*., 2006; Jeevan *et al*., 2012) were all around reported in the writing. Be that as it may, investigation of pathogenic organisms as the potential nano-factories has more enthusiasm for the natural union of nanoparticles. Since, the nanoparticles were set up by organic strategies, proteins were utilized as topping operators holding fast.

Biosynthesized or green nanoparticles are picking up force in the field of farming for irritation (Chutao *et al*., 2007; Jeevan *et al*. 2012; Mouchet *et al*., 2008) and infection administration (Sangappa and Thiagarajan, 2012; Krishnaraj *et al*., 2010). The most regularly utilized proteins as a part of the combination of nanoparticles are ferritin and apoferritin. The extracellular biosynthesis of AgNPs using the filamentous fungus *A. fumigatus* and entomopathogenic fungi *B. bassiana* was investigated by Bhainsa and Souza (2006), Ingle *et al.* (2008) and entomopathogenic fungi *Beauveria bassiana* by Prabakaran *et al.* (2016).

Henceforth, these strategies have been changed for the bioreduction utilizing organisms, including *Fusarium acuminatum* (Ingle *et al*., 2008) and *Penicillium fellutanum* (Kathiresan *et al*., 2009). The additional cell proteins delivered by the growth cause a decrease and later they go about as topping specialists (Ahmad *et al*., 2003). Regardless of these noteworthy results, there are no reports accessible for orchestrating silver nanoparticles utilizing *Beauveria bassiana* entomotoxic proteins and the point by point system have not been clarified. Keeping this lacuna in our mind, the present study was conceived to investigate the entomo-pathogenic organisms associated in the forest ecosystem, its duplication under laboratory condition, and quickly incorporating AgNPs utilizing *B. bassiana.*

MATERIALS AND METHODS

Beauveria bassiana conidial suspension preparation

The *Beauveria bassiana* Bals. (Ascomycota: Hypocreales) fungal isolates were developed in Sabouraud Dextrose Agar (SDA) at 28^0C for 8-10 days. Spores were collected by scrapping the media surface and watery arrangements were readied. The spore suspensions were then separated by a few layers of muslin fabric to evacuate mycelial mats. The convergence of spores in the last suspension was dictated by haemocytometry (Tomson, 2013).

Estimation of spore concentration

One milliliter of the refined entomopathogenic fungal suspension was weakened with water containing 0.1% wetting operator (Tween-80). The spores were checked in around 25 of 1/400 mm^2 utilizing a hemocytometer. Spore suspensions of craved fixation were set up from the stock with reasonable weakening with sanitized refined water.

Biogenesis of silver nanoparticles using fungus

Silver nitrate ($AgNO_3$) was obtained from HiMedia, Mumbai, India (99.9 %). All glasswares were washed with deionised water and dried in an oven at 50-60^0C before use. The broth used for reduction of $AgNO_3$ molecules to Ag^0 was prepared by taking 100 mL of fungus culture in a 250 mL Erlenmeyer flask. This solution was added to 50 mL of 10^{-3} M $AgNO_3$ aqueous solution (Tomson, 2013).

Characterization of silver nanoparticles

UV-Vis spectral analysis

Bio-reduction of $AgNO_3$-particles in a protein arrangement was observed by intermittent inspecting of aliquots (2 mL) of the fluid substance and measuring the

UV- visible spectrum of the arrangement. UV-noticeable spectra of these aliquots were checked as a component of time of response on the example.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

For Fourier Transform Infrared (FT-IR) spectroscopy estimations, bio-reduced silver nitrate solution drop covered on Si (III) substrates was done on a Perkin-Elmer, spectrum (Japan) model RX-I, in the diffuse reflection mode at a determination of 4 $cm^{-1.}$

High-Performance Liquid Chromatography (HPLC)

The *B. bassiana* culture filtrate and biosynthesized silver nanoparticles were examined on a HPLC (Shimadzu LC/10 AD, Japan) outfitted with an injector (20 µl circle) and C-18 column (5µm molecule size), (250 mm x 4.6 mm I.D) utilizing CH_3OH/H_2O (1:1 v/v) at a flow rate of 0.8 ml min-1with identification inclination from 3% MeOH (Himedia, India) and expanded up to 70% MeOH by UV absorption at 280 nm utilizing an indicator (SPD-10A/UV-Vis). Purification was executed by analytical HPLC (Shimadzu) utilizing an LC/10 AD ODS, 250 mm X 4.6 mm segment, MeOH/ H_2O linear elution inclination beginning from 3% MeOH amid five minutes and expanded up to 70% MeOH in thirty minutes with a stream rate of 1.0 mL $min^{-2.}$

TEM analysis of silver nanoparticles

For Transmission Electron Microscopy (TEM) analysis, dried nanoparticles were set onto carbon covered copper matrices and estimations were tackled a JEOL model 3010 and Philips CM-200, Japan instrument worked at an accelerating voltage of 120 kV. The size and state of bio-reduced nanoparticles were physically translated independently by watching 100 particles haphazardly as indicated by the shapes and sizes.

RESULTS AND DISCUSSION

Selection of *Beauveria bassiana* strains and culture

The four strains of *B. bassiana* were selected by the analyzing the sporulation capacity of the fungi in potato dextrose broth. Well sporulated strains were selected and represented as A (KFRI 330), B (KFRI 332), C (KFRI 351) and D (KFRI 352). Spore count was recorded using a haemocytometer (Table 1).

Biogenesis of silver nanoparticles using fungus

The bio-silver nanoparticles were synthesized using the strains of *B. bassiana* culture filtrate after 48 hours by the addition of silver nitrate solution (Figure 1). In UV-Visible spectroscopy, the synthesized silver nanoparticles' Surface Plasmon Resonance (SPR) bands occur in the range of 429- to 440 nm in an aqueous medium. As the bio-reduced silver nanoparticles were stable, highly intensified SPR bands initially occurred at 440 nm after 48 hours at room temperature and initially turned the turbid colored solution into brown. After completion of the reaction of the silver ions with the *B. bassiana*, the silver nanoparticles solution was tested for stability. It was observed that nanoparticles solution was stable for more than 60 days with little aggregation (Figure 1).

FT-IR spectroscopy

The silver nanoparticles (AgNPs) synthesized using the *B. bassiana* were subjected to FT-IR analysis to identify the bio-molecules stabilizing the nanoparticles in solution and also to provide a clue as to what the reducing agent might be. The silver nanoparticles synthesized using *B. bassiana* protein fraction showed strong bonds at 1638 (Plate 1) (Table 2) (Tomson, 2013). This band corresponds to the amide II bands of polypeptide/protein. As indicated by the FT-IR data, the functional group responsible for the reduction of Ag^+ was secondary amines (1636 and 1435cm^{-1}).

FT-IR studies confirmed an amine group from amino acid residue and showed a well-built binding

Table 1. Spore count of different strains of *B. bassiana*

B. bassiana strain	Spore count
KFRI 330 - A	$4.2x10^8$
KFRI 332 - B	$3.8x10^8$
KFRI 351 - C	$5.5x10^8$
KFRI 352 - D	$4.8x10^8$

Table 2. FT-IR analysis of *B. bassiana* culture extract and biosynthesized silver nanoparticles media

KFRI 330 - A		Nanoparticles	
Frequency (cm^{-1})	**Functional group**	**Frequency (cm^{-1})**	**Functional group**
3449.17	OH/NH Alcohol/Amide	3450.98	OH/NH Alcohol/Amide
2079.77	NH with CO Secondary amides	2075.57	NH with CO Secondary amides
1638.51	NH amide	1636.78	NH amide
482.02	Unknown	566.24	C-Br Alkyl halide
KFRI 332- B		**Nanoparticles**	
3449.17	OH/NH Alcohol/Amide	3455.85	OH/NH Alcohol/Amide
1637.56	NH Amide	2078.92	NH with CO Secondary amides
1084.24	CO/CN Alcohol/Amide	1637.73	NH amide
474.23	Unknown	567.40	C-Br Alkyl halide
KFRI 351- C		**Nanoparticles**	
3452.31	OH/NH Alcohol/Amide	3454..0	OH/NH Alcohol/Amide
2086.94	NH with CO Secondary amides	2078.75	NH with CO Secondary amides
1637.80	NH amide	1638.24	NH amide
524.14	C-Br Alkyl halide	588.19	C-Br Alkyl halide
KFRI 352- D		**Nanoparticles**	
3453.13	OH/NH Alcohol/Amide	3451.09	OH/NH Alcohol/Amide
2079.46	NH with CO Secondary amides	2078.46	NH with CO Secondary amides
1638.42	NH amide	1637.36	NH amide
570.79	C-Br Alkyl halide	575.40	C-Br Alkyl halide

potential with metal; this suggested the arrangement of a layer covering metal nanoparticles and acting as a capping agent to stop agglomeration and providing strength in the medium. These results confirmed the presence of proteins performing as reducing and stabilizing agents (Prabakaran *et al*., 2016). Therefore, it is evident that these amines act as a reducing agent and also as a stabilizing or capping agent. Proteins play a major role in the reduction of silver ions by oxidation. These findings are in accordance with the results of Sadowski *et al.* (2008) in the fungus mediated synthesis of AgNPs (Tomson, 2013)

HPLC analysis

HPLC analysis showed the peaks representing compound in the culture medium (Plate 2- 1,2,3 and 4) was absent in the nanoparticles synthesized medium (Plate 2- a,b,c and d). Hence, it clearly proved that the reducing agent acts on silver nitrates and produce nanoparticles as well as a capping agent for stable nanoparticles. Also, the compound present in the each strain of *B. bassiana* were varied (Plate 2). The results obtained from the culture filtrate of fungal strains indicated the common compounds produced by the fungus in the medium. After synthesis of bio-nanomaterial there found changes in the chromatogram, i.e. some peaks were absent or the peaks got reduced. That change clearly indicated the involvement of compounds produced in the fungal medium for nanoparticle synthesis.

TEM analyses

TEM analyses of the synthesized nanoparticles were clearly distinguishable owing to their size difference. From the TEM image, the size of the synthesized silver nanoparticles was measured 4 from70

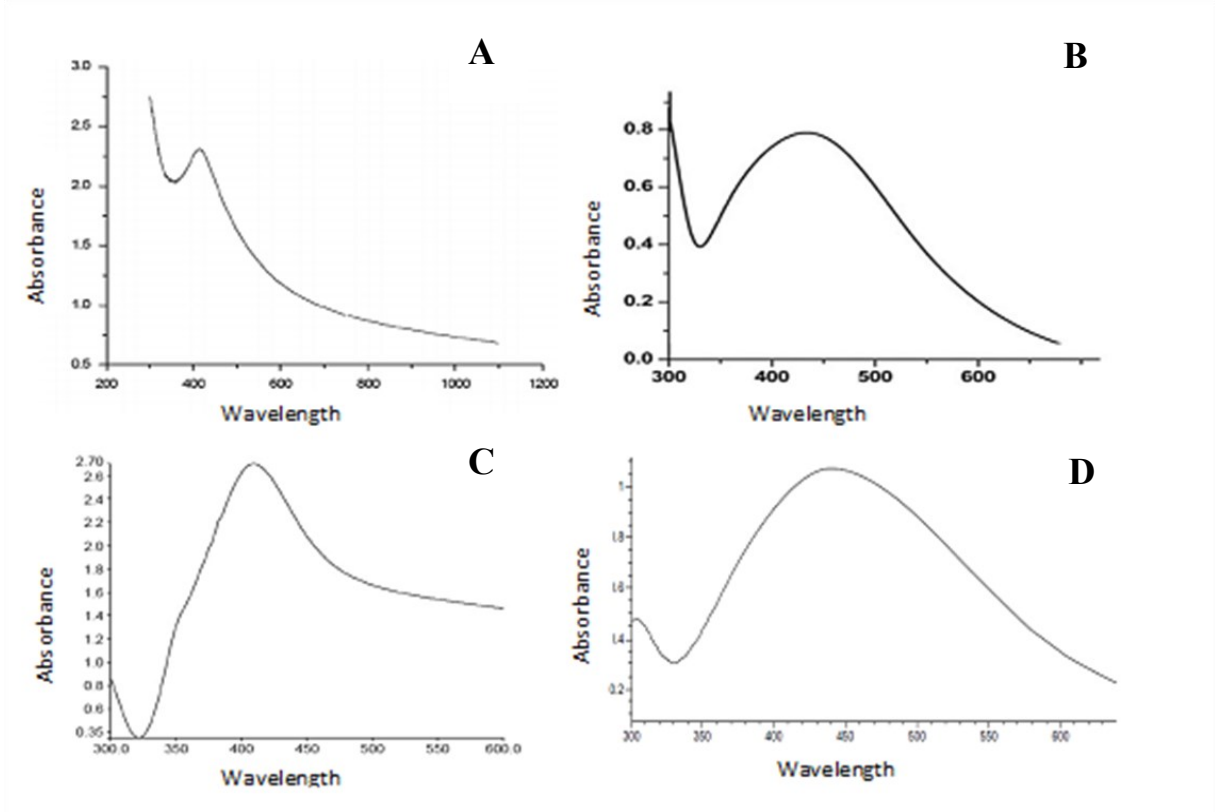

Figure 1. UV-Visible spectroscopy analysis of *B. bassiana* strains mediated biosynthesized silver nanoparticles (wavelength nm)

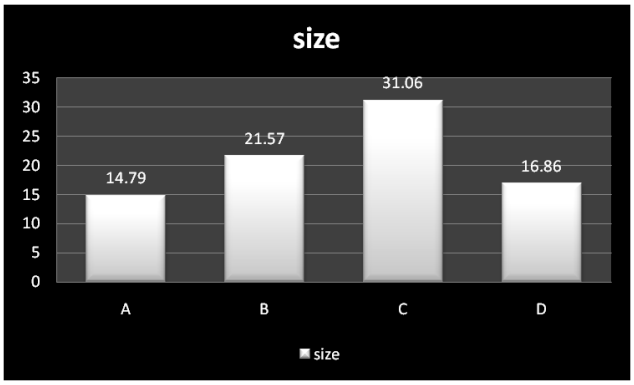

Figure 2. Different size silver nanoparticles produced by *B. bassiana strains*

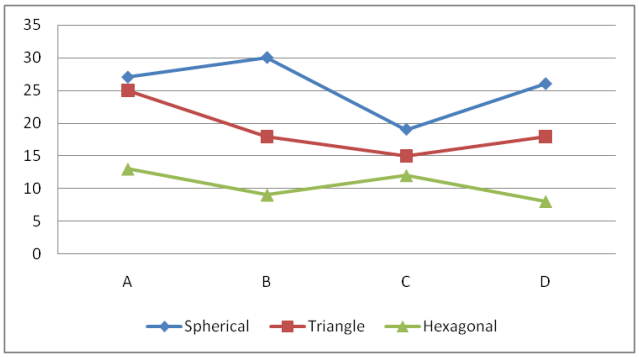

Figure 3. Spherical, triangular and hexagonal shape of silver nanoparticles reduced by *B. bassiana* strains

nm (Plate 3) with more number of spherical (42%) rather than triangular (38%) and hexagonal (20%) shapes (Figure 2 and 3). The nanoparticles obtained were highly crystalline in nature. The low magnification TEM image clearly showed a number of silver nanoparticles of a range of size and shapes.

The structural features of the individual silver nanoparticles are more clearly seen in the higher magnification TEM images. The particles were predominantly spherical, rather than hexagonal and triangular in shape, ranging in size from 4 nm – 12 nm. Similarly, the regenerative capability of biological systems coupled with the discovery that fungi such as *B. bassiana* are capable of hydrolyzing metal complexes that they never encounter during their growth cycle showed enormous promise for development, particularly the large-scale synthesis of metal oxide materials. The possible mechanism of biosynthesis of nanoparticles by

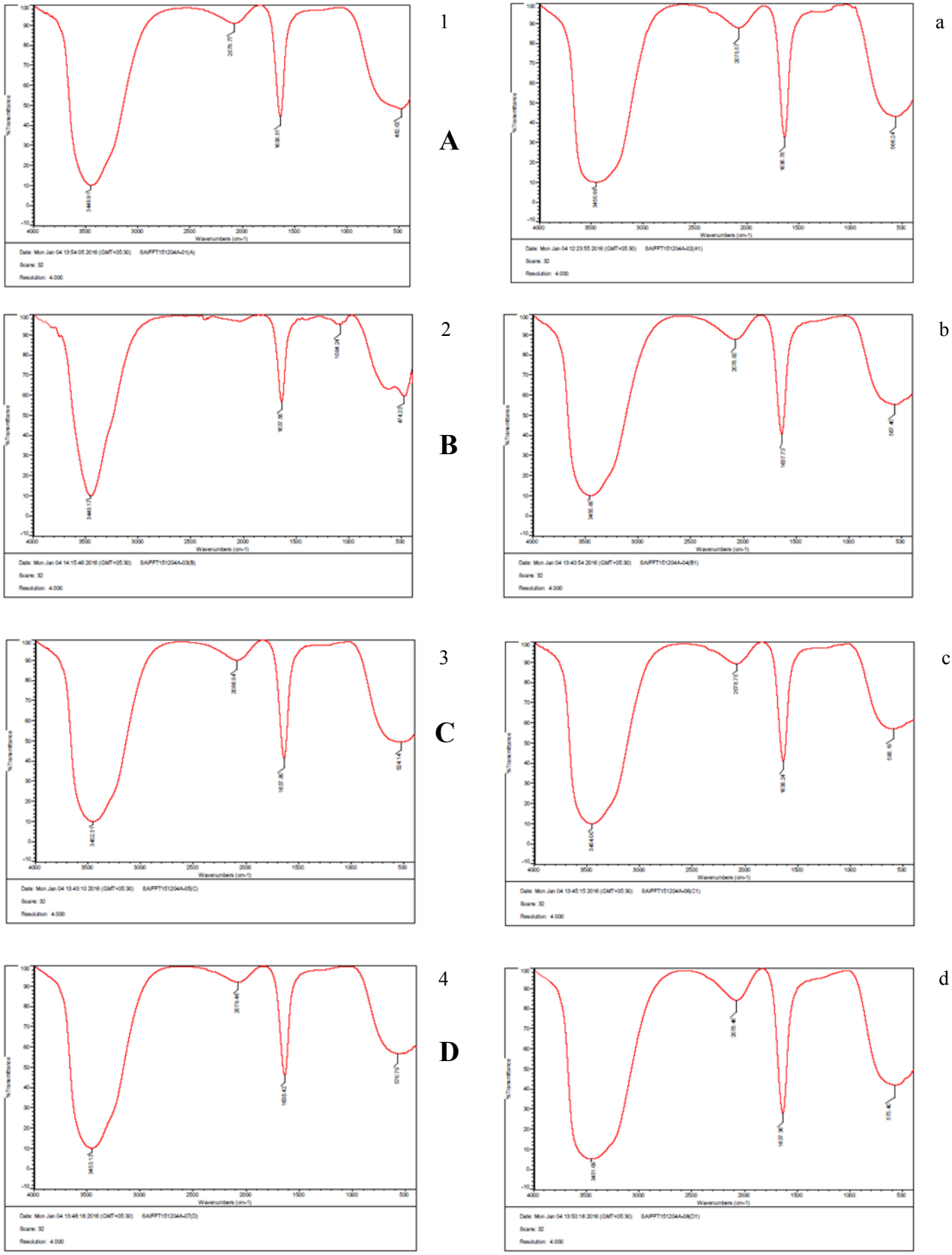

Plate 1. FT-IR analysis of *B. bassiana* culture extract (1,2,3,4) and biosynthesized silver nanoparticles media (a,b,c and d)

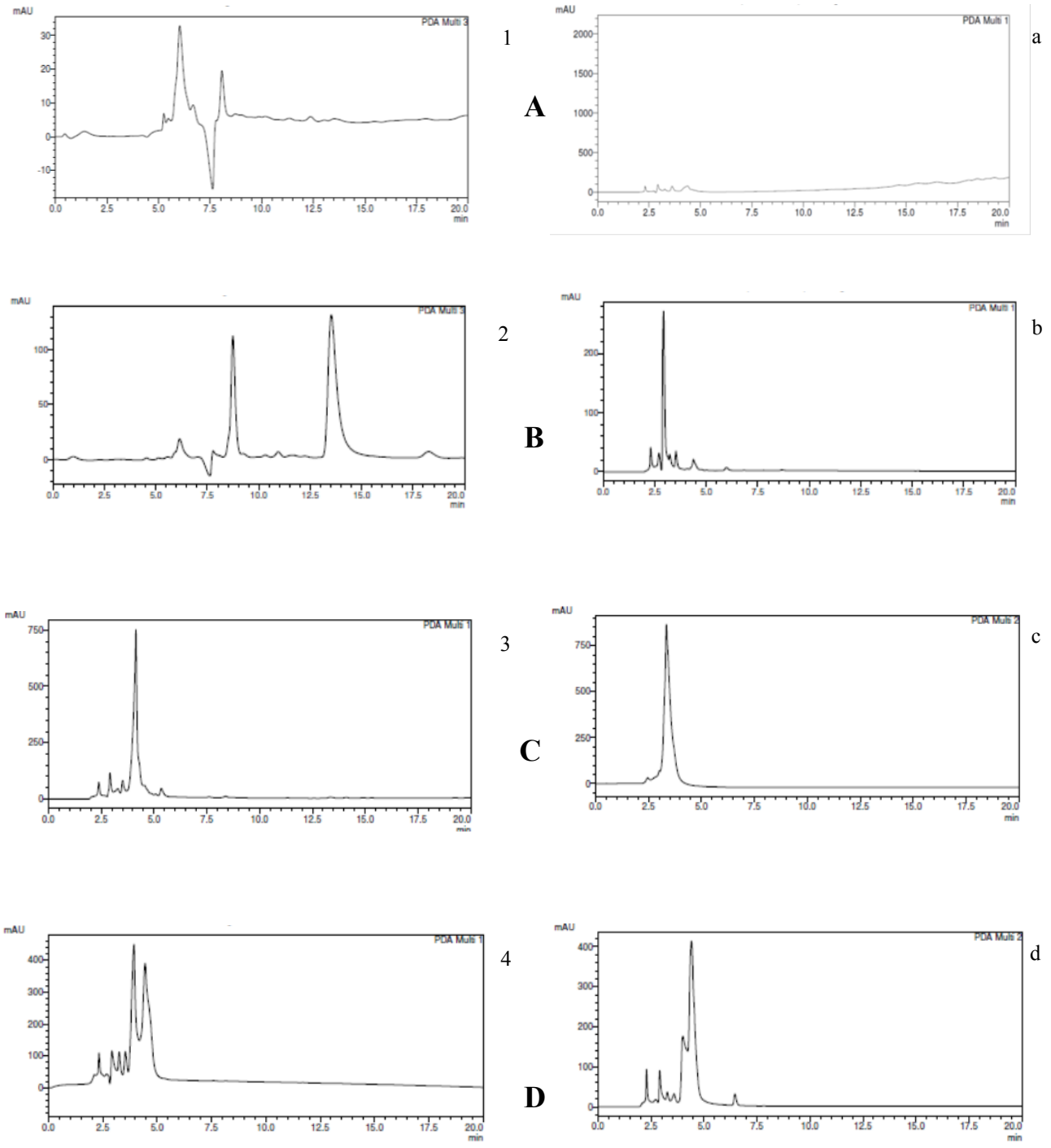

Plate 2. HPLC analysis of *B. bassiana* strains culture extract (1,2,3,4) and biosynthesized silver nanoparticles media (a,b,c,d)

the biological system was reductases and any other equivalent reductants as reported earlier by Krishnaraj *et al.* (2010).

TEM images of silver nanoparticles, it was noted that the particles are uniform size 4-12 nm. Bhainsa and Souza (2006) reported that the biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus* the shape and size of the nanoparticles were spherical in shape, sizes of the silver nano-particles were found to be in the range of 5–25 nm. The silver nanoparticles have an

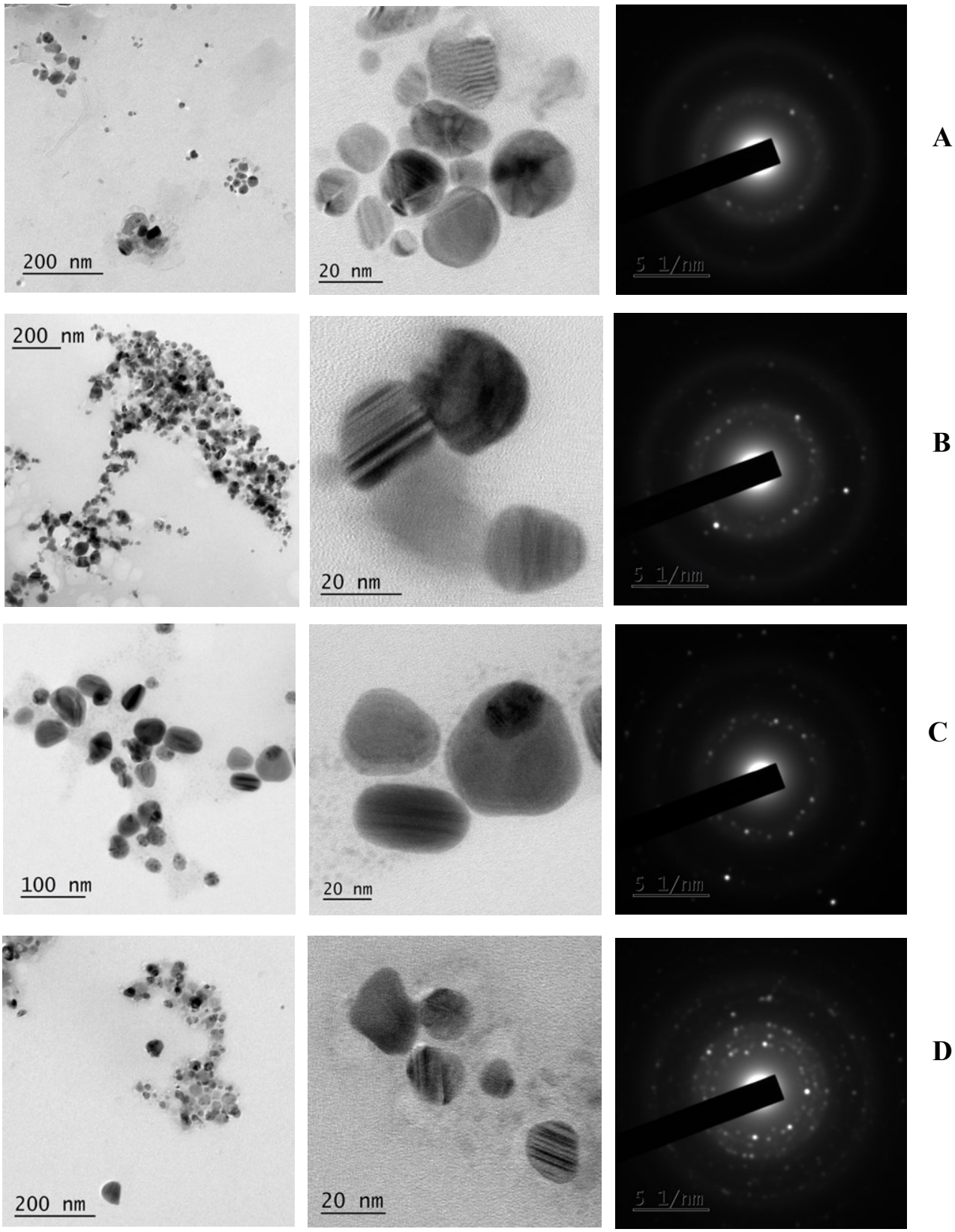

Plate 3. TEM analysis of *B. bassiana* mediated biosynthesized silver nanoparticles

inclination to form spherical structures that are nanocrystalline in nature. Variation in shape and size of nanoparticles synthesized by biological systems is common (Prabakaran *et al*., 2016).

Extracellular synthesized nanoparticles were stabilized by proteins and reducing agents secreted by the fungus. It has been reported that some high-molecular-weight proteins, including NADH-dependent reductase, are released by fungal biomass in nanoparticles synthesis and stabilization (Prabakaran *et al*., 2016). Our findings are correlated with the reports of Goel *et al.* (2001) and Prabakaran *et al*. (2016) they showed the proteins are capable of binding to nanoparticles through free amine groups or cysteine residues in the proteins and electrostatic attraction of negatively charged carboxylate groups in enzymes present in the cell wall of fungi.

CONCLUSION

The utilization and application of nanomaterials in the current field of science are increasing and it gaining its momentum. Biological agents have the ability to produce nano-silver by reduction of a silver atom to nanoparticles, decrease the chemical effects on the environment. The synthesized silver nanoparticles were size from 4 nm – 12 nm and spherical in shape. Here the fungus *B. bassiana* is a good agent to produce silver nanoparticles in minimum time. The size is greatly varied according to different strains of *B. bassiana.*

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Conflict of interest

The authors declare that they have no conflict of interest.

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