

BANANA PSEUDOSTEM-MEDIATED GREEN SYNTHESIS OF SILVER NANOPARTICLES: CHARACTERIZATION AND EVALUATION OF ANTIFUNGAL ACTIVITY

N.A. Musmade¹, Lalit Mahatma², R. D. Pawar³, K. P. Suthar⁴, A. M. Langhi⁵, S. V. Nalawade⁶, D. D. Gaikwad⁷

¹Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari-396450, Gujarat, 0000-0002-6144-6642, musmadenarayan@gmail.com

²Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, 0000-0003-0466-9695, mahatmalalit@yahoo.co.in

³Department of Horticulture, N.M. College of Agriculture, Navsari Agricultural University, 0000-0001-9414-1717, ravipawar44@gmail.com

⁴Department of Agricultural Botany, N.M. College of Agriculture, Navsari Agricultural University, 0000-0001-8113-1077, amollanghi@gmail.com

⁵Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, 0009-0001-4908-9320, suraj11n@gmail.com

⁶Department of Agronomy, N.M. College of Agriculture, Navsari Agricultural University, 0000-0003-3348-0103, gaikwad.dd@gmail.com

⁷Department of Biotechnology, N.M. College of Agriculture, Navsari Agricultural University, 0000-0002-0195-8187, kirnsuthar@nau.in

ABSTRACT

The present study reports an eco-friendly, cost-efficient, rapid, and easy method for synthesization of silver nanoparticles (AgNPs) using banana pseudo-stem extract (BSE) as a reducing and capping agent. The formation of AgNPs was confirmed by changing their colour to dark brown due to the surface Plasmon resonance (SPR) phenomenon. The different factors affecting silver reduction were investigated. The optimal synthesis conditions for the AgNPs were determined by varying parameters such as the pH of the AgNO₃, the AgNO₃ concentration, the BSE concentration, the temperature, and the reaction time. The optimum conditions were AgNO₃ (2.0 mM), BSE (2%), pH (11), temperature (80°C for 20 min), and incubation time (6 h). The UV-vis spectrum of AgNPs revealed a characteristic surface Plasmon resonance (SPR) peak at 404.5 nm. Silver nanoparticles were characterized. X-ray diffraction revealed their crystalline nature. The scanning electron microscope revealed near-spherical, monodisperse nanoparticles, with an average size of 50-92 nm. Fourier transform infrared spectroscopy affirmed the role of BSE as a reducing and capping agent of silver ions. The fungicidal activity of green-synthesized BSE-AgNPs at different concentrations (10, 50, 100, 150, 200 and 250 ppm) was tested against two soil-borne plant pathogens, viz., *Sclerotium rolfsii* Sacc and *Pythium aphanidermatum* (Edson) Fitzp. The result showed that BSE-AgNPs at 250 ppm concentration showed cent per cent inhibition against *S. rolfsii*, whereas BSE-AgNPs at 300 ppm showed cent per cent inhibition against *P. aphanidermatum*.

KEYWORDS: Green synthesis, silver nanoparticles, banana pseudostem extract, antifungal activity, SEM, FTIR, XRD.

1. INTRODUCTION

Nanotechnology is an innovative and emerging discipline in the field of science and technology. With its broad application, it's now becoming a key part of life sciences, including approaches that focus on phytopathogens for disease management. Agrochemical application against phytopathogens is not sustainable anymore because of nontarget effects and environmental hazards. These agrochemicals are conventionally applied to crops either by spraying, drenching, or broadcasting. They show problems like leaching, degradation by photolysis, hydrolysis, and microbial degradation; only a very low concentration of chemicals, which is much below the required minimum effective concentration, reaches the target site of crops. Therefore, repeated applications are necessary for effective control, which might cause unfavourable effects such as soil and water pollution. Under these circumstances, the character of nanoparticles (NPs), nano-emulsions, and nano-formulations makes them efficient nano-pesticides to focus on in a very efficient way, showing higher solubility, permeability and stability [8].

Nanotechnological applicability in crop disease protection offers great promise in the management of pathogens. AgNPs are very effective against phytopathogens with low toxicity and lead to a broad range of applicability in pesticide activity. It is efficiently used for site-targeted delivery of important agrochemical products and diagnostic tools in the case of prior detection of plant diseases [3]. Nanoparticles have the prospect to increase food quality, global food production, plant protection, detection and regulation of plant diseases, monitoring of plant growth, and pest control for "sustainable agricultural development" [13, 14, 24].

The banana (*Musa paradisiaca* L.), which belongs to the Musaceae family, is native to the Indo-Malaya region of Asia. Bananas are produced in large quantities in the tropical and subtropical regions of the world. The crop is of prime importance to the people living in the areas in which it grows, as it provides a major portion of their annual income and is a primary source of food. The production of bananas has considerable economic importance. After harvesting, however, a large amount of pseudostem residue is left behind in plantation soil to be used as organic material. It has been estimated that a few tonnes per hectare of banana pseudostem are produced annually. Biosynthesis of AgNPs using the commercially

economic and abundantly available banana pseudostem as the biomaterial. Phytochemical studies of banana pseudostem extract (BSE) showed the presence of flavonoids, polyphenols and their derivatives, which are largely responsible for antioxidant and free radical-scavenging properties [2, 5, 26].

This work aims to use BSE as a low-cost and eco-friendly environmental approach to the green synthesization of BSE-AgNPs. The effect of synthesis parameters such as silver ion concentration, BSE concentration, temperature, and reaction time has been investigated, and BSE-AgNPs were characterized by UV-vis spectroscopy, SEM, XRD, and FTIR. The synthesized BSE-AgNPs were also tested for antifungal activity.

Recent reviews describe plant-mediated AgNPs synthesis as a promising approach for managing phytopathogens, while emphasizing that synthesis conditions, nanoparticle properties and biological responses vary with the biological source and formulation [32–34]. Accordingly, the present work evaluates banana pseudostem extract as a synthesis medium and assesses antifungal activity under in vitro conditions.

2. MATERIALS AND METHODS

2.1 Preparation of aqueous BSE

The fresh and healthy pseudostem of the banana sword sucker was collected from the college farm of ACHF, NAU, Navsari. BSE was prepared by boiling 100 g of freshly cut pseudostem pieces in 100 ml of double-distilled water (1:1 w/v) at 60°C for 20 min. The extracts were filtered through a 0.2 µm nylon mesh followed by Whatman No. 1 filter paper using the Millipore vacuum filtration unit. The filtered extract was subjected to centrifugation at 10,000 rpm for 5 min. After centrifugation, the supernatant was collected and stored at 4°C as a stock solution for the green synthesization of BSE-AgNPs.

2.2 Synthesization of BSE-AgNPs

BSE-AgNPs were prepared by mixing different concentrations of BSE (1, 2, 5, and 10%) and AgNO₃ solution (0.5, 1.0, 1.5, and 2.0 mM), and the final volume of the solutions was made 100 mL with continuous stirring, followed by heating the mixture at 80°C for 20 min in a water bath for reduction of Ag⁺ ions. The synthesization of nanoparticles was indicated by the colour change of the solution. The reaction mixtures were monitored at different time intervals and the nanoparticles that were formed were characterized further.

2.3 Optimization of different parameters

The effect of pH on nanoparticle synthesis was determined by adjusting the pH of the reaction mixtures (2% BSE, 2.0 mM AgNO₃) to 5, 7, 9, 11 and 13. The pH of the reaction mixture was set by using freshly prepared 0.1 N HCl and 0.1 N NaOH solutions. The effect of the silver salt was determined by varying the concentration of AgNO₃ (0.5, 1.0, 1.5 and 2.0 mM) at 2% BSE. The BSE was varied (1, 2, 5 and 10%) while keeping the AgNO₃ concentration at a level of 2.0 mM. To study the effect of different incubation temperatures on AgNPs synthesization, the reaction mixtures containing 2% BSE and 2.0 mM AgNO₃ concentration at pH 11 were incubated at 40, 60 and 80°C in a water bath for 10 and 20 min, and the absorption spectra of the aqueous colloidal suspensions were recorded at each temperature and incubation period.

2.4 Characterization of BSE-AgNPs

The BSE-AgNPs were characterized by visual observation and spectroscopic methods. In visual observation, the colour change of the solution was confirmed by the naked eye. The AgNPs in solution were identified by using a UV-visible spectrometer (SHIMADZU UV-2600 spectrometer) in the wavelength (λ) range 300–800 nm. The surface chemistry of BSE-AgNPs and biomolecules in BSE was characterized by using Fourier transform infrared spectroscopy. The FTIR spectra were collected at a resolution of 4 cm⁻¹ in the transmission mode (4000–400 cm⁻¹) on an instrument made by Spectrum GX, Perkin Elmer, the U.S.A., having a range of 10,000 cm⁻¹ to 370 cm⁻¹, and reflectance measurements were carried out by IR Quant spectrum search software. The shape, size and surface of synthesized BSE-AgNPs were analyzed by a scanning electron microscope (Carl Zeiss Microscopy, Germany), and high-resolution images were observed at different magnifications and the images were captured. The phase distribution, purity and crystallinity of the synthesized nanoparticles were confirmed by X-ray diffraction (XRD) (Miniflex Serial No. 2005G303). The XRD pattern was taken in the 2θ range of 20°–80° in a fixed time mode at room temperature. The intensity and crystalline nature of AgNPs were measured using Match software version 3.11.3.192.

2.5 In-Vitro antifungal activity of BSE-AgNPs

The in vitro antifungal activity of BSE-AgNPs was evaluated against *S. rolfsii* and *P. aphanidermatum* using a poisoned food technique on PDA medium. The different concentrations of AgNPs, viz., 10, 50, 100, 150, 200, 250 and 300 ppm, were prepared in sterilized PDA medium, poured into sterilized Petri plates, and allowed to solidify. The pure 7-day-old cultures of *S. rolfsii* and *P. aphanidermatum* were taken and cut into 5 mm disc using a sterilized cork borer inside the laminar airflow and transferred onto the center of the Petri plates using a heat-sterilized inoculation needle. Petri plates poured with PDA medium without BSE-AgNPs and inoculated with fungal culture served as the control. Each concentration of BSE-AgNPs was repeated four times and incubated at 27°C in an incubator and observed periodically till the growth of the test pathogen covered the whole Petri plate in the control treatment. The mycelial growth of test pathogens was then measured, and per cent growth inhibitions over control were calculated using the formula given below

$$I = \frac{(C-T)}{C} \times 100$$

Where I= Per cent inhibition

C = Radial growth in control
T = Radial growth in treatment (AgNPs)

3. RESULTS AND DISCUSSION

3.1 Visual observation

The reduction of silver ions and the synthesization of AgNPs is a natural process that starts with the addition of BSE into the AgNO₃ solution. The initial colour of AgNO₃ solution in different concentrations and pH was different, ranging from milky white to light straw colour. With the addition of BSE, the colour of the solution changed gradually to dark brown, which also varied in different concentrations and pH. Colour changes were noticed till the first hour, then it became stable. Among the different combinations with different pH, AgNO₃ molarity and BSE concentrations, the maximum colour change was observed in AgNO₃ solution at pH 11 and 13, which was dark brown. With the decrease in pH, the intensity of the colour was reduced. Among the different treatments, maximum colour changes were observed in pH 11 and 13 of 2.0 mM AgNO₃. Whereas the minimum colour change was in pH 5 of 0.5 mM AgNO₃. The different colour changes observed were light brown to dark brown [5]. This colour change could easily be visualized and differentiated by the naked eye, making the monitoring process of synthesization of AgNPs easy. The initial colour of BSE was light brown, which was due to the presence of alkaloids, flavonoids, saponins, enzymes, steroids, etc. BSE acts as a reducing and capping agent of (Ag⁺) to the silver atom (Ag⁰) [5, 7, 21].

3.2 UV-vis spectrophotometer

Monitoring the process of the reduction of silver ions to AgNPs was applied in this study by UV-vis spectroscopy. UV-vis spectroscopy might be used to detect size- and shape-controlled AgNPs in aqueous suspensions [29]. UV-vis spectroscopy was used to follow the reaction process and characterize the optical properties of produced nanoparticles. The formation and optimization of AgNPs were monitored using UV-vis spectroscopy by measuring the absorbance in the range of 300–800 nm. The green-synthesized nanoparticles showed a sharp peak around 400–448 nm, indicating the formation of silver nanoparticles, which was identified as the “surface Plasmon resonance band,” and this band is ascribed to excitation of valence electrons [5, 30, 31]. SPR of BSE-AgNPs (Figure 1) showed a characteristic peak at λ_{max} 404.5 nm, maximum absorbance 1.617 and area under absorbance wavelength curve (AUAWC) 96.756 in comparison with BSE and 2.0 mM AgNO₃ did not show a peak. The position of the absorption band also mainly depends upon the dielectric constant of the medium and surface adsorbed species. The shape of the band was symmetrical, suggesting uniform scattering of spherical-shaped nanoparticles [1, 6, 21].

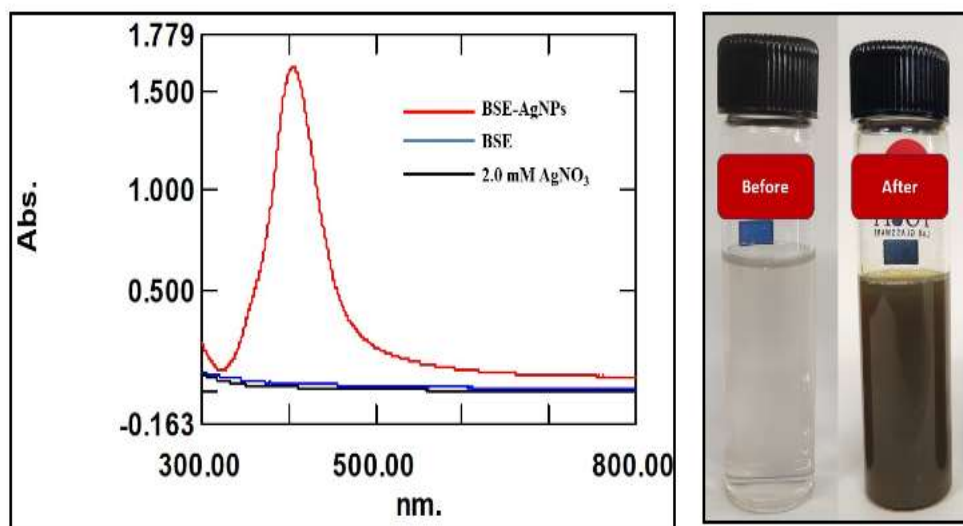


Figure 1. UV-vis spectra of synthesized BSE-AgNPs

3.3. Effect of pH

Research work involves the issue of synthesizing AgNPs at different pHs (5, 7, 9, 11 and 13). Figure 2 indicates that the synthesization of BSE-AgNPs was affected by pH and shows the synthesization of BSE-AgNPs using a UV-vis spectrophotometer [7]. It was observed that pH increased from 5 to 11, maximum absorbance increased with increasing pH up to 11 and again reduced at 13 pH, and λ_{max} shifted to a lower value. The narrow absorbance was observed at 11 pH; it indicates that lower pH 5-9 and higher pH (13) suppress the formation of the nanoparticles due to lower availability of functional groups of BSE. This indicates that 11 pH was the most favorable pH for the synthesization of BSE-AgNPs [19, 21].

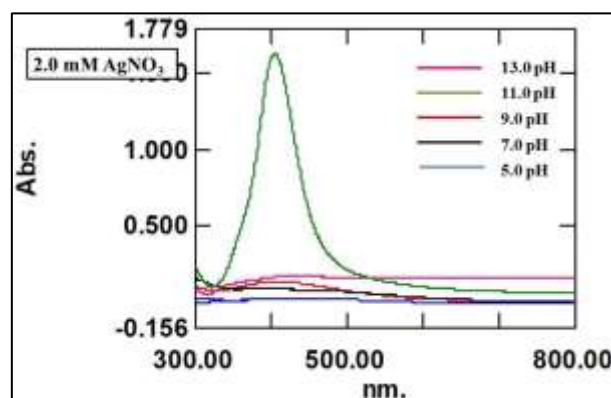


Figure 2. UV-vis spectra of varied pH of 2.0 mM AgNO₃ on synthesis of BSE-AgNPs by using 2% BSE

3.4 Effect of silver nitrate concentration

The optimization of AgNO₃ was carried out at different concentrations of AgNO₃ solution of 11 pH (0.5, 1.0, 1.5 and 2.0 mM) to maximize the yield of AgNPs at 2% concentrations of BSE. It was observed (Figure 3) that the peak intensity increased with the increase in AgNO₃ concentration from 0.5 mM to 2.0 mM, indicating a faster rate of reduction with increased concentration of metal precursor [19, 21, 25]. The UV-vis absorption spectra showed high absorbance when the concentration of AgNO₃ solution was 2.0 mM; this indicates that 2.0 mM AgNO₃ concentration was the most favorable for the synthesis of BSE-AgNPs. The maximum absorbance increased with increasing the concentration of AgNO₃ and the λ_{max} shifts to higher to lower wavelengths with an increase of silver nitrate concentration [10,17,18].

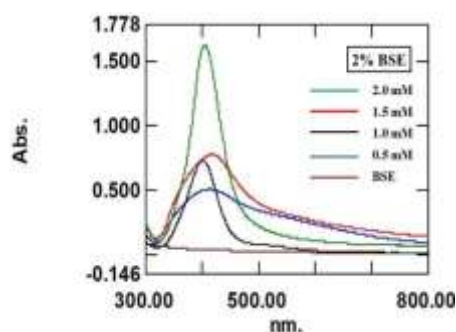


Figure 3. UV-vis spectra of different AgNO₃ concentrations of 11 pH on synthesis of BSE-AgNPs by using 2% BSE

3.5. Effect of BSE

To study the influence of BSE content on the synthesis of BSE-AgNPs, nanoparticles were prepared by adding 1.0%, 2.0%, 5.0% and 10.0% extract in 2.0 mM AgNO₃ concentration of 11 pH showed in fig. 4. The UV-vis absorption spectra showed high absorbance when the concentration of BSE was 2.0%. It is noted that 2.0% concentration of BSE was the most suitable for the synthesis of BSE-AgNPs [5].

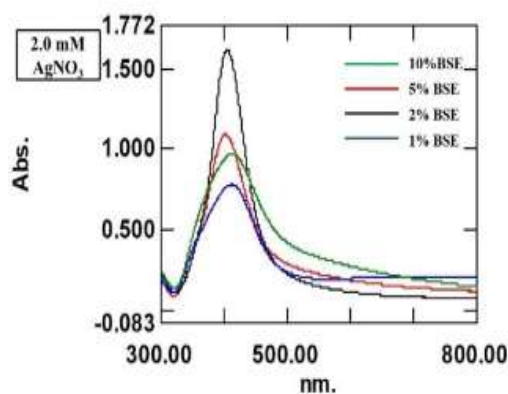


Figure 4. UV-vis spectra of different BSE concentration on synthesis of BSE-AgNPs at 2 mM AgNO₃ at pH 11

3.6 Effect of temperature

The temperature was one of the factors influencing the process of silver reduction during the synthesization of AgNPs. It was investigated at 2% BSE and 2.0 mM AgNO₃ concentration at pH 11 by varying different temperatures 40, 60 and 80°C incubated in a water bath for 10 and 20 min. The absorbance of the resulting solution was measured by a UV-vis spectrophotometer. Synthesization of BSE-AgNPs was affected by temperature and observed (Figure 5) that as the temperature raises from 40 to 80°C and 10 and 20 min incubation the maximum absorbance was increased with increasing temperatures and λ_{max} shift from higher to lower which indicates that the size of the synthesized nanoparticles decreases with increasing temperature [10,23], which may be due to the faster reaction rate at a higher temperature and the colour change happened faster. At high temperatures, the kinetic energy of the molecules increases, and silver ions get consumed faster, thus leaving less possibility for particle size growth [18,20,27]. The BSE-AgNPs showed higher absorbance at 80°C for 20 min. This is indicated that 80°C for 20 min was the most favourable temperature for the synthesization of BSE-AgNPs.

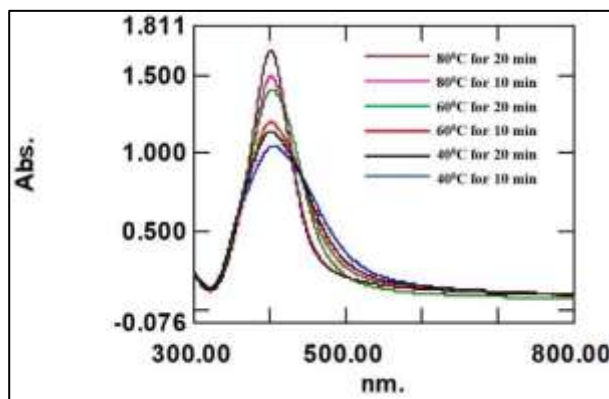


Figure 5. Effect of temperatures on synthesization of BSE-AgNPs using 2% BSE and 2.0 mM AgNO₃ conc. of 11 pH

3.7 Characterization of silver nanoparticles

Scanning Electron Microscopy

The scanning electron micrograph reveals the morphology of AgNPs, it was observed that they were nearly spherical with a smooth surface Figure6. The particle size of the BSE-AgNPs ranged from 50 to 92 nm. The morphological study also revealed that powder from BSE-AgNPs particles was slightly agglomerated[5].

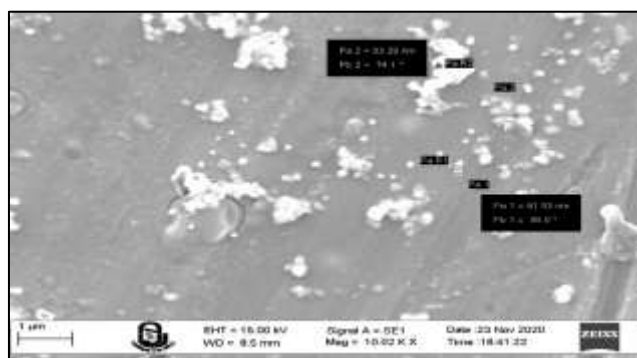


Figure 6. Scanning electron microscopy image of BSE-AgNPs

X-ray Diffraction Analysis of BSE-AgNPs

The XRD was widely used for the identification of unknown crystalline materials (e.g., minerals, inorganic compounds, etc.) as well as the determination of the orientation of a single crystal and crystal structure of an unknown material. It also measured the size, shape and internal stress of small crystalline regions [4,18]. The XRD patterns of synthesized BSE-AgNPs (Figure 7) showed Bragg reflection peaks at 38.064°, 44.24°, 64.35° and 77.28° in the 2 θ range between 20°–80° which could be indexed to the (111), (200), (202) and (311) planes of face-centered cubic (fcc) crystal, respectively. These Bragg peaks might have resulted from the capping agent (BSE) present in the nanoparticles, revealing that the synthesized AgNPs were composed of pure crystalline silver. The peak corresponding to the (111) plane was more intense than the other planes, suggesting that the (111) plane was in the predominant orientation. The XRD pattern of BSE-AgNPs showed the crystalline nature of the polymeric nanoparticles [6,12,21].

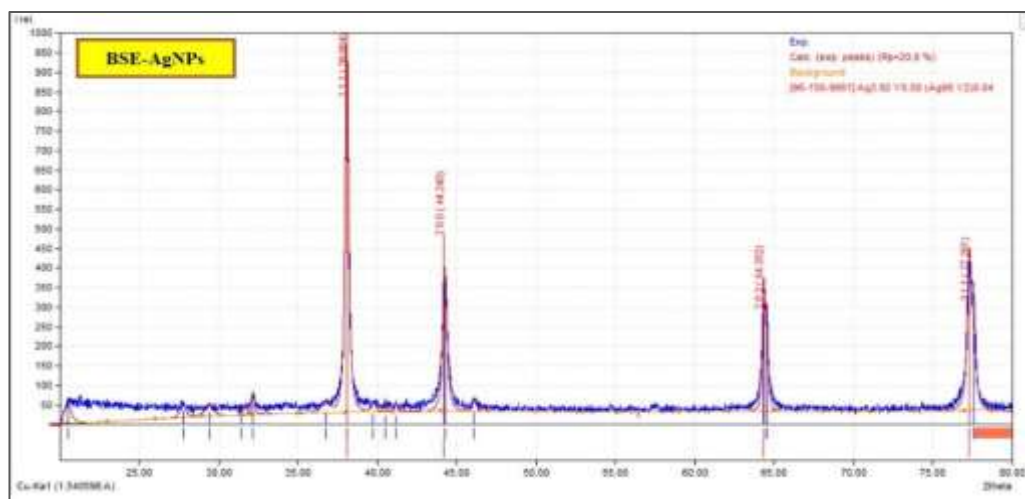


Figure 7. Powder XRD spectra of synthesized BSE-AgNPs

FTIR of BSE-AgNPs

For the synthesization of BSE-AgNPs, major factors responsible for the biological reduction of Ag^+ to Ago present in the BSE were identified using FTIR spectroscopy. The FTIR spectrum of BSE-AgNPs presented in Figure 8 showed the presence of distinct peaks in the range of 3427.55, 2923.72, 1633.48, 1384.16, 1160.60, 1111.93, 1058.21, 898.03 and 595.83. The strong, intense peak located at around 1384.16 cm^{-1} ($-\text{CH}_3$ bending) could be assigned to the carboxyl group. The medium bands appear at 1058.21 cm^{-1} (C-O-C stretching) and 1633.48 cm^{-1} ($-\text{C}=\text{O}$ stretching) were due to fatty acid and flavanone, respectively. The weak absorption peaks at 1111.93 cm^{-1} and 2923.72 cm^{-1} ($-\text{CH}_2$ stretching) were attributed to the amide bond of proteins and carboxylic acids present in BSE. Also, there was a broad peak located at 3427.55 ($-\text{OH}$ stretching), which could be assigned to the O-H stretching vibration, indicating the presence of alcohols and phenols. As per the FTIR results, it was understood that some proteins and metabolites such as flavones, polyphenols, etc., present in the BSE might be responsible for the reducing and capping of AgNPs [5,22,31].

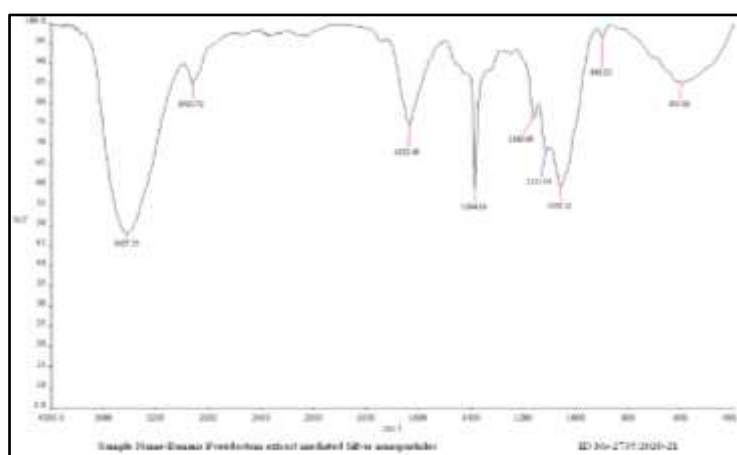


Figure 8. FTIR Spectra of BSE-AgNPs

3.8 In-vitro efficacy of silver nanoparticles against fungi

In this study, data presented in Table 1 and Figures 9 & 10 for optimization of BSE-AgNPs dose were tested with different concentrations 10, 50, 100, 150, 200, 250, and 300 ppm against both pathogens *S. rolfsii* and *P. aphanidermatum*. The per cent growth of inhibition was recorded 0, 1.76, 10.88, 39.12, 69.12 at 10, 50, 100, 150, 200 ppm concentrations while 100 per cent inhibition was observed in 250 and 300 ppm concentrations against *S. rolfsii* and *P. aphanidermatum* showed 0, 0, 6.47, 36.76, 71.76, 91.76 and 100 per cent respectively compared with the control. The BSE-AgNPs showed 100 per cent growth inhibition against *S. rolfsii* and *P. aphanidermatum* at 250 and 300 ppm concentrations, respectively. Recent literature likewise reports antifungal effects of biologically synthesized AgNPs against plant-pathogenic fungi, but also identifies formulation-dependent activity and the need to assess phytotoxicity and environmental fate before agricultural deployment [32–35]. Therefore, the inhibition observed here supports further investigation of BSE-AgNPs; it does not by itself establish field efficacy, crop safety or suitability as a pesticide replacement. The AgNPs can be used as alternatives to chemical pesticides that affect fungal cells by attacking their membranes; thus, disrupting the membrane potential and causing cell death. Membrane damage due to peroxidation was also reported in fungi via AgNPs activity that might be a major cause of AgNPs leading to cell death; hence are better fungicides [9,11,15]

Table 1: In vitro efficacy of synthesized BSE-AgNPs against fungi

Concentrations of BSE-AgNPs (ppm)	% growth of inhibition of fungi on PDA	
	<i>S. rolfsii</i>	<i>P. aphanidermatum</i>
10	0.00	0.00
50	1.76	0.00
100	10.88	6.47
150	39.12	36.76
200	69.12	71.76
250	100.0	91.76
300	100.0	100.0
Control	0.00	0.00

**Figure 9. In vitro efficacy of BSE-AgNPs against *S. rolfsii*****Figure 10. In-vitro efficacy of BSE-AgNPs against *P. aphanidermatum***

4. Conclusion

Banana pseudostem extract (BSE), an abundant agricultural waste biomass, was successfully employed as a biological reducing and stabilizing agent for the rapid synthesis of silver nanoparticles (BSE-AgNPs). The synthesized nanoparticles were systematically characterized using UV–visible spectroscopy, FTIR spectroscopy, XRD, and SEM. The results confirmed the formation of crystalline, predominantly near-spherical nanoparticles with a relatively smooth surface and particle sizes ranging from 50 to 92 nm. The reduction of Ag^+ ions to metallic Ag^0 was indicated by the characteristic change in colour of the reaction mixture from light brown to dark brown and was further supported by the appearance of a surface plasmon resonance (SPR) band at 404.5 nm. Optimization of the synthesis conditions revealed that an alkaline pH of 11 was favourable for efficient BSE-AgNP formation under the conditions investigated. Furthermore, the synthesized BSE-AgNPs exhibited antifungal activity against the phytopathogenic fungi *Sclerotium rolfsii* and *Pythium aphanidermatum*, highlighting their potential for further investigation as a nanoparticle-based approach to plant disease management. Overall, the study demonstrates the potential of banana pseudostem biomass for the sustainable biosynthesis of AgNPs and provides an approach for the valorization of agricultural waste into value-added nanomaterials. However,

further studies addressing nanoparticle stability, reproducibility, phytotoxicity, environmental fate, non-target effects, formulation performance, and greenhouse and field efficacy are necessary to establish their safety, consistency, and practical applicability in agricultural systems.

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