A Novel Entomopathogenic Actinobacteria Mediated Silver Nanoparticles Characterization and Their Insecticidal, Antibacterial and Cytotoxic Activities

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A NOVEL ENTOMOPATHOGENIC ACTINOBACTERIA MEDIATED SILVER NANOPARTICLES CHARACTERIZATION AND THEIR INSECTICIDAL, ANTIBACTERIAL AND CYTOTOXIC ACTIVITIES

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Abstract

Nanomaterials were highly inspired in the field of nanotechnology especially bio synthesized silver nanoparticles (AgNPs) have effectively attracted worldwide that helps to improve in the field of medical science and human health. Actinabacteria mediated nanoparticle were widely used to control insect pests, treatment of antibiotic resistant bacteria, cancer and other diseases due to their potential pharmacological properties compared with other chemical drugs. The present investigation an entomopathogenic actinobacteria *Actinokineospora fastidiosa* was isolated and synthesis of AgNPs and tested their mosquito larvicidal, antibacterial and anticancer activity. Moreover, the outcome of biosynthesized AgNPs was characterized by UV-visible spectroscopy, X-ray Diffraction (XRD), Fourier transforms infrared spectroscopy (FT-IR), Transmission electron microscope (TEM), scanning electron microscope (SEM) with energy dispersive X-ray spectroscopy

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(EDX) and zeta potential analysis. The characterized AgNPs showed a potent larvicidal activity against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* the obtained LC_{50} values is 9.86 ppm, 8.50 ppm and 8.29 ppm respectively. However, at 25 ppm concentration, AgNPs showed highest ovicidal activity (100 %, 100 % and 97.77 % against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* respectively) and oviposition deterrence activity was 100 %, 100 % and 98.66 % were achieved against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* respectively. The enzymes of α, β esterase and Glutathione-S-transferase level were increased due to detoxification process against AgNPs. The histopathological results of AgNPs showed series damage in epithelial cells and brush border cell of mosquito larval gut. In addition, actinobacterial mediated AgNPs was tested against different human pathogenic bacteria. The obtained results showed biosynthesized AgNPs revealed remarkable antibacterial activity against all tested pathogens. The lowest mortality was observed on non-target organisms of *Artemia salina* at highest concentration of AgNPs. Finally, *in vitro* toxicity study showed that biosynthesized AgNPs potential anticancer activity and induced reactive oxygen species (ROS) production in human cervical cancer (HeLa) cell line at an inhibitory concentration (IC_{50}) range 42.37 µg/mL. Therefore, it can be concluded that biosynthesized AgNPs using a novel stains *Actinokineospora fastidiosa* potentially influenced the vector mosquito and bacterial diseases management and pharmacological applications in eco-friendly manner.

Keywords: *Actinokineospora fastidiosa*, silver nanoparticle, optimization, larvicidal, antimicrobial, non-target organisms

1. Introduction

Recently, World health organization (WHO) reported that about 2100 million of peoples are at risk of vector bore disease such as malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya, yellow fever and etc., which affects the current civilization in

terms of economic loss, morbidity and mortality (Ali and Venkatesalu 2020). The major vector mosquitoes of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were important carriers of transmitting many deadly diseases and nuisance to public health (Balaraju et al. 2009) (Sukumaran and Maheswaran 2020). *A. stephensi* is the most important vector of malaria fever in India and other West Asian countries. Malaria alone affects 36% of the people in worldwide i.e. 2020 million in 107 countries and territories of tropical and subtropical regions (Ali and Venkatesalu 2020). In recent years 1.4 billion peoples in 73 countries were life threatened by lymphatic filariasis, formerly named as elephantiasis caused by *C. quinquefasciatus*. In other hand 120 million peoples are infected with about 40 million disfigured and incapacitated by mosquito vector disease (Kalaimurugan et al. 2019). *A. aegypti* is the major viral vector for Chikungunya, Dengue, Dengue hemorrhagic fever, Yellow fever, Zika virus and etc. Nearly, 2500 million peoples were at risk of dengue viral infection i.e., two-fifth of the world's population and there may be 50 million cases were reported in dengue fever in every year (Moulin et al. 2016). These are the thought-provoking issues for public health in worldwide, and causes social and economic impact on worldwide (Benelli and Mehlhorn 2016). Vector-borne diseases were tenacious due to resistant in mosquitoes since the excessive application of chemical insecticides and pesticides (Enayati et al. 2003) and lack of effective vaccines (Kumar et al. 2011). In modern years, frequent usage of synthetic chemical insecticides for controlling vector mosquitoes has disrupted natural systems and causes to resurgence in mosquito populations leading to major outbreaks of mosquito borne diseases. Variety of control measures like physical, chemical and biological methods were available to controlling mosquitoes. Bio control programs were an alternate way to stabilize the life frightening insects in an ecofriendly approach. Hence, larvicides play an important role in combating mosquitoes from their breeding sites (Maheswaran and Ignacimuthu, 2013).

The wide range of biological resources were available for biosynthesis of nanoparticles using bacteria, fungi and plants (Nadagouda et al. 2009). In this aspect actinobacterium were more suitable for the production of nanometals and well known their supreme ability for the production of many active molecules with several biological applications (Manivasagan et al. 2013b). *Streptomyces* sp. was considered more significant due to the approximate production of 50-55 % of antibiotics by itself (Manivasagan et al. 2014). Earlier reports of nanoparticle synthesis using *Streptomyces viridogens* (Balagurunathan et al. 2011), *Streptomyces naganishii* (Shanmugasundaram et al. 2013), *Streptomyces hygroscopicus* (Sadhasivam et al. 2010), *Streptomyces sp.* (Karthik et al. 2014), *Nocardia farcinica* (Oza et al. 2012), *Thermomonospora sp.* (Ahmad et al. 2003b), *Nocardiopsis sp*. (Manivasagan et al. 2013a) and *Rhodococcus sp*. (Ahmad et al. 2003a). Rapid growth in the field of nanoparticles (NPs) research due to the development and incorporation of nanocomposites having a wide range of applications into products and various technologies (Moghimi et al. 2001) and also the AgNPs were most important vehicles for drug delivery to small cell wall (Rastegari et al. 2019). Rising resistance in pathogenic bacteria against certain antimicrobial agents was one of the major problems in the world. Effective treatment of a disease demands the development of new potential source of novel drugs (Kirtiwar et al. 2018). Green and chemically synthesized AgNPs could modulate the antibacterial activity against pathogenic bacteria, antioxidant activity, DNA cleavage mechanism and apoptosis. Hence, the current study was designed to evaluate newly isolated actinobacteria mediated AgNPs were tested against eggs, larvae and adults of *A. aegypti, A. stephensi* and *C. quinquefasciatus.* In addition, actinobacteria mediated synthesized AgNPs was tested for their anti-cancer on human cervical cancer cell line (HeLa) and apoptotic morphological analysis and non-target organisms.

2. MATERIALS AND METHODS

2.1. Mosquito rearing

The larvae of *A. stephensi, A. aegypti* and *C. quinquefasciatus* were collected from paddy fields and stagnant water bodies of Sadayampatti village, Madurai, Tamil Nadu, India to start the colony. The larvae were transferred in laboratory conditions at (RT: 26 ± 2 °C, and 70–85 % relative humidity RH) and photoperiod of 14:10 h (light/dark). Around 200 larvae were reared under relaxed conditions (1 larva/ 5 mL) in each rearing tray (40 x 30 x 8 cm) with 1 L of tap water and water was restored every 2–3 days. The breeding tray was kept closed with a muslin cloth to prevent the entering of foreign mosquitoes. Larvae were fed a diet of Brewers yeast, dog biscuits and algae collected from ponds in a ratio of 3:1:1, respectively (Maheswaran and Ignacimuthu, 2015a; Maheswaran and Ignacimuthu, 2015b). Pupae were collected daily and transferred to glass beakers containing 500 mL of water that placed in a mosquito cage (45×45×40 cm) for adult emergence. Adult mosquitoes were maintained with 10 % sucrose and periodically blood-fed on chicken. After 3 days, ovitrap was kept inside the mosquito cages for egg laying then eggs were collected and transferred to enamel trays. After egg hatching, similar developmental stages of mosquito larvae (F1 Generation) were used for bioassays. Two developmental stages, larvae and adult females, were continuously available for the experiments (Maheswaran and Ignacimuthu 2012).

2.2. Isolation and Identification of potential actinobacterium

Soil samples were collected from different place of Western Ghats of Tamil Nadu, India. Samples were brought to the laboratory using ice cold pack and stored at 4 °C. Isolation of actinobacterial strains from soil using starch casein agar with addition of Nystatin (10 µg/mL) and Nalidixic acid (20 µg/mL) were used to avoid unnecessary microbial growth on the culture plate. Serial dilution, spread plate and streak plate technique were used for isolation and separation of different bacterial strains based on their morphology and pigment

production according to the Bergey's systematic bacteriology manual (Deepika and Kannabiran 2010). All the experimental chemicals were purchased form Himedia (Mumbai).

Each bacterial isolate was inoculated in 50 mL of starch casein broth medium. The inoculated flasks were kept in a shaking incubator at 120 rpm and 28±2 °C for 7-10 days of incubation period. After incubation period cultures were centrifuged at 10,000 rpm for 20 min, supernatants were filtered through sterile mesh line cloth (0.2 μm pore size). Preliminary screening of larvicidal activity was evaluated by following the procedure of WHO with minor variation (WHO 2005). Five batches of 20 number of *C. quinquefasciatus* larvae (IV instar) were introduced in 199 mL of distilled water and 1.0 mL of desired actinobacterial cell free filtrate. The larval mortality was calculated after 24 h of exposure period. The percentage of mortality rate was calculated using Abbott's formula (Abbott 1925).

2.3. Biosynthesis of AgNPs

In 250 mL Erlenmeyer flasks, 100 mL of 1 mM $AgNO_3$ and 100 mL of cell free filtrate of actinobacterial (CHI-10) strain were added then flasks were incubated in a rotary shaker at 28±2 °C and 200 rpm for 120 h for synthesis of AgNPs (Abd-Elnaby et al. 2016). The reduction of silver ions was continuously monitored visually based on colour change from pale yellow to brown, which was confirmed the biosynthesis of AgNPs in the solution mixer (Ramesh Kumar et al. 2014). Subsequently, the reduction of silver ions in the inoculated flask which is primarily confirmed using UV–Vis spectrophotometer. After preliminary confirmation of actinobacteria mediated synthesized AgNPs were purified and separated by ultra-centrifugation then pellets were dried at 60 °C up to 24 h (hours) and stored at 4 °C for further analysis. After centrifugation, the quantity of AgNPs was weighed per 100 ml. The various optimization parameters such as pH (2.0, 4.0, 6.0 and 8.0), Metal concentration ratio (1.0, 2.0, 3.0, 4.0 and 5.0 mM), Time (5, 10, 15, 20 and 25 h), Aqueous cell-free filtrate (1.0, 2.0, 3.0 and 4.0 mL) and Temperature (10, 20, 30, 40, 50 and 60 °C) all

absorbance maxima reaction synthesized AgNPs were monitored and measured by UV–Vis spectrophotometer at 420 nm.

2.4. UV- Vis spectrophotometer analysis

The bio reduction of silver ions was inspected by color change from pale yellow to brown color. In additional that were confirmed by peaks obtained in a correspondence range of spectrum value of actinobacteria mediated synthesized AgNPs solution by using UV–Vis spectrophotometer (UV-1800) (Shimadzu, Japan) which, essential to validate the formation of nano metal provides the surface resonance exists for the nanometals. An aliquot of the tested solution was load in a cuvette (quartz) and monitored the scanning range 200 to 800 nm at room temperature (Singh et al. 2014).

2.5. X-Ray Diffraction (XRD) analysis

X-Ray Diffraction studies performed assist the crystalline phases present in a nanomaterial and thereby reveal chemical composition information about nanometals (Rigaku Ultima 4). The scan was measured by voltage at 40 kV and 30 mA with Cu-Kα radiation in the 2Θ range from 10° to 80°.

2.6. Fourier Transform-Infrared Spectroscopy (FT-IR) analysis

The FT-IR analysis was to determine the bio transformed molecules found in the extracellular from effective actinobacterium strain. The synthesized AgNPs suspension was centrifuged at 10,000 rpm for 10 min and dried sample was subjected to analysis Perkin Elmer one FT-IR spectrophotometer in the range from 4000 to 400 cm^{-1} at resolution of 4 cm^{-1}.

2.7. TEM and SEM-EDX analysis

Transmission Electron Microscopy (TEM) is a significant characterization for obtained assessable measures of particle, size distribution, and morphology. This action was

performed by casting a drop of AgNPs was transferred on to a carbon coated grid and allowed to dry prior to measurements (Hitachi H-500) at voltage of 100kV.

Scanning Electron Microscopy (SEM) analysis achieved by interaction of electrons with atoms in the synthesized NPs produces the surface topography and composition of the AgNPs. The elemental composition of the NPs was obtained through Energy Dispersive X-Ray Spectroscopy (EDX) analysis conjunction with SEM (EDS- Jeol, JFSM 6380). The potential stability of AgNPs was explored by using a Malvern Zetasizer Nano ZS, UK).

2.8. Larvicidal activity

Actinobacterium mediated synthesized AgNPs were tested for larvicidal activity against fourth instar larvae of *A. aegypti, A. stephensi* and *C. quinquefasciatus* at 5, 10, 15, 20, 25 ppm concentrations by WHO (WHO 2005) method with some modifications. 0.05 % of Dimethyl sulfoxide (DMSO) was used as negative control, Temephos (0.025 %) used as a positive control. The Mortality and survival rate were calculated after 24 h exposure period. Mortality percentage was calculated by Abbott formula (Abbott 1925).

2.9. Ovicidal Activity

The ovicidal activity was evaluated by the protocol of Shoukat et al., (2020). Thirty freshly laid eggs of *A. aegypti, A. stephensi* and *C. quinquefasciatus* were treated with synthesized AgNPs at 5, 10, 15, 20 and 25 ppm concentrations. Each experimental concentration was replicated 5 times. DMSO (250 ppm) was used as a control. After 48 h of treatment egg hatchability was recorded until 72 h. After treatment the eggs from each concentration were separately transferred to distilled water cups for observing hatching assessment under dissection microscope. The non-hatched eggs with unopened opercula were

counted in each experimental group then percentage ovicidal activity was determined by using the following formula:

$$\% \text{ of egg mortality} = \frac{\text{No. of hatched larvae}}{\text{Total No. of eggs}} \text{X } 100$$

2.10. Oviposition deterrent activity

The effect of synthesized AgNPs on egg-laying of *A. aegypti A. stephensi* and *C. quinquefasciatus* was tested the procedure of Prajapati et al., (2005). 25 gravid females were fed on chicken blood (1–7 day old) and 50 males were instantaneously introduced in oviposition cages (33x30x30 cm). 10 % of sucrose solution was provided for all times. The cages contained plastic cups (100 mL) containing 5, 10, 15, 20 and 25 ppm of AgNPs and 250 ppm of DMSO used as control with five replicates. The numbers of eggs and egg rafts were counted on the 7th day after treatment. The percentage of effective repellency was and statistically analyzed by Tukey's test using SPSS 21.0 software.

$$\text{ER } (\%) = \frac{\text{NC - NT}}{\text{NC}} \text{ X } 100 \ (\%)$$

Where ER=percent effective repellency; NC=number of eggs in control; and NT=number of eggs in treatment.

2.11. Extraction of crude enzyme

Different enzymatic assays were performed in *in-vivo* condition. After 24 h, of experimental period survived larvae were subjected to crude enzyme extraction for determine the level of α and β esterase and Glutathione-S-transferase (GST). The extraction method was performed according to Maheswaran and Ignacimuthu, (2012) with minor modification. A batches of 25 each larval species were homogenized individually in 1 mL of 0.1 M potassium phosphate buffer (PBS) (pH 7.2) using a glass homogenizer immersed in the ice cold box.

215 Tissue homogenates were centrifuged at 4 °C and 12,000 rpm for 15 min. After that
216 supernatants were collected in new tubes and kept on -20 °C for to study the different enzyme
217 activities.

218 **2.12. α and β esterase activity**

219 For esterase activity, 20 µl of α-naphthyl acetate (1 mL of 30 mM α-naphthyl acetate in
220 acetone in 99 mL of 0.02 M PBS, pH 7.2) and 200 µg β-naphthyl acetate (prepared as for α-
221 naphthyl acetate solution) were added into 200 µL of homogenate. The enzyme reaction
222 allows to running 2 minutes at 27±2 °C before the addition of 50 µL of fast blue stain solution
223 for to stop the enzymatic reaction. Absorbance value was measured at 570 nm (Matowo et al.
224 2010).

225 **2.13. Glutathione-S-transferase activity**

226 Glutathione-S-transferase enzyme assay was estimated following the method of Habig et
227 al., (1974). Five replicates of each treated group homogenate (100 µL) were placed in a
228 96 well plate, and then add 50 µL of 2 mM glutathione followed by add 50 µL of 1 mM
229 1-chloro-2, 4- dinitrobenzene (CDNB). The reaction mixture was incubated for 30
230 minutes at 27±2 °C. Enzyme activity was measured in a spectrophotometer at 340 nm.

231 **2.14. Histopathology studies**

232 After 24 h of experimental period treated and control larvae were dissected and
233 midgut was removed out then washed with 0.9 % of physiological saline and fixed with 10 %
234 formalin solution for histological studies. Tissues were embedded with paraffin wax;
235 transverse sections (5 µm) of paraffin embedded tissue were sectioned through microtome
236 (Leica-cryocut1800, Germany). The mounted glass slides were stained with hematoxylin and

eosin. Morphological changes were observed through light microscopy (Lawrence and Mayo: NLCD-307) with the magnification at 10X (Shu et al. 2018).

2.15. Antimicrobial activity of the synthesized AgNPs

The actinobacterial mediated synthesized AgNPs was tested against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae* by agar well diffusion method with 5, 10, 15 and 20 ppm concentrations (Perez et al. 1990). Each concentration repeated three times. 20 ppm of DMSO and Kanamycin was served as a negative and positive control. After 24 h zone of inhibition was measured.

2.16. Cytotoxicity assay

The synthesized AgNPs using actinobacterium was evaluated against Human cervical cancer cell line (HeLa) by MTT (3-(4, 5-dimethyl-thiazol2-yl)-2, 5-diphenyl tetrazolium bromide) method (Palanivel et al. 2013). HeLa cells were attained from National Centre for Cell Science, Pune, India and sustained as a monolayer culture in Dulbecco's Modified Eagle Medium, complemented with 10 % Fetal Bovine Serum, with humidified atmosphere at 37 °C and 5 % CO_2. Cells were counted on a Z2 Coulter Counter (Beckman Coulter, USA) and seeded in Petri dishes and 96-well culture plates in a desired concentration. After 24 h incubation, Cells were treated with 0, 2, 4, 8, 16, 32, 64, 128, 256, 512 µg/mL concentrations of synthesized AgNPs with three replicates. Percentage of growth inhibition was calculated by using the following formula.

2.17. Determination of apoptotic morphological changes

Acridine orange (AO) and ethidium bromide (EBr) stains were used to determine apoptotic cells validations. The cells (3 x 10^4/well) were cultured in 6-well plate treated with

AgNPs for 24 hrs. Then cells were fixed in methanol: glacial acetic acid (3:1) for 30 min at 4 ºC. Subsequently, cells were washed twice in PBS followed by stained with 1:1 ratio of AO/EBr for 30 min at 37 ºC. After staining cells were washed with PBS twice and observed under a floid cell imaging station (Invitrogen, USA). The number of cells showing features of apoptosis was counted as a function of the total number of cells present in the field.

2.18. Toxicity of actinobacteria mediated AgNPs on non-target organism

The toxicity of synthesised AgNPs was tested on non-target organism of Brine shrimp *Artemia salina* (L.). The brine shrimp cysts were procured from Sagar Aquarium, Gujarat, India and the culture condition was maintained (Ragavendran et al. 2017). Briefly, encysted *A. salina* was hydrated with distilled water at 4 °C for 12 h and then floating cysts were separated into the conical flask. Subsequently, sinking cysts were collected using a buchner funnel and washed with cold distilled water. Nearly, 2 g of *A. salina* cysts were added in 1.0 L of seawater in a glass jar at 25-28 °C. For successive hatching, culture condition was maintained with pH 8 and constant lighting provided by A1500 lmx (daylight) fluorescent lamps. Aeration was provided using air pump and after 36 h, hatching of *A. salina* was noticed. The adult brine shrimp were exposed to AgNPs at varies concentration of 5, 4, 3, 2 and 1 ppm for a period of 24 h with three replications along with negative control (Apu et al. 2010)(Amutha et al. 2019). The mortality rate was calculated using standard formula,

3. Results and Discussion

3.1. Isolation and identification of Actinobacteria

Biosynthesized nanoparticles using actinobacteria have produced large number of potential secondary metabolites, enzymes, proteins and it's very easy to manipulate large quantity as well as recovery of AgNPs (Sundaravadivelan and Padmanabhan 2014). Totally

50 actinobacterial strains were isolated from different soil samples collected from Western Ghats of Tamil Nadu, India. Among them actinobacterial strain (CHI-10) showed potential larvicidal activity against *C. quinquefasciatus* (86.66 %) compared with other strains (Table 1). Hence, present study was aimed to synthesis AgNPs using newly isolated *Actinokineospora fastidiosa* (CHI-10) (MN337968) and evaluated their bio efficacy.

3.2. Biosynthesis of AgNPs production

The optical properties and bioreduction of synthesized Ag+ ions were visually confirmed turn of color pale yellow to dark brown due to the reaction of cell free filtrate of *A. fastidiosa* (CHI-10) was added into the $AgNO_3$ solution. The pale yellow colour was gradually changed to dark brown colour indicates the presence of nitrate reductase enzyme that confirmed the formation of AgNPs (Yassin et al. 2017)(Manimegalai et al. 2020). AgNPs have an absorbance spectrum exhibited a peak at 420nm due to the surface plasmon resonance (Fig. 1). The dry weight of biosynthesized AgNPs was 58mg/100ml. No color change was observed in culture supernatant without $AgNO_3$. Our results were positively correlates with earlier reports of Singh et al., (2015). They observed 420-450 nm using *Penicillium* sp. by Maliszewska et al., (2009) on *Streptomyces aegyptia* mediated AgNPs showed absorption peak at 415 nm (El-Naggar et al. 2014).

Different environmental conditions and physicochemical properties were involved in size and shape of synthesized AgNPs, with respect to high yield. The actinobacteria mediated AgNPs was optimized by following factors, wherein the optimum pH was found to be 8.0, 5 mM of $AgNO_3$ concentration, 6 mL (cell free filtrate) + 44 mL of $AgNO_3$, temperature of 60 °C and time at 24 h were noticed for the production of most favorable factors for synthesis of AgNPs (Fig. 2). Similarly, Adiguzel et al., (2018) reported that AgNPs were synthesized by using cell lysate of *Streptomyces* sp., the optimum of pH, $AgNO_3$ and cell lysate concentration was found to be pH 9.0, 1 mM $AgNO_3$ and 2-fold diluted cell lysate. Our

observations corroborate with similar findings of biosynthesized AgNPs using *Acinetobacter calcoaceticus* and optimized to produce AgNPs within 24 h. Furthermore, the achieved monodisperse spherical nanoparticles of 8–12 nm were achieved with 0.7 mM $AgNO_3$ at 70 °C (Singh et al. 2013). Those synthesized NPs possessed strong antibacterial activity.

3.3. XRD analysis *A. fastidiosa* mediated AgNPs

The attained XRD peaks of *A. fastidiosa* mediated AgNPs at 2θ values of 31.83°, 45.65°, 57.12° and 76.69° it could be attributed to (111), (200), (220) and (311), respectively and the obtained plane was confirming the crystalline nature of the AgNPs (Fig. 3). The average size of the AgNPs was determined using the Debye Scherrer's equation

Where D is the crystalline size of NPs, k denotes the shape constant of geometric factor (0.9 to 1), λ represents wavelength of the X-ray sources (0.1541 nm), β is the line broadening at half maximum intensity and θ is the Braggs angle. The average particle size was calculated to be 73.03 nm. The ability of the AgNPs over the range 5- 100 nm were highly toxic to bacterial strains with lowering particle size (Agnihotri et al. 2014). The spurious diffraction peaks were detected due to the presence of small particles in the medium and values were positively correlated with *Streptomyces grieseorubens* mediated AgNPs proved as antimicrobial agent against pathogenic microorganisms (Vidhyashree and Sudha Lakshmi 2015).

3.4. FTIR analysis of *A. fastidiosa* mediated AgNPs

FTIR analysis used to detect biomolecule present in the filtrate and also engrossment of biomolecule responsible for synthesis and stabilization of AgNPs (Dhanasekaran and Thangaraj 2013). FT-IR analysis of *A. fastidiosa* mediated AgNPs showed intense absorption bands at 3421.71 cm^{-1}, 2957.37 cm^{-1}, 2953.74 cm^{-1}, 2853.74 cm^{-1}, 1713.18 cm^{-1}, 1638.65 cm^{-1}, 1452.65 cm^{-1} and 1384.86 cm^{-1} (Fig. 4). The absorbance bands

situated between 3000–3600 cm^{-1} assigned to the stretching vibrations of hydroxyl groups and amine groups where N-H was characterized by primary and secondary amines of amino acids, peptides, proteins, etc. (Hamouda et al., 2019, Yana et al., 2013). The band in 1638.65 cm^{-1} shows amines and amides (C-N stretch and NH out plane) region which may involve in stabilizing nanoparticles by proteins (Castro et al. 2013). The presence of amide linkage of protein possessed the greater potential to join silver and subsequently forming protein covering around AgNPs to prevent agglomeration of the medium it could be contributed stabilization of the AgNPs (Shanmuganathan et al. 2018). Furthermore, the peak located at 1452.65 cm^{-1} is recognized to the vibration of proteins as being stabilizing agent via free amine groups or cysteine groups (Adina et al. 2010). Our resultant bands were similar to that preparation of nanoparticles using plant extracts (Marimuthu et al. 2012) and microorganism (Naveen et al. 2010).

3.5. SEM-EDX and TEM analysis of *A. fastidiosa* mediated AgNPs

The surface morphology and topography of AgNPs were analyzed through the SEM imaging technique. Synthesized nanoparticles were irregular in shape, as look like colloidal form which means particles that can be uniformly dispersed within a solution (Fig. 5a). Green synthesized AgNPs using *Artemisia nilagirica* possessed varied size and shape, interestingly those nanoparticles showed significant mosquitocidal activity (Nalini et al. 2017). In the present investigation elemental analysis was done by EDX, which possessed strong signals in the silver region that confirmed formation of *A. fastidiosa* mediated AgNPs (Fig. 5b). Other EDX peak O and Cu were corresponded to the X-ray emission from protein molecules presence in the cell free filtrate that can bind with nanoparticles either through free amino or cysteine residues (Elbeshehy et al. 2015). TEM image was confirmed mono disperse of AgNPs that appeared as spherical in shape and size distribution with the range of 19–41 nm. Similar size of synthesized nanoparticles (< 100 nm) exhibited antibacterial and cytotoxic

activity against colon adenocarcinoma cell line (Awad et al. 2016). The selected area electron diffraction (SEAD) pattern of a mono dispersed nanoparticle proposed single crystalline of nanoparticle (Fig. 6). The bio-compatibility and the bio-safety of the nanotechnology depend on the unique properties such as particle size, shape and morphology (Dakhlaoui et al. 2009). The biologically synthesized nanoparticles with size depended (45-95 nm) and effective *H. pylori* strains and exhibits lesser toxicity on mammalian cells (Saravanan et al. 2017). Correspondingly, our results were coincides with previous findings of mono dispersed spherical nanoparticles with a size of 20 nm exhibited better growth inhibition on bacterial strains and ability to scavenge DPPH radicals capability (Elemike et al. 2017). Different particles size of synthesized AgNPs (10-40 nm) were effective against *Staphylococcus aureus* and *Escherichia coli* and non-toxic on cell growth (Abdel-Mohsen et al. 2013). In this study Zeta potential value -13.8 mV was observed on *A. fastidiosa* mediated AgNPs (Fig. 7) and correlated with similar reports (Wypij et al. 2018)(Prakasham et al. 2012). Zeta potential values should be more positive than +30 mV or more negative than −30 mV was considered to be stable (Saeb et al. 2014). Zeta potential analysis as an important characteristic feature of synthesis of AgNPs, since it gives saturation solubility and dissolution velocity, physical stability, or even biological performances (Abdelmoteleb et al. 2018).

3.6. Larvicidal activity of *A. fastidiosa* mediated AgNPs

Larvicidal effect of *A. fastidiosa* mediated AgNPs against *A. aegypti, A. stephensi* and *C. quinquefasciatus*. The highest percentage of mortality was found against the larvae of *C. quinquefasciatus* (100.00 %) and *A. stephensi* (100.00 %) followed by *A. aegpyti* (98.66 %) at 1000 ppm concentration. The obtained LC_{50} value was 8.29 ppm, 8.50 ppm and 9.86 ppm against *C. quinquefasciatus, A. stephensi* and *A. aegypti.* The silver nitrate ($AgNO_3$) alone showed moderate larvicidal activity (Table 2). The influence of $AgNO_3$ on larvicidal activity overcome with coupling of silver ions coated with biomolecules becomes more

385 biocompatible (Rajput et al. 2020). Silver ions coated with bioorganic compounds that makes
386 them insight of pesticidal applications. Furthermore, uses of biomolecules involved in the
387 formation of nanoparticles which possess lethality on mosquito larvae and little or no effect
388 on non-target organisms (Pirtarighat et al. 2019).

389 Our results were corroborates with earlier report of synthesized AgNPS using
390 *Euphorbia hirta* against *A. stephensi* (Priyadarshini et al. 2012). Similar larvicidal activities
391 against *C. quinquefasciatus* and *C. gelidus* using *Ficus racemosa* mediated AgNPs with the
392 LC_{50} value of 67.72 mg/L and 63.70 mg/L against *C. quinquefasciatus* and *C. gelidus*
393 (Velayutham et al. 2013). The biologically synthesized AgNPs were highly toxic to *A.*
394 *aegypti* and *A. stephensi* and 100 % mortality was at 1.0 % of AgNPs (Nalini et al. 2017).
395 The actinobacteria of $KA_1$3-3 and $KA_2$5-A mediated AgNPs showed 100 % mortality against
396 *C. quinquefasciatus* (Rajesh et al. 2015). Similar agreement with the impact of *Streptomyces*
397 *citreofluorescens* mediated nanoparticles tested and obtained LC_{50} value was 122.6 µL/mL
398 and 60.0 µL/mL against *A. stephensi* and *C. quinquefasciatus* (Singh and Prakash 2012). The
399 actinobacterial strains showed most significant activity against *A. aegypti* and *A. stephensi* at
400 different concentrations (Balakrishnan et al. 2017).

401 The possible mechanism to implicates the larvicidal activity of AgNPs, due to silver
402 interact with sulfur ions it causes denaturation of the protein molecules, furthermore,
403 interaction of silver phosphorus complex in a DNA structure leads to the alteration of cellular
404 organelles and enzymes that causes to cell death (Choi et al. 2008). In other potential impact
405 of AgNPs that collapse cellular transport system in order to reduces cellular membrane
406 permeability and reduction in ATP synthesis which ends with cellular damage (Sap-Lam et
407 al. 2010). The present findings showed that AgNPs could bring a very promising target tool
408 which can be used for vector mosquito management.

409 **3.7. Ovicidal activity**

The results of ovicidal activity on eggs of *A. aegypti, C. quinquefasciatus* and *A. stephensi* treated against AgNPs (Table 3). The LC_{50} values of 6.95, 6.74, and 9.14 ppm on the eggs of *A. aegypti, A. stephensi* and *C. quinquefasciatus*, respectively. All the eggs were hatched in control group. Luz et al., (2007) investigated that ovicidal activity of 21 fungal strains was treated on the eggs of *A. aegypti*. They observed that high numbers of ovicidal activity was obtained on eggs (≥70 %) during 25 days of exposure. Sun et al., (2017) tested ovicidal and insecticidal activities of pyriproxyfen against *Plutella xylostella*, *Myzus persicae* and *Helicoverpa armigera*. They found that moderate to high insecticidal activity against *P. xylostella* and *M. persicae* and highest ovicidal activity at 600 µg/mL concentration against *H. armigera*; especially isolated compounds of 5j, 5o, 5p, 5q, and 5s were inflicted 100 % ovicidal activity. (Benelli and Govindarajan 2017) Benelli and Govindarajan examined NPs synthesized from biological by products that showed high toxicity on eggs and larvae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* with the LC_{50} values was 12.45, 13.58 and 14.79 µg/mL respectively. No egg hatchability was noted post-treatment of 40, 50 and 60 µg/mL concentrations. The ovicidal action was associated with the ability of biomolecules were interacted with precipitate proteins. Especially in eggs, a coating of active bio molecule-protein complexes were bound on the eggshells and prevent hatching (Borges et al. 2019).

3.8. Oviposition deterrent activity

The potential oviposition deterrent activity of 100.00 %, 100.00 % and 98.66 % was observed against females of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* at 25 ppm concentration of AgNPs (Table 3). Cent percent egg laid was observed in control group. Similarly, Arjunan et al., (2012) noticed that bio synthesized AgNPs treated against *A. aegypti, C. quinquefasciatus* and *A. stephensi* was achieved 36 % of eggs laid at 0.1 ppm concentration. Karthik et al., (2011) noticed that extracts of marine actinobacterial strains LK-1 and LK-3 showed high oviposition repellence against females of *C. tritaeniorhynchus*

435 and *C. gelidus.* Our results were positively corroborates with the findings of El-Gendy and
436 Shaalan, (2012) studied that extraction of essential oils obtained from *Matricaria recutita*,
437 *Sesamum indicum*, *Simmondsia chinensis* and *Zingiber officinalis* treated against *C. pipiens.*
438 They obtained various degrees of oviposition repellency ranging from 48.73-100 % on all the
439 tested oils. Swathi et al., (2010) examined ethanolic extract from *Pongamia pinnata*, *Coleus*
440 *forskohlii*, and *Datura stramonium* reduced egg laying capacity against *A. aegypti,*
441 *C. quinquefasciatus* at 0.1% concentration. Eden et al., (2020)studied active component of
442 geraniol and citronellol isolated from *Cymbopogon winterianus* showed 78 % and 77.33 %
443 oviposition repellency against *A. aegypti*. Reegan et al., (2015) reported that hexane extract
444 of *Limonia acidissima* showed 100 % oviposition deterrent effect against *C. quinquefasciatus*
445 and *A. aegypti* adult females at 500 ppm concentrations. In addition methanol extract of
446 *Bryopsis pennata* showed strongest oviposition deterrent effect of 100.00% was achieved
447 against *A. aegypti* and *A. albopictus* (Yu et al. 2015). The extract of *A. indica* exhibited
448 highest oviposition deterrent index (ODI) 71.48% against *C. quinquefasciatus* at both 0.4 and
449 0.5% concentrations (Fatima et al. 2011).

450 **3.9. α, β esterase and Glutathione-S-transferase activity**

451 In insects, usually, external stress factors that leads to resistance are morphological,
452 physiological, biochemical and behavioral. In this scenario important to find and designated
453 the metabolic variations in the mosquito resistance (Montella et al. 2012). The enzymatic
454 activity of α esterase level was increased (0.49±0.060, 0.59±0.009 and 0.51±0.057 µg napthol
455 produced/min/mg larval protein) and β esterase level was also increased (0.52±0.095,
456 0.57±0.062 and 0.49±0.172 µg napthol produced/min/mg larval protein) at 25 ppm
457 concentration of AgNPs against larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*,
458 respectively. Subsequently GST level was increased in *A. aegypti*, *A. stephensi* and *C.*
459 *quinquefasciatus* (0.71±0.98, 0.73±0.09 and 0.69±1.15 µmol/min/mg larval protein)

460 compared to control group (Table 4). The α- and β-esterase were important detoxification
461 enzyme in the insect biology to defend itself against various poisonous allelochemicals and
462 pesticides. In the present investigation α-and β-esterase enzyme level were quantitatively
463 increased in treated group due to the inhibition of enzyme activity on fourth instar larvae *A.*
464 *aegypti, A. stephensi* and *C. quinquefasciatus*. The results indicated that *A. fastidiosa*
465 mediated synthesized AgNPs proved the ability for inhibition of detoxifying enzyme activity
466 on target organisms without developing any resistance. When increase the total amount of
467 esterase activity indicates regulatory genes modification or regulatory loci joint with
468 structural genes, which leads to change the enzyme synthesis in the organism which causes
469 cell death (Stankovic and Kostic 2017). GST is a key biomarker enzyme to perceive whether
470 organism developed a resistance or susceptibility upon exposed to the certain insecticides.
471 Therefore, GST plays a major significant role in the detoxification process. Naturally
472 established insecticides were involved in the inhibition of these enzymes, may reactant in
473 metabolic disparity, impairment of growth and induction of mortality. Fouad et al., (2018)
474 reported that green synthesis of AgNPs using *Cassia fistula* were effective against the larvae
475 and pupae of *A. albopictus* and *C. pipiens*. The synthesized AgNPs caused normal
476 detoxifications enzymes of Acetylcholinesterase and α- and β-carboxylesterase activity on the
477 larvae of *A. albopictus* and *C. pipiens* compared to control larval groups. Parthiban et al.,
478 (2019) evaluated biochemical parameters on *A. aegypti* treated with AgNPs synthesized by
479 using *Annona reticulata*. The enzymatic activity of acetylcholinesterase, α-and β-
480 carboxylesterase and GST enzymes were significantly reduced in the treated larvae of *A.*
481 *aegypti* compared to control group.

482 **3.10. Histology studies**

483 The results of AgNPs treated larval midgut tissue of *A. aegypti, A. stephensi* and *C.*
484 *quinquefasciatus* showed disrupted cell and organelle integrity. Additionally, epithelial cells

were damaged and appendages of the cell arrangement appeared in vacuolization with destroyed intercellular membranes also noticed. Subsequently, degeneration of nuclei, brush border cells and microvilli were seriously damaged with consequence of nanoparticles exposed larvae as compared to control (Fig. 8). Our result accordance well with previous findings of synthesized AgNPs using *Aquilaria sinensis* against *Aedes albopictus* and observed severe cell damage in epithelial and brush border cells (Ga'al et al. 2018). The toxicity and histopathological study of AgNPs using *Matricharia chamomella* showed significant tissue damage on midgut of *C. quinquefasciatus* (Almehmadi 2011) .

3.11. Antibacterial activity of *A. fastidiosa* mediated AgNPs

Antibacterial activity of *A. fastidiosa* mediated AgNPs was investigated against *S. aureus, B. cereus, B. subtilis, P. aeruginosa, E. coli* and *K. pneumoniae* by agar well diffusion method. The present investigation *A. fastidiosa* mediated AgNPs showed acceptable anti-bacterial activity against all tested pathogens. The Zone of inhibition was obtained from *K. pneumoniae* (21.1 mm) followed by *B. subtilis* (20.3 mm), *S. aureus* (14.6 mm), *B. cereus* (16.0 mm), *E. coli* (12.3 mm) *P. aeruginosa* (10.3 mm) at 20 ppm concentration (Fig. 9 and, Table. 5). Similar results of antibacterial activity of biosynthesized AgNPs using acidophilic actinobacteria toward gram positive and gram negative bacteria was documented (Buszewski et al. 2018). Biologically synthesized AgNPs using actinobacteria inhibits the growth of *P. aeruginosa* (10 mm) followed by *S. aureus*, *B. subtilis* and *P. mirabilis* (all 8 mm) (Railean-Plugaru et al. 2016). The active mechanism of inhibitory action of synthesized AgNPs on microorganisms through electrostatic attraction between the positive exciting nanoparticles and negative exciting cell membrane of the microorganism (Hamouda et al. 2001). Based on the results, our study has demonstrated that AgNPs synthesized using *A. fastidiosa* have potential anti-bacterial activity by inhibiting microbial growth.

509 Anti-bacterial activity of AgNPs mainly affects the cellular biomolecules like proteins
510 and enzymes which important to produce ATP that becomes inactivated form (Yamanaka et
511 al. 2005). In addition, AgNPs pass through the inner membrane by inhibiting the enzymatic
512 respiratory system of the microbes and generate over production of ROS which arrest the
513 DNA replication or destroyed the cell membrane to end with cellular apoptosis (Yamanaka et
514 al. 2005)(Palza 2015). Biologically synthesized AgNPs released silver ions inside the
515 bacterial cells and enhancing their bactericidal activity on pathogenic bacteria (Morones et al.
516 2005) (Park et al. 2011).

517 **3.12. Effect of AgNPs on cytotoxic and Apoptotic morphological changes**

518 The MTT assay of *A. fastidiosa* mediated AgNPs showed significant cytotoxicity on
519 HeLa cell line. MTT assay decreased by living cells and the resultant formazan product was
520 proportional to the cell viability. The cell viability of biosynthesized AgNPs treatment (2, 4,
521 8, 16, 32, 64, 128, 256, 512 µg/mL for 24 h) on HeLa cells that induced a dose-dependent
522 cytotoxicity with IC_{50} value of 42.37 µg/mL, whereas Paclitaxel shows IC_{50} value of 32.76
523 µg/mL (Fig. 10 and 11). Our results suggested that cytotoxicity was greatly increased using
524 biosynthesized AgNPs treated HeLa cells due to its potential anti-carcinogenic effect. These
525 results were well consistent with previous findings of AgNPs synthesized using *Phoenix*
526 *dactylifera* (Oves et al. 2018). They observed dose-dependent inhibition of cell proliferation
527 was achieved at 29.6 µg/mL concentration. The cytotoxicity of synthesized nanoparticles
528 using *Streptomyces* sp. was treated against A549 adenocarcinoma lung cancer cell line that
529 showed 83.23% activity at 100 µl with IC_{50} value of 50 µl (Saravana Kumar et al. 2015). The
530 relevant mechanism of apoptosis induced by AgNPs may occur due to mitochondria
531 determines the viable and non-viable of the cells by playing a central role in apoptotic cell
532 death signaling and controlling the cellular energy metabolism, contribution of excessive
533 ROS generation, and release of apoptotic factors into the cytosol (Al-sheddi et al. 2018).

534 Moreover, our results also agree with Wypij et al., (2018) synthesized AgNPs from
535 *Streptomyces calidiresistens* IF 11 and IF 17 strains exhibited a potential cytotoxicity against
536 HeLa cell line at 28.5 and 53.8 μg/mL^{-1}, respectively. Similarly, *Streptomyces* sp. mediated
537 biosynthesized NPs was reported as an anticancer agent, when employed against human
538 osteosarcoma cell line (Shanmugasundaram and Balagurunathan 2017). Based on our results,
539 current study suggested that biosynthesized AgNPs proved direct and dose dependent
540 apoptotic action on HeLa cells. Biosynthesized AgNPs induce apoptosis in HeLa cells was
541 also determined by the dual staining method using AO/EtBr. This result provided supportive
542 microscopic evidence of apoptotic property of AgNPs. AO/EtBr staining categorizes viable
543 cells seen uniform bright green nuclei and non-viable cells with orange to red nuclei after
544 EtBr intercalating with DNA. Imbalance between deoxyribonuclease (DNase) and enzymes
545 righty responsible to maintain DNA integrity as consequences in chromatin condensation and
546 cell death was occur during apoptosis (Buttacavoli et al. 2018) (Nakkala et al. 2018). The
547 results obtained from AO/EtBr staining were accessible in control cells fluoresced brightly
548 with green nuclei and exhibited normal cell morphology (Fig. 12). In addition, at 42.37
549 μg/mL AgNPs exposure revealed an orange luminescent with apoptotic body formation when
550 compared to control cells. Following AgNPs exposure, cells yield to apoptosis indicating its
551 anti-carcinogenic effect. In a recent study, synthesized AgNPs from the *Streptomyces rochei*
552 showed similar dose dependent apoptotic action (Abd-Elnaby et al. 2016)(Hamed et al.
553 2020).

554 **3.10. Effect of biosynthesized AgNPs on non-targeted organisms**

555 The results of brine shrimp toxicity were expressed in Table 6 and Fig 13. The *A.*
556 *fastidiosa* mediated synthesized AgNPs caused mortality of 13.0 % at highest concentration
557 of 5 ppm concentration followed by 10.0 % and 6.0 % at 4 and 3 ppm concentration. No
558 mortality was observed at 2 and 1 ppm concentrations. When treated with $AgNo_3$ 56.6 %

559 mortality was observed at 5 ppm concentration followed 43.3, 30.0, 26.6, and 16.0 % at 4, 3,
560 2 and 1 ppm concentrations, respectively. Fate and behavior of AgNPs and Ag+ ions were
561 dependent on the test media and should be considered a crucial factor in evaluating NP
562 toxicity (Lekamge et al. 2018). It was observed that the increase in mortality rate was dose
563 dependent manner. The biologically synthesized AgNPs using Oscillatoria sp. was tested and
564 observed dose dependent effect against brine shrimp (Adebayo-Tayo et al. 2019). The present
565 findings were corroborated well with previous report of AgNPs concentration were increased,
566 subsequently it increases the mortality rate and DNA damage in *Artemia nauplii* (Arulvasu et
567 al. 2014). Based on this study we suggest understanding the prospective impacts of these *A.*
568 *fastidiosa* mediated nanomaterials can preserve the aquatic environment while also advancing
569 medical and environmental technology.

570 **4. Conclusion**

571 Nano medicines research an important new prospect to develop unique techniques and
572 used to treat against several human diseases. Combining nanoparticle with antibiotics not
573 only reduces the toxicity of both agents towards human cells by minimizing the requirement
574 for high dosages. In the present piece of work, we have successfully synthesized silver
575 nanomaterial using newly isolated novel strain *Actinokineospora fastidiosa* and demonstrated
576 their potential applications against important pathogens and cancer cell line. In this study,
577 adopted a nanoparticles preparation method and synthesis was very easy, rapid and
578 environmentally friendly without any involvement of energy consuming steps.
579 Biosynthesized nano materials were applied for several biological activities like anticancer
580 activity on cervical cancer cell line (HeLa), antimicrobial activity treated against *E. coli*, *P.*
581 *aeruginosa*, *B. cereus* and *S. aureus*. Biosynthesized AgNPs possess strong larvicidal activity
582 treated agonist *A. aegypti, A. stephensi* and *C. quinquefasciatus*. The biochemical parameters
583 of α, β esterase and Glutathione-S-transferase enzymes were significantly increased due to

584 detoxifying the effect of AgNPs. Overall, our findings we conclude that biosynthesized
585 AgNPs were biocompatible and an effectively influenced on pest management and it showed
586 bio efficacy of microbial infections and cancer treatment in eco friendly manner. This
587 biosynthesized AgNPs could also serve as an advantage for the treatment of cancer especially
588 cervical cancer.

589 **Credit author statement**

590 Krishnan Raguvaran: Investigation, Writing - original draft, Manickam Kalpana &
591 Thulasiraman Manimegalai: Investigation, Writing - review & editing, Rengasamy
592 Balakrishnan: Cytotoxicity investigation. Rajan Maheswaran: Conceptualization,
593 Supervision.

594 **Ethical approval**

595 Not Applicable

596 **Competing interests**

597 The authors declare that they have no competing interests.

598 **Consent to participate**

599 Not Applicable

600 **Consent to publish**

601 We confirm that if the paper is accepted, it could be published in this prestigious journal.

602 **Availability of Date and materials**

603 The datasets used or analysed during the study are available from the corresponding author
604 on reasonable request.

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Figures

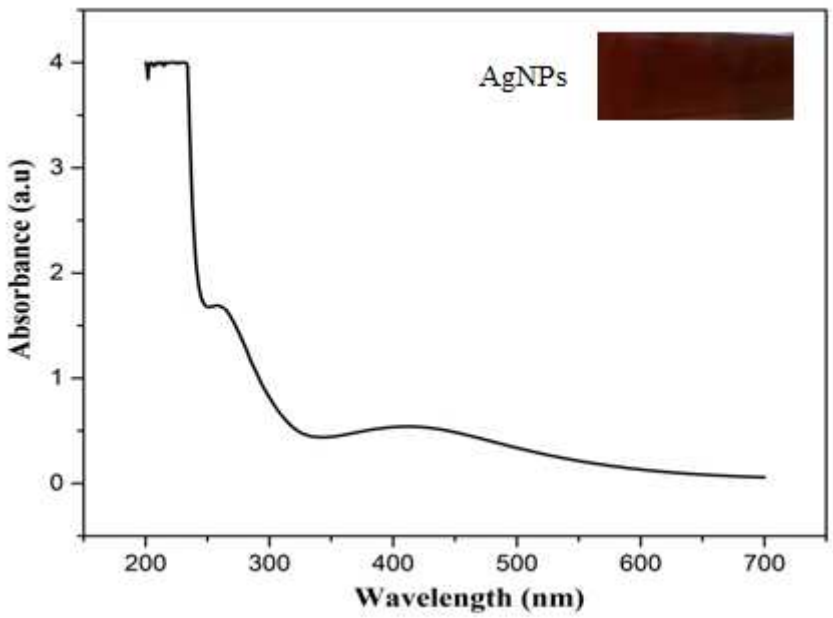

Figure 1

UV–Vis absorption of AgNPs synthesized using actinobacterium CHI-10

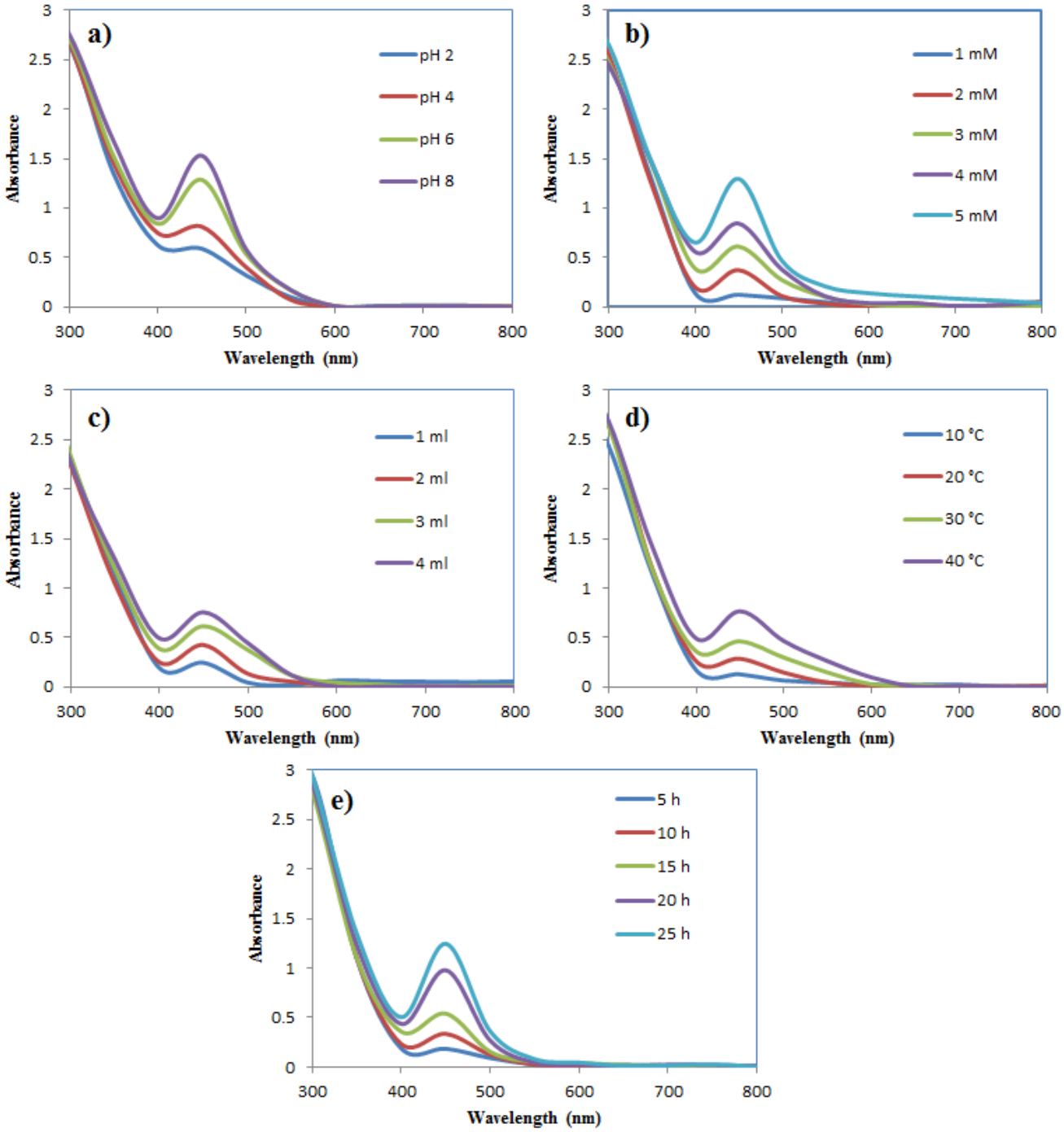

Figure 2

UV–Vis absorption spectra of optimized AgNPs (a) pH, (b) Concentration of Silver nitrate solution (mM), (c) Ratios of Cell free extract (mL), (d) Temperature (e) Incubation time

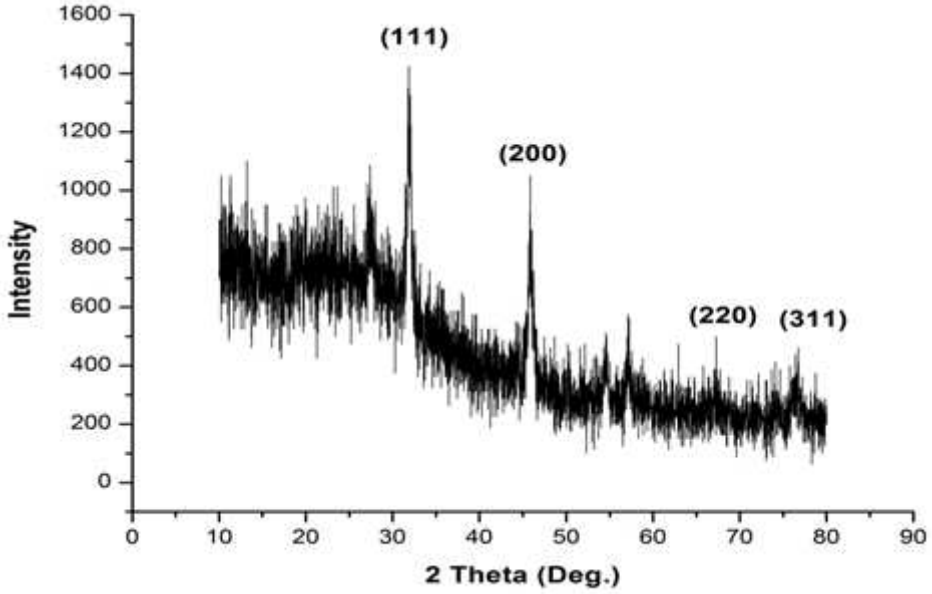

Figure 3

XRD image of AgNPs synthesized using actinobacterium CHI-10

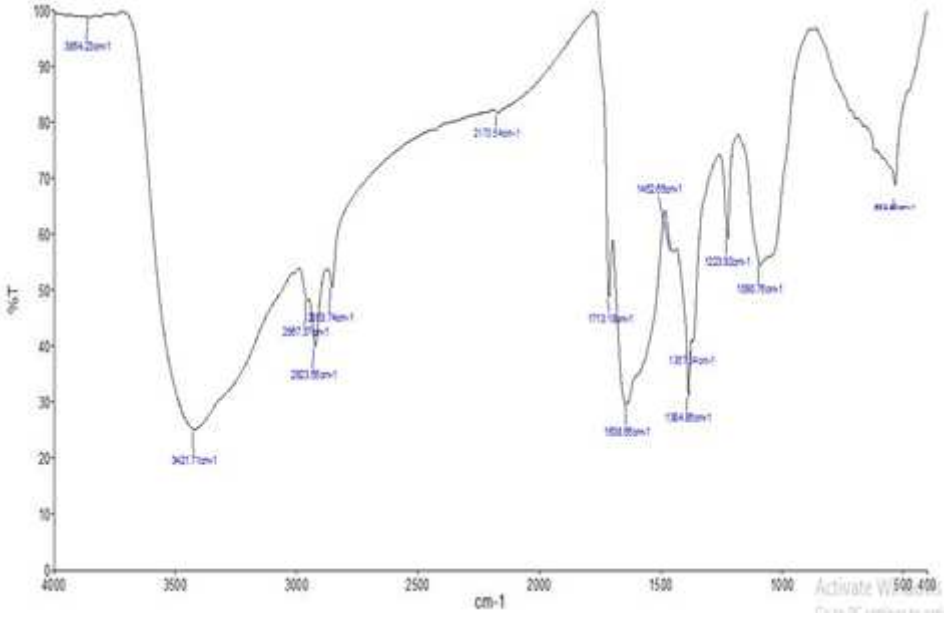

Figure 4

FT-IR spectrum of biosynthesized AgNPs

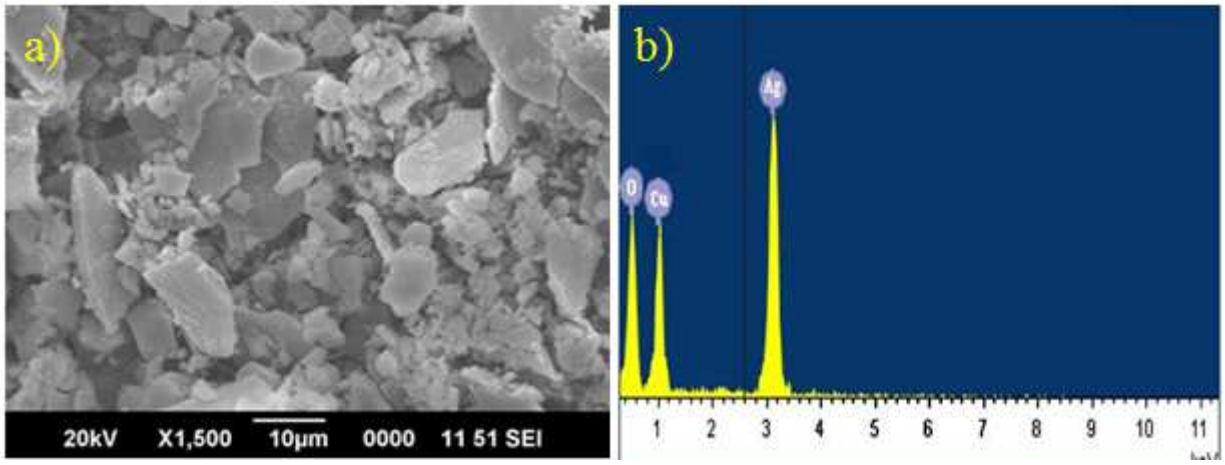

Figure 5

a) SEM image of biosynthesized AgNPs (b) EDX image represented chemical composition of the synthesized AgNPs.

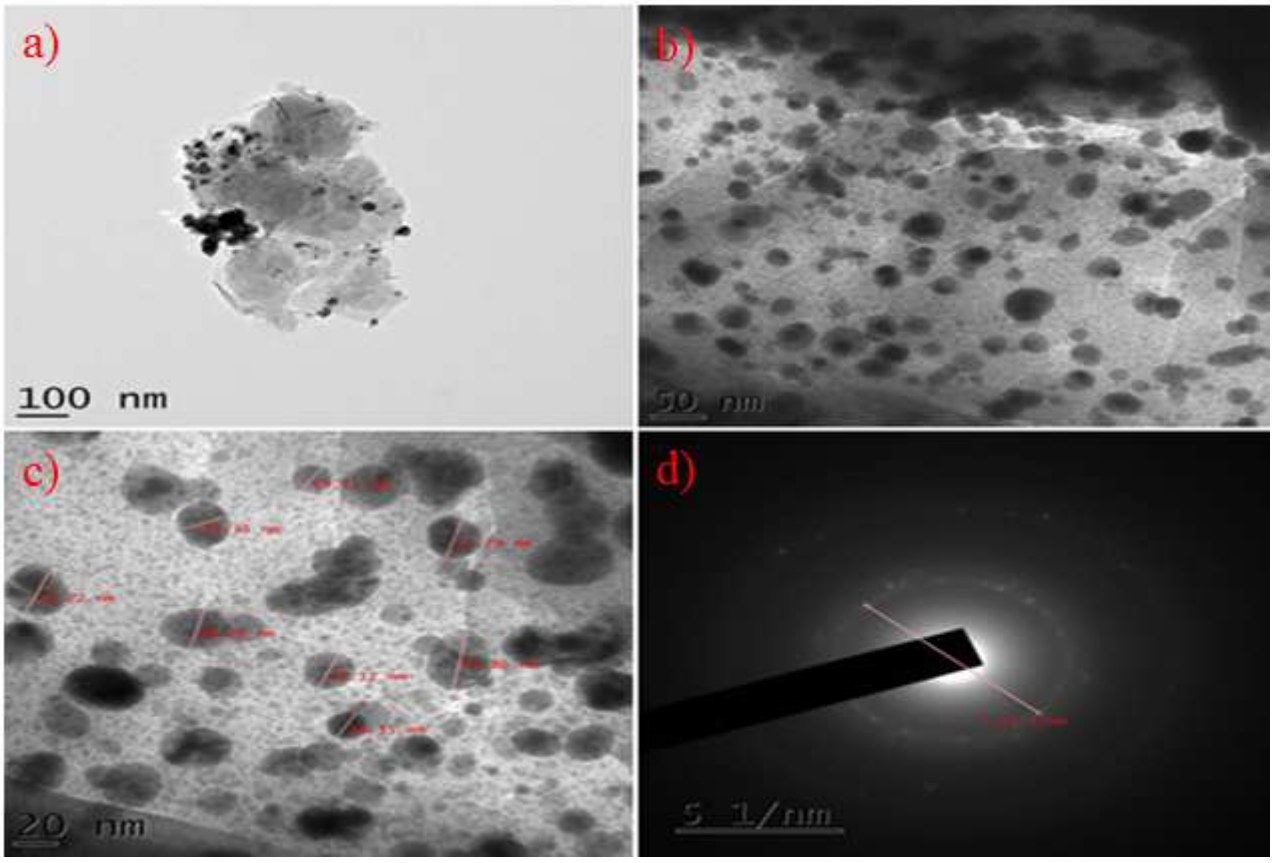

Figure 6

TEM image of AgNPs at (a) 100-nm scale, (b) 50-nm scale, (c) 20-nm scale and (d) corresponding SAED pattern for AgNPs.

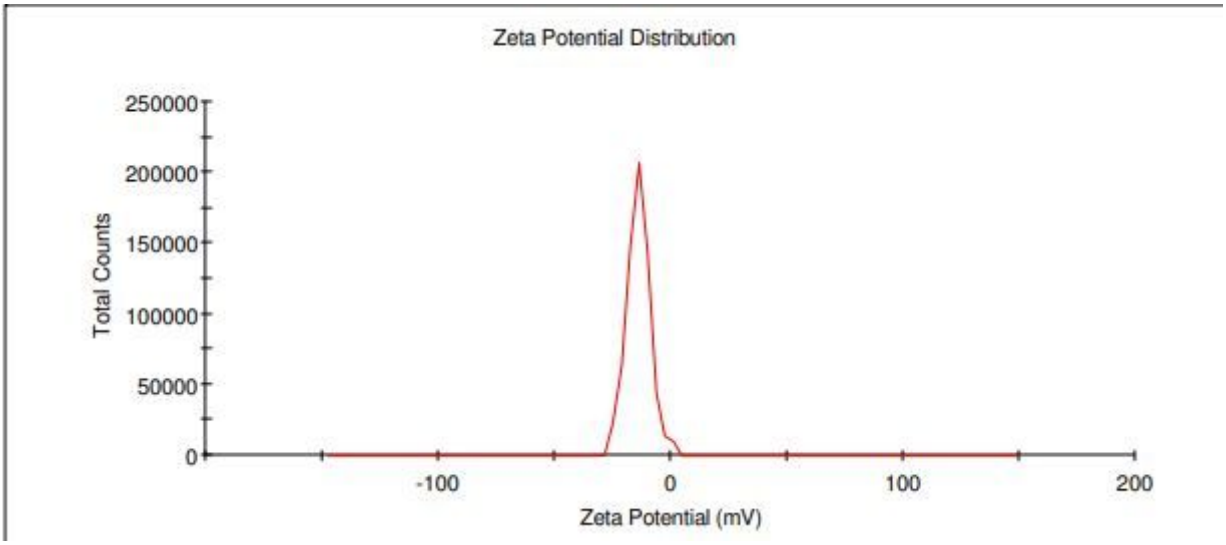

Figure 7

Zeta potential of biosynthesized AgNPs using actinobacterium CHI-10

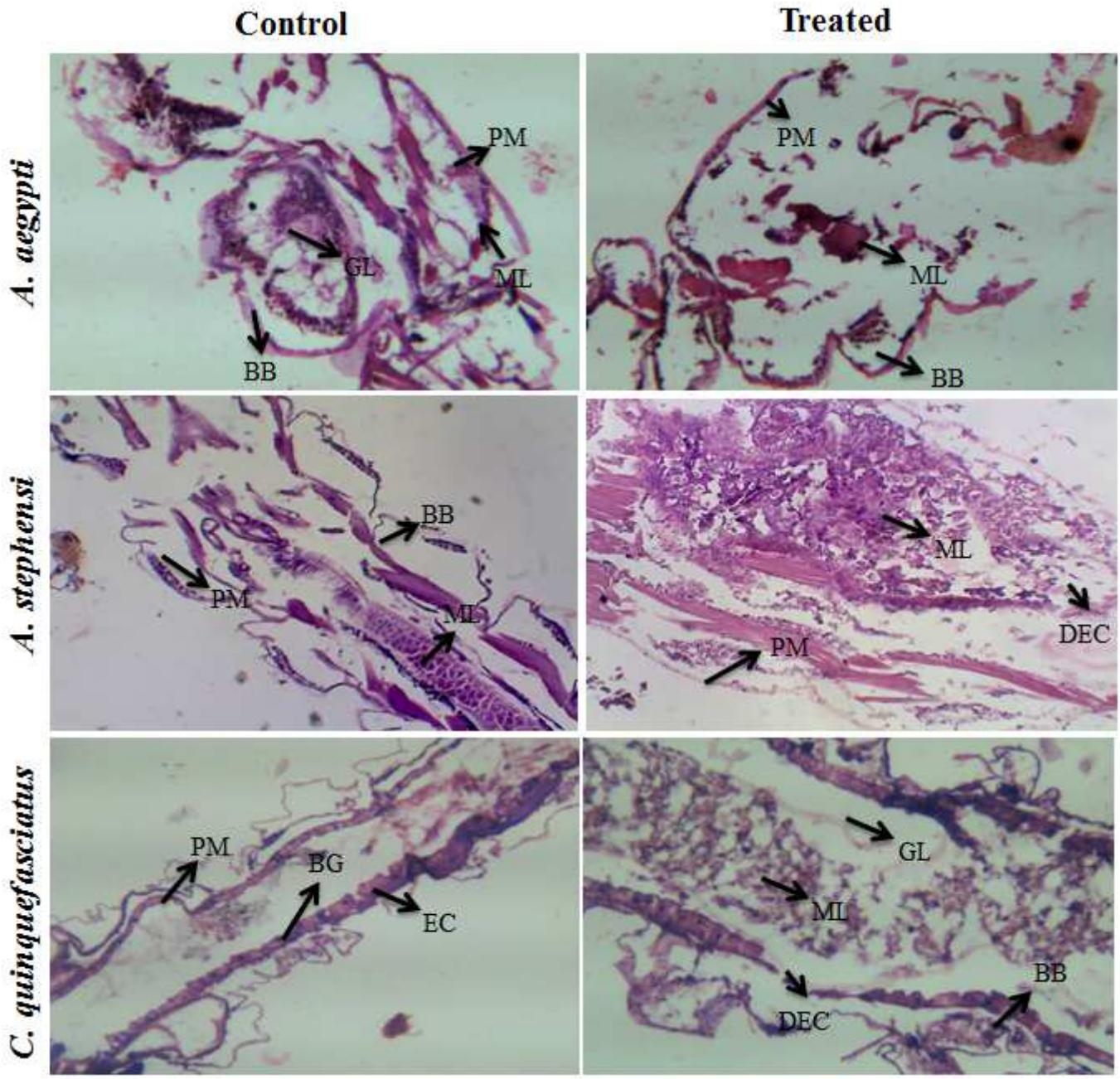

Figure 8

Histological image of the midgut region of control and treated larvae of A. aegypti, A. stephensi and C. quinquefasciatus treated with AgNPs. BB: Brush border; BG: Basophilic granules; PM: Peritrophic membrane; ML: muscle layer; EP: Epithelial cells; DEP: Destructed epithelial cells.

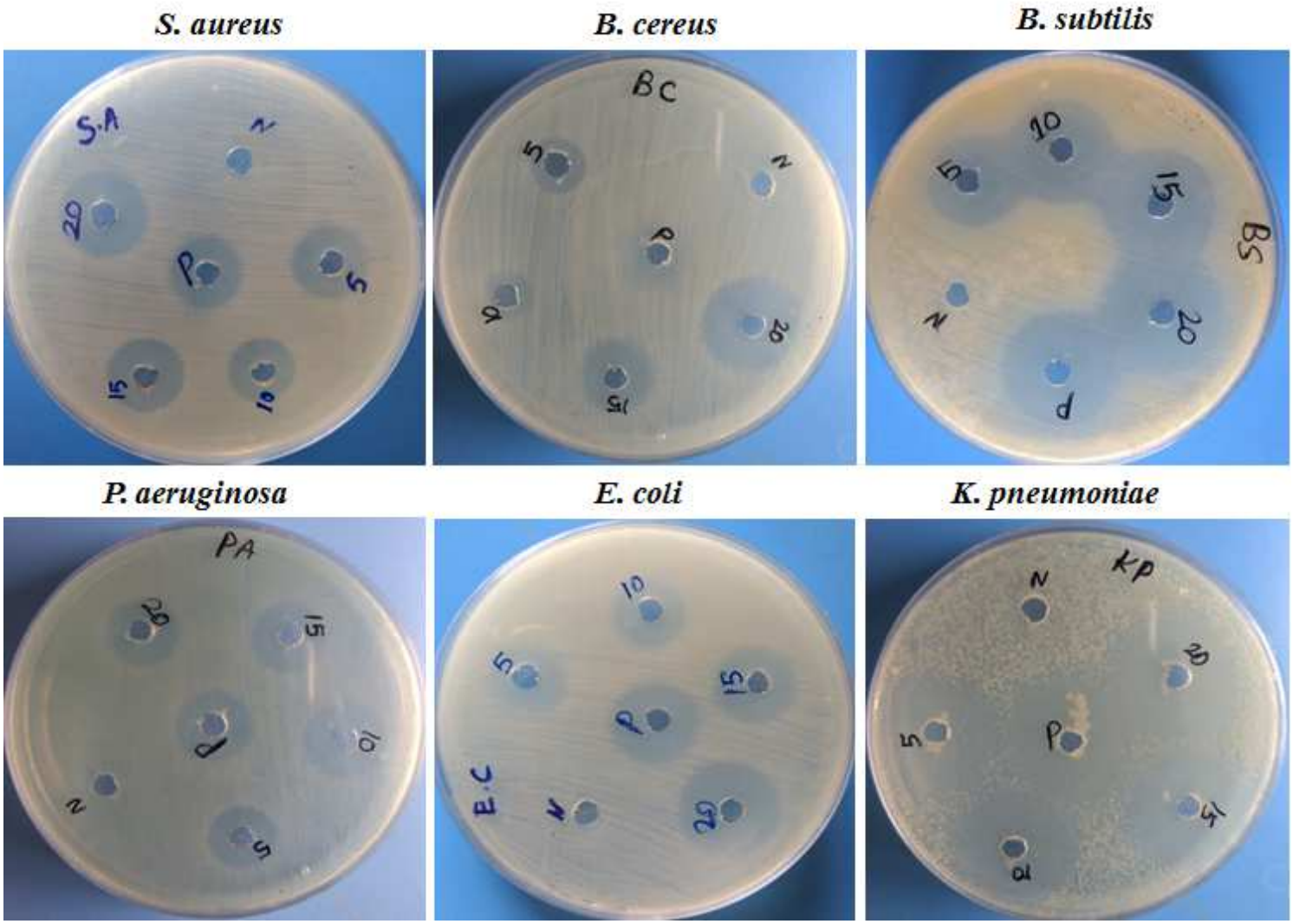

Figure 9

Inhibition zone of AgNPs treated against bacterial pathogens

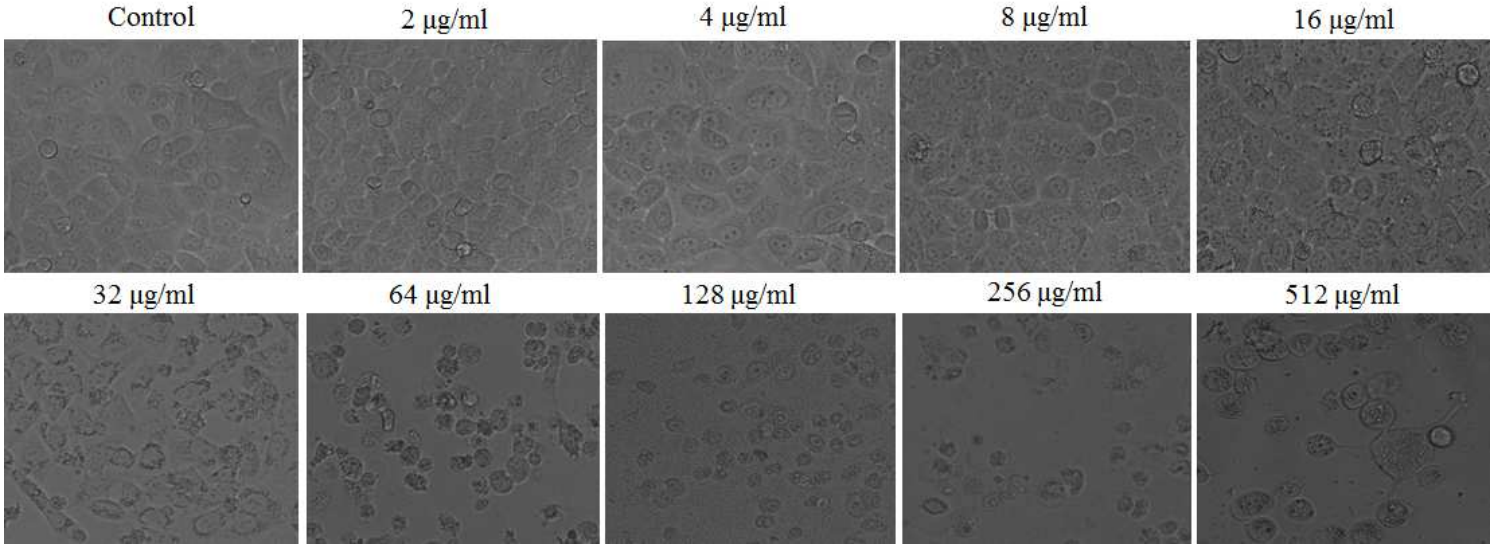

Figure 10

Growth inhibitory potential of AgNPs against HeLa cancer cell line, cells were treated with varying concentrations of AgNPs (0–512 µg/ ml) for 24 h, and the growth-inhibiting potential of AgNPs was determined by MTT assay.

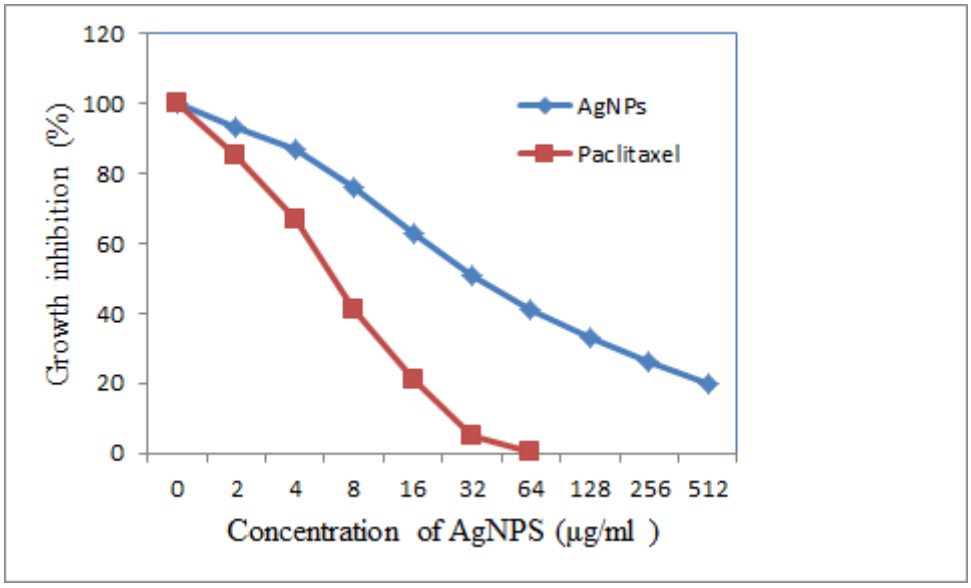

Figure 11

Cell viability assay (MTT assay) was performed by comparative analysis between AgNPs and Paclitaxel expressing HeLa cancer cell line.

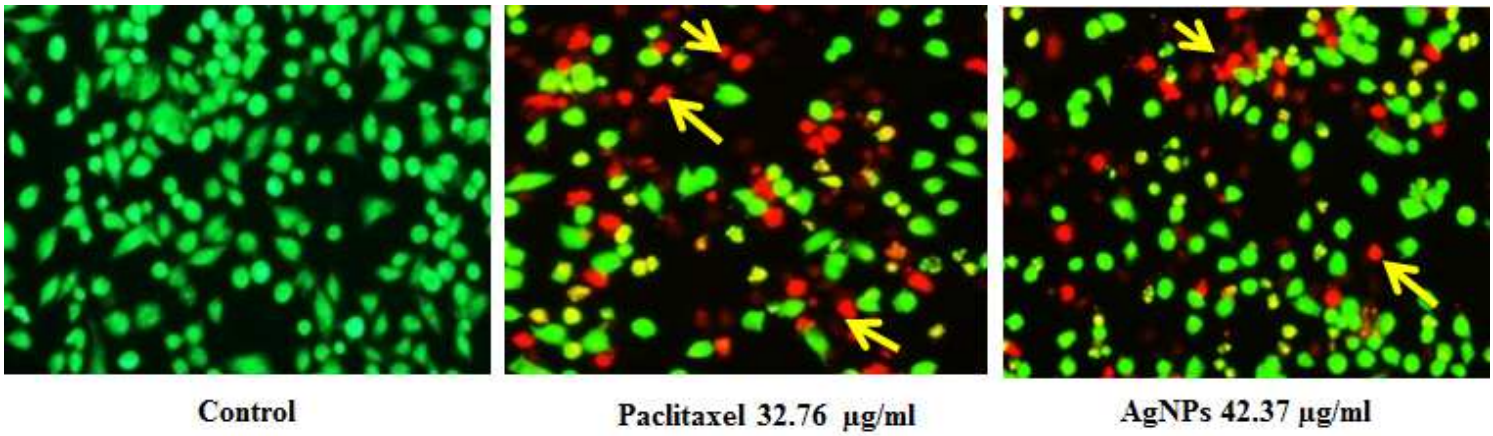

Figure 12

AO/EtBr staining of MCF-7 cells.

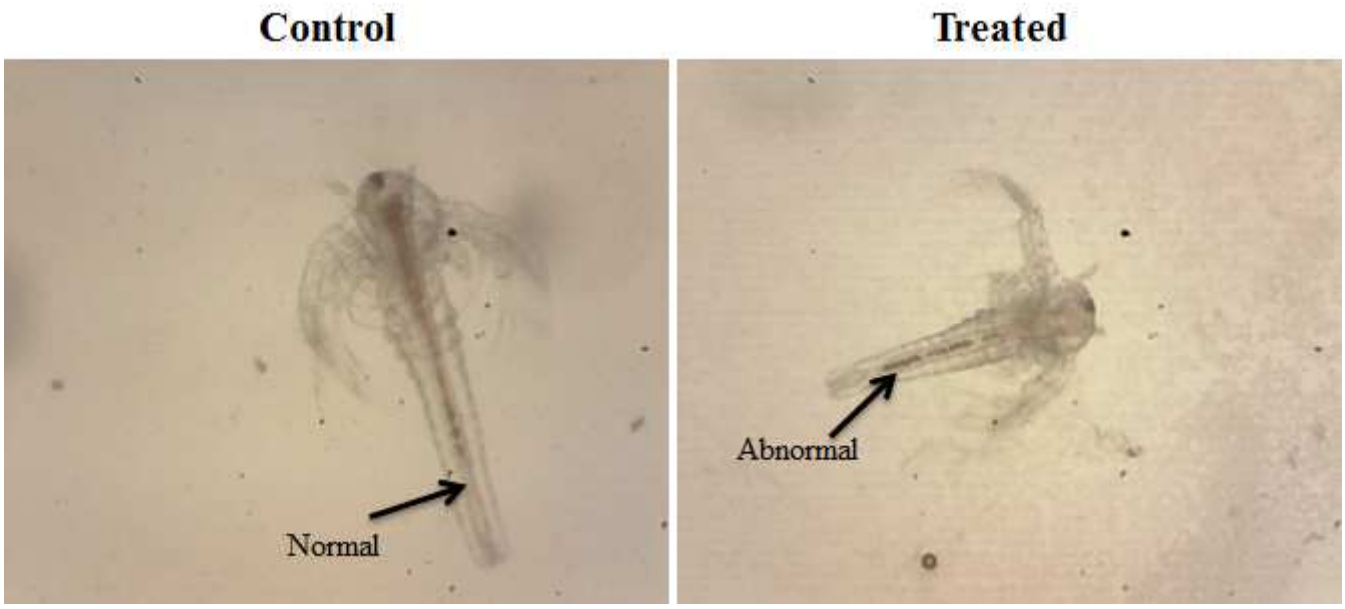

Figure 13

Effect of AgNPs synthesized using actinobacterium CHI-10 treated against brine shrimp. Arrow indicates the empty guts in the control group of larvae. AgNPs is visible as a dark line inside the gut of treated group.