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Antimicrobial Activity of Green Synthesized Silver Nanoparticles Room Sprayer from Arachis hypogaea Root Nodules

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Abstract

Our research provides an update vision about the recent development in nanodrug by using eco-friendly and cost-effective silver nanoparticles from A. hypogaea root nodules for the first time. We also characterized these silver nanoparticles from various spectroscopic and microscopic methods. Their stability makes the present method a viable alternative to chemical and physical methods. Because of the least specificity of the reaction parameters, this process can be explored for large-scale synthesis of silver nanoparticles. Antimicrobial activity of silver nanoparticles room sprayer showed promising antimicrobial activity against S. aureus, E.coli and A. niger in the 100 ppm concentration and in future this kind of eco-friendly and cost effective silver nanoparticles room sprayer is needed to control many dread full diseases in the present scenario instead of using commercially available room sprayers. The investigation features a productive method to acquire bionanoaerosol that can be used against pathogenic microorganisms, thus contributing to solve this globally serious concern, especially given their being a limited choice of nanoantibiotic treatment.

Keywords: Nano sprayers, CFU, phytochemicals, AFM , Arachis hypogaea

1. Introduction

Nanotechnology is an important field of modern research dealing with synthesis, strategy and manipulation of particle’s structure ranging from approximately 1 to 100 nm in size. Within this size range all the properties (chemical, physical and biological) changes in fundamental ways of both individual atoms/molecules and their corresponding bulk [1]. Several methods have been used to synthesize NPs, including chemical reduction [2], electrochemical, photochemical [3] and physical methods, such as physical vapor condensation [4]. Production of nanoparticles due to has properties of chemical and electrical unusual is interestingly. Metals such as silver, which have a resonance of strong surface plasmon, are very important in the synthesis of nanoparticles [5,6].

Green synthesis of nanoparticle has thus gained the attention of scientists due to the cost effectiveness, eco-friendliness and easiness [7]. The advancement of green syntheses over chemical and physical methods is, environment friendly, cost effective and easily scaled up for large scale syntheses of nanoparticles, furthermore there is no need to use high temperature, pressure, energy and toxic chemicals [8].

Due to rapid industrialization and urbanization, our environment is undergoing great damage and a large amount of hazardous and unwanted chemical, gases or substances [1] are released, and so now it is our need to learn about the secrets that are present in the nature and its products which leads to the growth of advancements in the synthesis processes of nanoparticles [9].

A lot of literature has been reported to till date on biological syntheses of silver nanoparticles using microorganisms including bacteria, fungi and plants; because of their antioxidant or reducing properties typically responsible for the reduction of metal compounds in their respective nanoparticles [10]. That's why recently, has been considered by many researcher's biosynthesis of nanoparticles by plants fungi, bacteria as a biocompatible and green method. Using of leaves and fruits extract of plants can be a better substitute to previous methods of nanoparticle production due to low costs, safety, non-toxic and compatible with environment [10-13].

Nanoparticles (NP) or ultrafine particles are used in pharmaceutical industry, computers, fabrics, energy, defence etc [14-16]. Several physicochemical methods have been used to formulate AgNPs. However, these methods have many disadvantages as they include excessive consumption of energy and the use of chemicals to generate hazardous waste and may pose serious environmental health hazards [17]. Green synthesis (GS) method involves the plants and microorganisms to synthesis NP does not pollute the environment and is eco-friendly [18].

Arachis hypogaea is an important legume plant grown in Argentina, 98% of which is concentrated in the province of Córdoba. Argentina is the second largest peanut (PN) exporter in the world [19]. PN contains many nutritional phytocompounds (PC) which provide numerous health benefits such as antioxidant (ANT), anti-inflammatory (AIN), Anti-microbial (AMB) , antihelmintic (AH) and anticancer activity (ACA) [20,21]. However, there is no systemic evidence that has been reported till date for the GS of AgNPs using A. hypogea RN and its AMB activity. In view of this A. hypogea RN aqueous extract (AE) has been taken as a source of study to unravel its AgNPs synthesizing property and also to assess its Antimicrobial (AMB) property by colony forming unit (CFU).

2. Materials and methods

2.1. Chemicals

Silver nitrate (SN), Nutrient Agar (NA) and Potato dextrose Agar (PDA) was purchased from Hi Media, Chennai.

2. 2. Collection and processing of root nodules (RN)

Fresh plants of *A.hypogea* were collected from Polur village of Tiruvannamalai District, Tamil Nadu India, and were authentically identified by Prof. Dr. P. Jayaraman, PARC, West Tambaram, Chennai, India as *A. hypogea* (*Fabaceae*). From the fresh plants, RN alone was taken for the further study. The RN was washed with running tape water and distilled water (DW) thrice to remove the debris. The surface-sterilized RN were extracted using DW and the AgNPs were synthesized according to the method of Velu *et al.* (2015) [8].

2.3. Qualitative phytochemical (QP) and Characterization of AgNPs

QP analysis of *A. hypogea* RN AE was done according to the method of Manimekalai *et al*. (2016) [21]. Characterization of AgNPs was done by UV-vis (PHILIPS, PU 8620), FTIR (Agilet Technologies), XRD (X'Pert Pro), DLS (Malvern Instruments Ltd, V2.0) and AFM (EZ2-AFM).

2.4. AMB activity

To study the AMB effect of AgNPs room-sprayer (AgNPs-RS), a closed room of bathroom (BR) 12 feet (3.66m) X 12 feet (3.66m) dimension was selected. For the uniform exchange of air inside the BR small blower was kept. The microbe present in the air was analyzed by the petri plate kept in open condition. Biochemical test for identification of microbes was done by Obi *et al*. (2004) [10]. The microbe was identified as *S.aureus, E.coli* and *A.niger* and it was taken for the further study to assess its AMB activity using the different concentration (25, 50, 75 and 100 ppm) of AgNPs-RS. Antimicrobial (AMB) activity of *S. aureus*, *E.coli* and *A.niger* was done by CFU using AgNPs coated petriplates according to the method of Rubab Anjum *et al*. (2018) [10].

3. Results and discussion

3.1. Synthesis and characterization of AgNPs

The UV absorption showed a strong broad peak at 420 nm within 10 min, widening of peak showed that the particles were polydispersive (PD) in nature and the AgNPs were known to show a maximum absorption of UV-vis in the range of 400-450 nm at different time intervals due to SPR (Fig. 1).

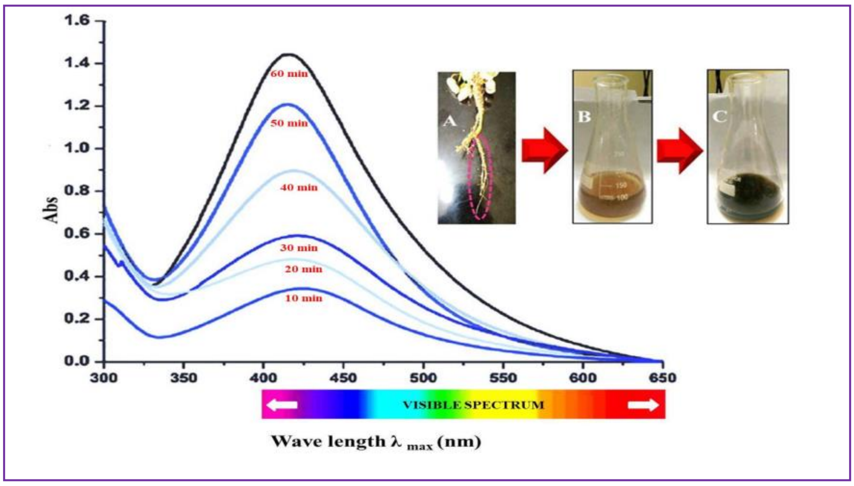

Figure 1: UV-vis absorption spectrum of A- *A. hypogaea* root nodules, B- *A. hypogaea* RN AE (dark brown colour) and C- SPR exhibited by green synthesized AgNPs (dark brownish black)

The appearance of dark brownish black is evidence of the formation of AgNPs (Fig. 1C). In this study, bioreduction of Ag^+ to Ag^0 was rapidly achieved within 10 minutes of incubation period. Zero Valente (ZV) of Ag metal aggregates around the secondary metabolities present in the AE which contains polyphenolic (PP) compounds which is responsible to form AgNPs (Fig. 2B). The UV spectral analysis predicts that NP are evenly distributed pseudoshere in shape, the size of NP was achieved by GS method which is relatively very slow and that is the main attraction of this GS method. Inconsistency in the Ag ion concentration and the AE influences the AgNPs synthesis. It is therefore suggested that natural GS by using AE acts as a bioreducing agent for the synthesis of AgNPs. The benefit of using this GS method when compared with other study such as physical and chemical synthesis method it does not need any catylatic substances to be added [11,12]. Studies revealed that AE containing polyphenols could favour the synthesis of NP much faster than other PC [13].

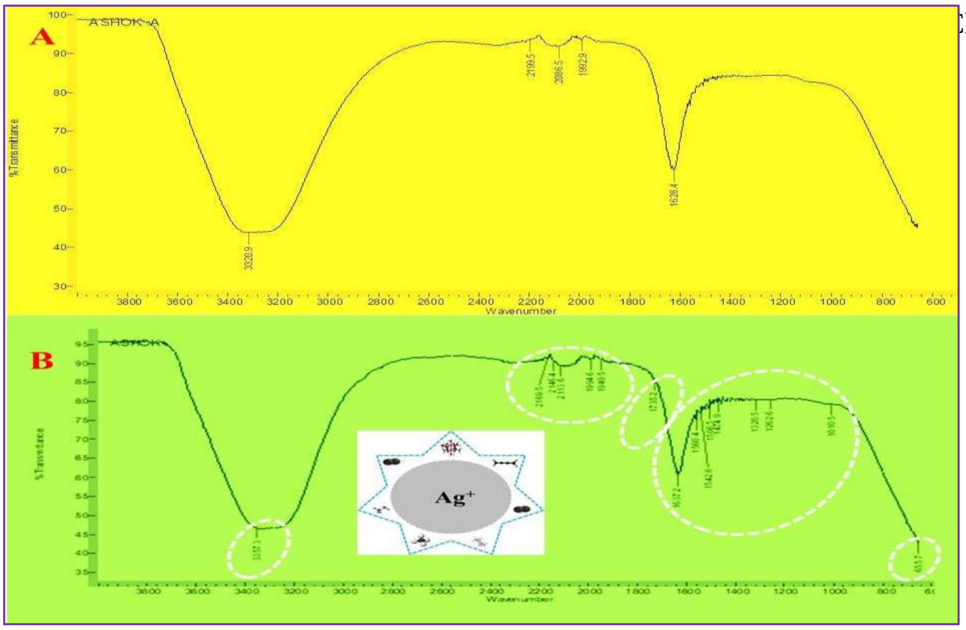

Figure 2: FTIR spectrum of A- *A. hypogaea* RN AE and B- Green synthesized AgNPs (doted white circle highlights the functional groups responsible for the bioreduction

AgNPs often have unexpected visible properties because they are small enough to confine their electrons and produce quantum effects. AgNPs appear dark brown to dark brownish black in solution this is due to the large surface area to volume ratio also reduces the incipient meeting temperature of NP.

QP analysis of *A. hypogea* RN AE showed the presence of carbohydrates, saponin, flavonoids, alkaloids, quinones, phenols, coumarins, acids, protein, cyanin and cardiac glycosides and on the other hand tannins, glycosides, terpenoids and triterpenoids are absent. The presence of polyphenol shows a wide biological activity such as AIN, ANT, AMB, neuroprotective effects, anti-diabetic (ADA), hepatoprotective, hypoglycemic and hypolipidemic activities [14]. PP compounds are widely present in all the plants and it exert multiple activity such as ACA, ANT, AIN, anti-ulcer, ADA, and AMB activity [15].

The IR spectrum (Fig. 2A and B) of AE and bioreduced AgNPs was obtained between the length range of 600-3800 cm^{-1}.The IR spectrum of synthesized by using *A. hypogaea* RN AE was characterized by peaks at 3320.9, 2199.5, 1992.9 and 1628.4 cm^{-1} corresponds to carboxylic acid with OH stretch (strong intensity), C≡C variable stretch, C=C=C allene stretch and aromatic C=C bending respectively. Nevertheless, the OH stretch and C≡C variable stretch are the main constituents of AE such as amino acids, ATP, vitamins, flavones and hormones. The IR spectrum of AE and AgNPs dispersion showed peaks at 3357.3 (OH stretch with strong intensity), 2160.5 cm^{-1} (C≡C variable stretch), 2146.4 cm^{-1} (C≡C variable stretch), 1735 cm^{-1} (C=O ketone stretch with strong intensity), 1637.2 cm^{-1} (C=C alkene with strong intensity), 1360.4 cm^{-1} (C-F stretch with strong intensity) and 655.7 cm^{-1} (C-Br stretch with strong intensity). The peak at 3357.3 cm^{-1} was attributed to the O-H vibration of alcohol groups. The exact mechanism of the bioreduced AgNPs can be illustrated as follows:

Active constituents (OH group) + Ag^{+} $\rightarrow$ Active constituents with = O group + AgNPs

DLS size distribution of AgNPs (Fig. 3A) showed the size distribution in the range of 60 to 150 nm. The calculated average size distribution of AgNPs was found to 74 nm. The DLS analyser confirms that the particle size (PZ) is decreased when compared with the sharp peak of 420 nm in UV-vis spectra. The XRD pattern of the AgNPs from *A. hypogaea* RN AE is shown in figure 3B.

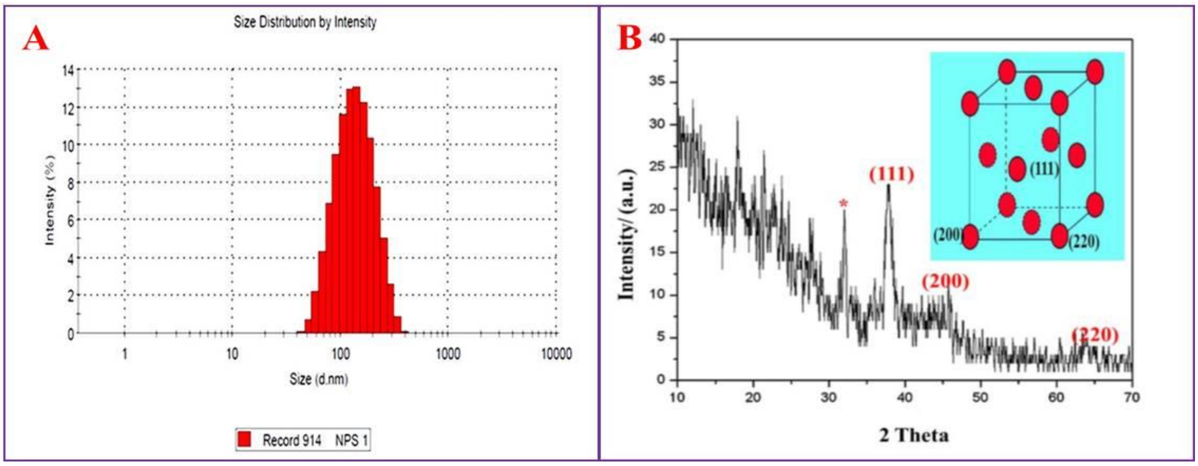

Figure 3**:** A- DLS analysis of synthesized AgNPs and B-XRD spectral image of AgNPs inserted with FCC lattice planes arrangement

AgNPs is a compatible with the FCC phase which can be indexed to the planes of (111), (200) and (220) respectively. The average PZ of the AgNPs was found to be 76 nm. The variation in peak is due to the BM present in the *A. hypogaea* RN AE.

AFM images of AgNPs formed by the film deposition on glass slides by a spin coater. The images are measured by AFM raster-scan probes under ambient conditions. The images found to be pseudoshere (Tractricoid) in shape with different sizes.

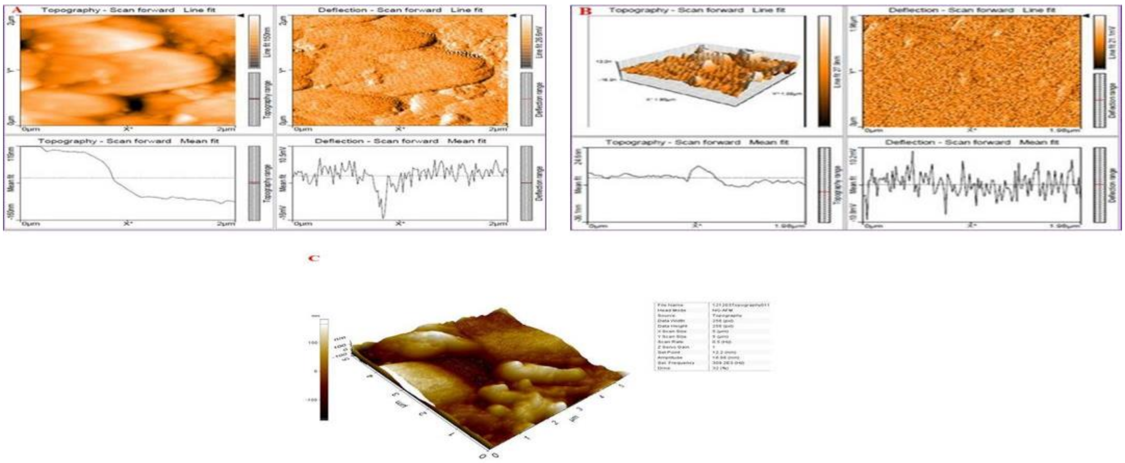

Figure 4: A -AFM image of AgNPs scan scale of 2 µm, B-AFM image of AgNPs scan scale of 1.98 µm and C-AFM image of AgNPs scan scale of 5 µm

In contrast, we see on Fig. 1 and 2 that the AgNPs width and height values, obtained from AFM images are almost larger than the particles size obtained by DLS and XRD. In fact a primary purpose of the introducing the AE to cause bioreduction of AgNPs by using 1mM SN have created aggregation, but with the introduction of AE, 1mM SN solution would coordinate with PP compounds present in AE and that might have covered ZV of Ag metal to aggregates around the secondary metabolites and this in turn is reflected as larger size (height and width) of the particles as shown in the fig. 4A, B and C. The size and shape of the AgNPs depends on the type of salt used such as chloride, lactate, halide and nitrate, Ag ions ratio, the reaction temperature, pH ionic strength media and stirring rate. Among the metal NP Ag are an arch product from the field of NT because of their unique property such as thermodynamic stability, good conductivity, catalytic and most importantly AMB activity [16]. QP analysis showed the presence of polyphenol which actively chelate and reduce metal ions into AgNPs. Mechanism involved in the synthesis of AgNPs using *A. hypogaea* RN AE undergoes three phases namely activation phase, growth phase and termination phase.

3.2. AMB activity by CFU using AgNPs-RS

The AMB effect of AgNPs-RS with 100 ppm alone showed higher AMB activity with 80% microbial reduction was observed with 24 hrs of exposure in BR as shown in the Fig. 5, 6, 7 and 8. The AgNPs-RS tested with 100 ppm concentration showed promising AMB efficiency of around 80% reduction in CFU within 24 hrs exposure.

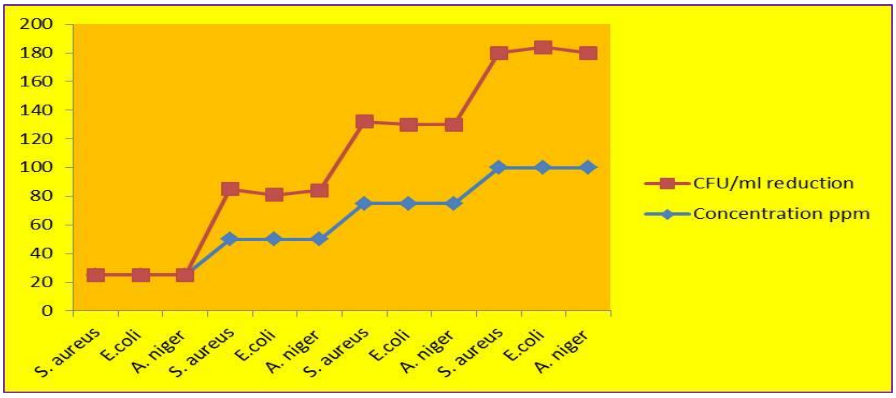

Figure 5: Antimicrobial activity of AgNPs-RS

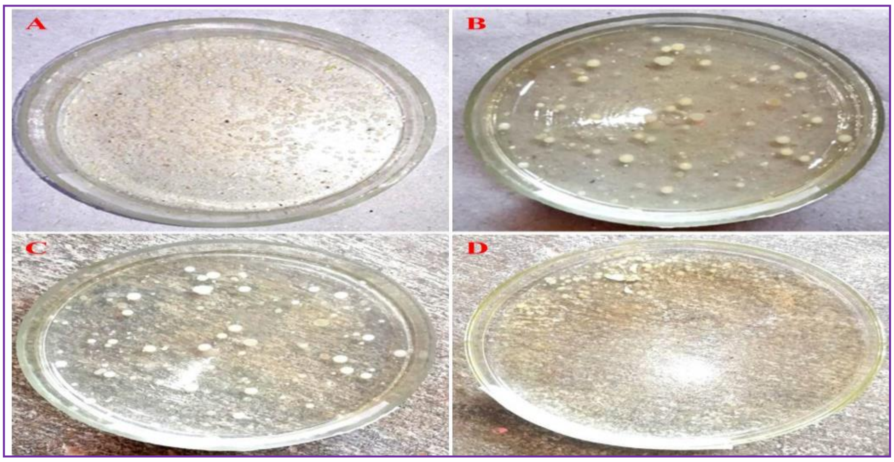

Figure 6: AMB activity of *S. aureus* using AgNPs-RS (A)- 25 ppm, (B)-50 ppm, (C)-75 ppm and (D)-100 ppm

If we prolonged the exposure time and concentration in future these kinds of AgNPs-RS may be highly helpful in eradicating many dread full diseases like SARS-COVID-19 with this continuation of our work we will do AgNPs-hand sanitizer to make environment microbe's free zone. This is the first work to report on *A. hypogaea* RN AE mediated synthesized AgNPs and to prepare AgNPs-RS to assess its AMB activity. Our results are in concordance with data from research carried out on antimicrobial activity of AgNPs showing pseudoshere in shape and size ranging from below 100 nm, as a function of strain [16].

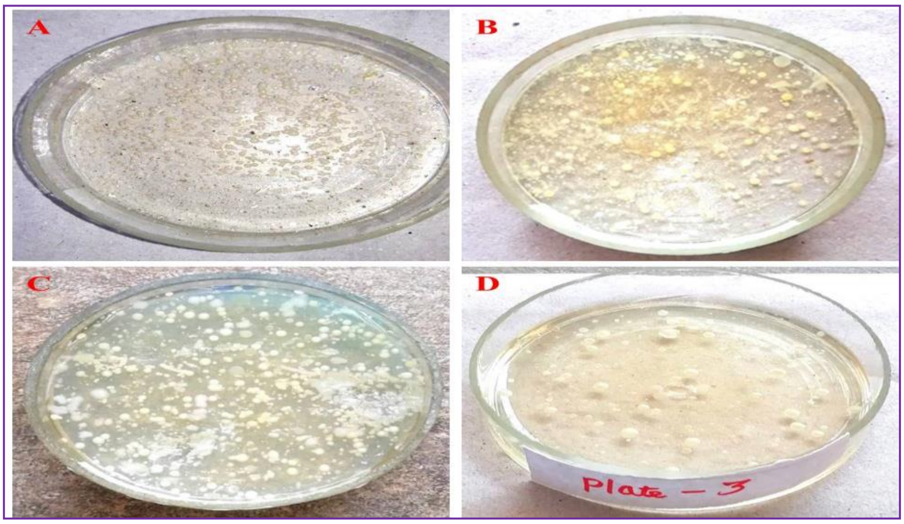

Figure 7: AMB activity of *E.coli* using AgNPs-RS (A)- 25 ppm, (B)-50 ppm, (C)-75 ppm and (D)-100 ppm

It is reported that AgNPs are very promising agent against Gram positive and Gram negative bacteria because of its significant antibacterial activity [17]. In our study AgNPs-RS could anchor to the cell wall subsequently penetrates it, thereby causing cytological changes in the cell membrane like the permeability of cell membrane and cell death (CD). The formation of free radicals by the AgNPs-RS may be considered to be another mechanism by which the cells die in our study. The interaction of the AgNPs-RS with sulphur and phosphorus of the DNA can lead to problems in the DNA replication of bacteria and thus terminate the microbes. Similar results were observed by John *et al*. (2020) [18] by using AgNPs against *Pseudomonas species* and he also stated that AgNPs might have penetrated the bacterial cell and caused the cell death. Ag being a soft acid can act on bases and destroy the DNA which leads to cell death of the bacteria [19]. The study focuses on effective strategies for obtaining bionanomaterial that can be used with a large number of drug resistant microbes, therefore helping to solve serious problems around the world.

4. Conclusion

In conclusion, the green synthesized AgNPs by the AE of *A. hypogaea* RN has been demonstrated for the first time. This approach towards the synthesis of AgNPs can be easily scaled up economically and eco-friendly. The AMB activity of AgNPs-room sprayer showed promising AMB activity against *S. aureus*, *E.coli* and *A. niger* in the 100 ppm concentration and eventually this kind of eco-friendly and cost-effective AgNPs-room sprayer is needed to control many dread full diseases in the present scenario instead of using commercially available room sprayers.

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