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Mangifera indica L. leaf assisted biosynthesis and characterization of silver nanoparticles and their antifungal activity against plant pathogen *Aspergillus niger* Tiegh.

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Abstract

The biosynthesis of silver nanoparticles is an emerging field in nanotechnology. There are several different methods to prepare silver nanoparticles depending upon their uses such as in the pharmaceutical and textile industries, and the field of medicine. One of the most important applications of silver nanoparticles is their antifungal activity against different plant pathogenic fungi. In this study, a cost-effective and environment-friendly method was used to synthesize silver nanoparticles using leaf extract of different varieties of *Mangifera indica* L. The varieties used in this study were Fajri, Malta, Sinduri, Sufaid Chaunsa, and Langra. The aqueous extract of the leaves of mango acted as reducing and stabilizing agents for the silver nanoparticles. The formation of silver nanoparticles was confirmed by the UV-Vis spectroscopy in which a surface plasmon resonance peak within the range of 448-454 nm was observed. FTIR analysis was also performed which showed the bands between 3738 cm^{-1} to 432 cm^{-1} which confirmed the presence of different chemical groups. Silver nanoparticles were further characterized by the scanning electron microscopy which revealed the sizes of the nanoparticles within the range of 12-235 nm. After characterization, the antifungal activity of biosynthesized silver nanoparticles was evaluated. From the results, it was concluded that all the varieties showed a greater inhibitory effect at the concentration of 100 mg/L, but it was observed that silver nanoparticles prepared by the leaf extract of cv. Fajri exhibited a greater inhibitory effect against *Aspergillus niger* having a percent inhibition of 79.8%.

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Introduction

Nanoscience is currently a rapidly developing technology (Mandal et al., 2006; Dan, 2020). It collectively defines technology and science involving nano-scale particles (nanoparticles), which increases the possibility of checking and regulating the interaction between synthetic materials and biological materials at the cellular level (Du et al., 2007). Particles in the 1-100 nm range are referred to as nanoparticles (Goralska et al., 2005). Nanoparticles have properties like chemical, optical, electrical, and many other physical properties that make them different from larger-sized particles (Yang et al., 2010; Khan et al., 2019).

Nanoparticles also play an important role in many industries and fields of medicine such as diagnostics, sensing, imaging, drug delivery, artificial implants, gene delivery, and tissue engineering (Morones et al., 2005; Silva et al., 2023). Different types of nanoparticles, i.e., silver, gold, nickel, copper,

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and silicon have been discovered. Silver nanoparticles are the most essential among all the above-mentioned nanoparticles due to their unique magnetic and optical properties (Zhang et al., 2016; Ali et al., 2023). Silver nanoparticles have also the highest electrical and magnetic conductivity as compared to that of other nanoparticles (Wiley et al., 2007). Because of their unique properties, silver nanoparticles are important in microelectronics, ink production, and medical imaging (Monteiro et al., 2009).

Silver nanoparticles have also been used in many consumer products such as plastics, pastes, food, textiles, and soaps for years (Dallas et al., 2011; Garcia et al., 2011; Zhang et al., 2016; Ali et al., 2023) due to their wide-ranging bactericidal and fungicidal activities (Ahamed et al., 2010; More et al., 2023). Silver nanoparticles may also act as a layer of moisture on different materials which are responsible for protective barriers against bacterial and fungal pathogens (Dallas et al., 2011; Bruna et al., 2021).

Silver has been used for the disinfection of many harmful microorganisms for years (Chambers et al., 1962; Kim et al., 2004; Dallas et al., 2011). Silver nanoparticles are the most effective nanoparticles against fungal pathogens. Antifungal properties of silver nanoparticles have been studied for several decades (Bruna et al., 2021). The primary requirement for the use of silver nanoparticles as an antifungal material is to gather information on their antifungal activity and compounds present in silver nanoparticles responsible for antifungal activity. To increase the effectiveness of disease suppression it is required to develop better application strategies (Jo et al., 2009).

Many methods of biosynthesizing silver nanoparticles have been discovered; various chemical, biological, and physical methods have been discovered (Vishwanath and Negi, 2021). Each method has different advantages and disadvantages including cost and particle size analysis. Silver nanoparticles are prepared with the help of many chemical methods by using various solutions (Ahmad et al., 2011; Nguyen et al. 2023).

Nowadays many environment-friendly methods have been discovered to produce nanoparticles in the field of nanotechnology (Bhattacharya et al., 2005; Beni and Jabbari, 2022). Among all the methods, the biological method is the most essential and cost-effective method to produce silver nanoparticles. In the biological method, silver nanoparticles can be synthesized using various living organisms including bacteria, yeasts, fungi, algae, and different plants (Sintubin et al., 2012; Pandit et al., 2022). The biological method produces wide-ranging silver nanoparticles which is an environment-friendly method as no harmful chemicals or solutions are used in this method. Also, using biological agents and reducing metal ions is faster and the easiest method by applying different temperature and pressure conditions (Thakkar et al., 2010). For the biosynthesis of nanoparticles, plant extract can be used. In biological methods, plant extract is mainly used to produce nanoparticles (Antunes Filho et al., 2023).

Mangifera indica L. (Mango) is an evergreen tree. The main component present in mango is mangiferin (Kumar et al., 2021). Mangiferin is a flavonoid that is used in pharmaceutical industries, since it consists of natural xanthone C-glycoside, and it is extracted at high concentrations from mango young leaves (El-Nashar et al., 2022). Mangiferin has several medicinal actions and possible health benefits. It has many antidiabetics, antifungal, antimicrobial, anti-inflammatory, antiviral, hypoglycemic, antiallergic, and anticancer activities (Imran et al., 2017).

The main objective of this study was to biosynthesize silver nanoparticles from the leaf extract of different varieties of *Mangifera indica* L., characterize, and study the antifungal properties of biosynthesized silver nanoparticles against *Aspergillus niger* Tiegh. *Aspergillus niger* is a fungus belonging to family Trichochomaceae.

Materials and Methods

Green synthesis of nanoparticles

Nanoparticles were synthesized by the leaf extract of different varieties of *Mangifera indica* L.

Preparation of plant material

Leaves of *Mangifera indica* L. cvs. Langra, Sufaid Chaunsa, Sinduri, Malta, and Fajri were collected from a garden of D.G. Khan, Pakistan. The leaves were brought to the laboratory and washed thoroughly with tap water to remove all dust particles on the leaves, and then rinsed with distilled water. The washed leaves were dried in a cool place for a few days. The dried leaves were ground into a fine powder with the help of an electric grinder, and then the powder was stored in an airtight jar for further use.

Preparation of plant extract

After crushing, 10 g of powdered leaves were taken and added to 100 mL of distilled water. The powder was boiled in 100 mL of distilled water on a hot plate for 20 minutes. The boiled mixture was cooled at room temperature and then filtered through Whatman No. 1 filter paper. The extract was stored at 4 °C for further use.

Silver nanoparticles synthesis

To reduce silver ions, an aliquot of 10 mL of leaf extract was mixed with 5 mL of 1 mM $AgNO_3$ aqueous solution in a glass vial and incubated in the dark at room temperature for 24 h. The color changed from light yellow to yellowish-brown, indicated the reduction of silver nitrate. The synthesized nanoparticles were centrifuged at 13000 rpm for 10 minutes, the supernatant discarded, and the pellet so formed was stored at 4 °C for further use.

Characterization of silver nanoparticles

After the preparation of AgNPs, the prepared particles were characterized using different techniques. The techniques that were used to characterize the particles were UV-vis spectroscopy, SEM, and FTIR. The UV-visible spectrum analysis was performed by using a UV-visible spectrophotometer in the range of 300 to 800 nm. Thereafter, scanning electron microscopy was performed. The sample was dried and then crushed into fine powder. A small amount of the sample was placed on the carbon-coated grid and the extra-sample was removed. It was performed to study the morphology of the synthesized particles. The EDX of the sample was also performed which indicated the presence of AgNPs in the sample. FTIR analysis was also carried out to determine the quality and consistency of a sample and the number of components in the mixture. For the FTIR analysis, the pellet was dried and crushed with KBr in an Eppendorf tube. The data obtained from the FTIR analysis was recorded in the form of wavelengths.

Antifungal activity of silver nanoparticles

The antifungal activity of silver nanoparticles was studied by preparing culture media which was inoculated under sterile conditions. For the preparation of culture media, all the glassware items were washed and sterilized in an autoclave machine at 121 ℃ and 15 psi pressure for 15-20 minutes and then oven-dried at 60 ℃ for 2 h.

Procurement of pathogen and preparation of culture media

Fungal pathogen *Aspergillus niger* Tiegh. was obtained from the fungal culture bank at the Institute of Agricultural Sciences, University of the Punjab, Lahore. For the inoculation of the fungal pathogen, the MEA media was prepared by adding 12 g malt extract in 15 g of agar in a small amount of distilled water and mixed well by heating. Then the final volume was increased to 1000 mL with the help of distilled water. Then the media was autoclaved at 121 ℃ and15 psi pressure for 15-20 minutes. Before inoculation, the plates were prepared, and media was poured into each Petri plate under aseptic conditions. After pouring the media into Petri plates it was inoculated with the fungal pathogen and the plates were covered and incubated at 37 ℃ for 24 h in an incubator.

Preparation of silver nanoparticles stock solution

Stock solutions (100 mg/L) of AgNPs were prepared in distilled water. Different concentrations of the stock solution were prepared. A solution of 80 mg/L was prepared by taking 8 mL of the solution from the stock solution and the final volume was raised to 10 mL by adding 2 mL distilled water, then a range of concentrations such as 60, 40, and 20 mg/L were prepared.

Application of nanoparticle solutions

The laminar airflow cabinet was sterilized with ethanol to prevent any microbial contamination. Then the UV light was switched on for 20 minutes to prevent any air contamination. Media was prepared for the treatment and all the glassware and media were autoclaved in an autoclave machine at 121 ℃ and 15 psi pressure. For the preparation of plates, 20 mL of the media were poured in each plate along with 3 mL of each silver nanoparticle solution. For each concentration, three plates were prepared.

Measurement of growth parameter

After 24 h of incubation, fungal growth was observed on Petri plates. Percent inhibition was measured. The size of the colony was measured with the help of the ImageJ software. The following formula was used to measure the zone of inhibition:

$$\text{Inhibition rate } (\%) = \frac{R - r}{r}$$

Where R is the radial growth of fungal mycelia on the control plate and r the radial growth of fungal mycelia on the plate treated with silver nanoparticles.

Statistical analysis

First, data was tested for normality. The SPSS software was used to perform statistical analyses, particularly for working out analysis of variance (ANOVA) of data for each parameter. Standard errors

were also worked out to assess variation in individual data recorded within each mean value.

Results

Characterization of silver nanoparticles

Characterization was performed by using various techniques, i.e., UV-visible spectroscopy, FTIR, and SEM which indicated the formation of silver nanoparticles. UV-Vis spectroscopy showed the spectrum formed by the silver nanoparticles. FTIR analysis indicated the attached groups with AgNPs. The SEM analysis showed the morphology of the formed nanoparticles.

UV-Visible spectroscopy

In this study, UV-visible spectroscopy was performed with the help of a UV-visible spectrophotometer. The color change of the extract resulted in the surface plasmon resonance by AgNPs (**Figure 1**) which showed different spectra at different wavelengths. Different varieties of *Mangifera indica* L. showed different spectra. Cultivars Fajri and Malta showed a spectrum at a wavelength of 448 nm, whereas cv. Sinduri showed a peak at 452 nm (**Figure 2**). The spectrum of cvs. Sufaid Chaunsa and Langra was observed at 454 nm and 446 nm, respectively. Cultivar Fajri showed the highest peak at 448 nm (**Figure 2**).

Figure 1. Leaf extract of *Mangifera indica* L. before and after the reaction with silver nitrate solution

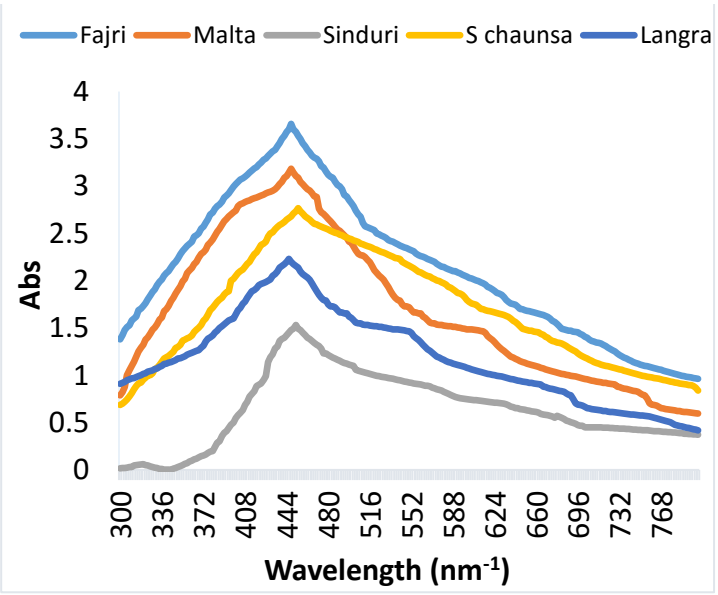

Figure 2. UV spectrum of green synthesized silver nanoparticles using leaf extract of different varieties of *Mangifera indica* L. within 300-800 nm

Fourier transform infrared spectroscopy

FTIR analysis of AgNPs synthesized by leaf extract of Mangifera indica L. cv. Fajri

Various absorption bands in the FTIR spectroscopy showed the presence of different chemical groups in the extract containing biosynthesized AgNPs with the help of leaf extract of cv. Fajri. Bands at 3738 cm^{-1} and 3415 cm^{-1} showed the presence of O-H group (**Figure 3a**). Bands at 2316.5 cm^{-1} and 2067 cm^{-1} revealed the presence of C=C and C≡C conjugated bonds. The bands at 1629 cm^{-1}, 1517 cm^{-1}, 1411 cm^{-1}, 1282 cm^{-1} and 1213 cm^{-1} corresponded to the presence of C=O, C-N, C=O, C-N amide III, respectively (**Figure 3a**). Bands formed at 1116 cm^{-1}, 1066 cm^{-1}, 985 cm^{-1}, 673 cm^{-1} and 597 cm^{-1} confirmed the presence of C-O-C, C=N and C-H groups.

FTIR analysis of AgNPs synthesized by leaf extract of Mangifera indica L. cv. Malta

Silver nanoparticles biosynthesized with the help of leaf extract of cv. Malta showed different bands recorded through FTIR spectroscopy. Bands at 3738 cm^{-1} and 3417 cm^{-1} showed presence of O-H group (**Figure 3b**). Bands at 2891 cm^{-1}, 2318 cm^{-1}, and 2071 cm^{-1} corresponded to presence of C=C conjugated bonds. The bands at 1629 cm^{-1}, 1517cm^{-1}, 1411 cm^{-1}, 1282 cm^{-1} and 1213 cm^{-1} corresponded to presence of C=O, C-N, C=O, C-N amide III, respectively. Bands formed at 1116 cm^{-1}, 1066 cm^{-1}, 985 cm^{-1}, 675 cm^{-1} and 597 cm^{-1} and 555 cm^{-1} confirmed the presence of C-O-C, C-O, C=C, C=N and C-H groups (**Figure 3b**).

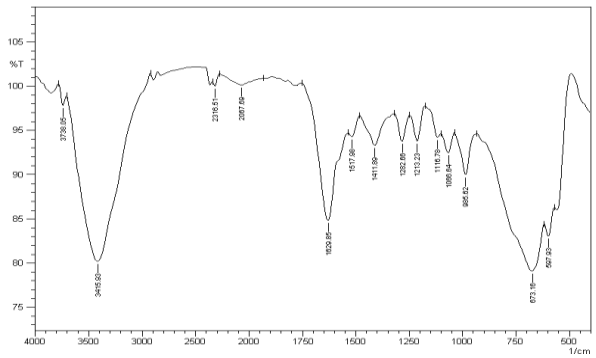

Figure 3a. Fourier transform infrared spectroscopy of AgNPs synthesized using *Mangifera indica* L. cv. Fajri leaf extract

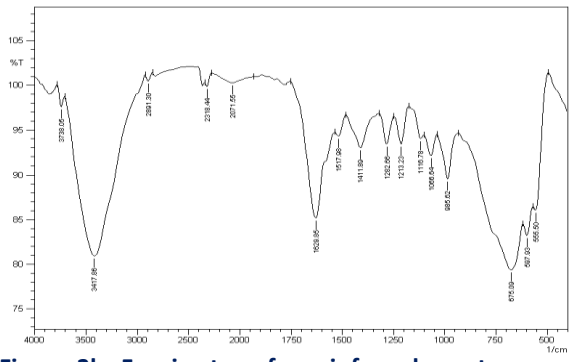

Figure 3b. Fourier transform infrared spectroscopy of AgNPs synthesized using *Mangifera indica* L. cv. Malta leaf extract

FTIR analysis of AgNPs synthesized by leaf extract of Mangifera indica L. cv. Sinduri

Silver nanoparticles biosynthesized with the help of leaf extract of cv. Sinduri showed different bands recorded through FTIR spectroscopy (**Figure 3c**). Bands at 3738 cm^{-1} and 3415 cm^{-1} showed the presence of O-H group. Bands at 2891 cm^{-1}, 2361 cm^{-1}, and 2067 cm^{-1} corresponded to the presence of C=C conjugated bonds. The bands at 1627 cm^{-1}, 1517cm^{-1}, 1411 cm^{-1}, 1282 cm^{-1} and 1213 cm^{-1} corresponded to the presence of C=O, C-N, C=O, C-N amide III, respectively. Bands formed at 1066 cm^{-1}, 985 cm^{-1}, 675 cm^{-1} and 597 cm^{-1} confirmed the presence of C-O-C, C=N and C-H groups (**Figure 3c**).

FTIR analysis of AgNPs synthesized by leaf extract of Mangifera indica L. cv. Sufaid Chaunsa

Silver nanoparticles biosynthesized using leaf extract of cv. Sufaid Chaunsa showed different bands of FTIR spectroscopy (**Figure 3d**). Bands at 3738 cm^{-1} and 3415 cm^{-1} showed the presence of O-H group, whereas bands at 2893 cm^{-1} and 2069 cm^{-1} corresponded to the presence of C=C and C≡C conjugated bonds. The bands at 1627 cm^{-1}, 1517 cm^{-1}, 1409 cm^{-1}, 1282 cm^{-1} and 1211 cm^{-1} corresponded to the presence of C=O, C-N, C=O, C-N amide III, respectively (**Figure 3d**). Bands formed at 1116 cm^{-1}, 1066 cm^{-1}, 985 cm^{-1}, 673 cm^{-1} and 597 cm^{-1} confirmed the presence of C-O-C, C=N and C-H groups.

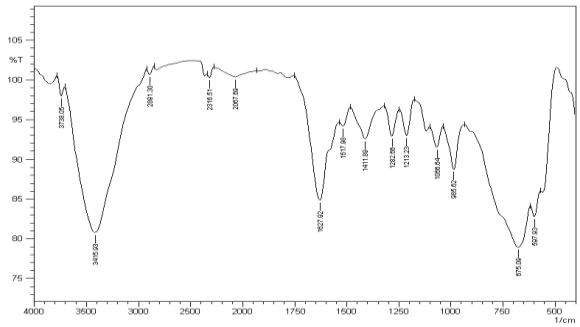

Figure 3c. Fourier transform infrared spectroscopy of AgNPs synthesized using *Mangifera indica* L. cv. Sinduri leaf extract

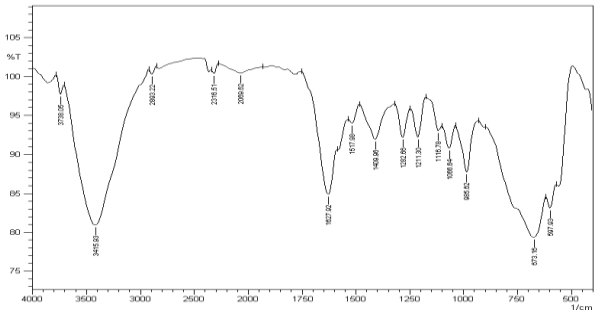

Figure 3d. Fourier transform infrared spectroscopy of AgNPs synthesized using *Mangifera indica* L. cv. Sufaid Chaunsa leaf extract

FTIR analysis of AgNPs synthesized by leaf extract of Mangifera indica L. cv. Langra

Silver nanoparticles biosynthesized using cv. Langra showed different bands of FTIR spectroscopy (**Figure 3e**). Bands at 3738 cm^{-1} and 3417 cm^{-1} showed the presence of O-H group, whereas those at 2891 cm^{-1}, 2314 cm^{-1} and 2071 cm^{-1} corresponded to the presence of C=C and C≡C conjugated bonds. The bands at 1629 cm^{-1}, 1517cm^{-1}, 1411 cm^{-1}, 1282 cm^{-1} and 1213 cm^{-1} corresponded to the presence of C=O, C-N, C=O, C-N amide III, respectively (**Figure 3e**). However, bands formed at 1116 cm^{-1}, 1066 cm^{-1}, 985 cm^{-1}, 675 cm^{-1} and 597 cm^{-1} and 432 cm^{-1} confirmed the presence of C-O-C, C-O, C=C, C=N and C-H groups.

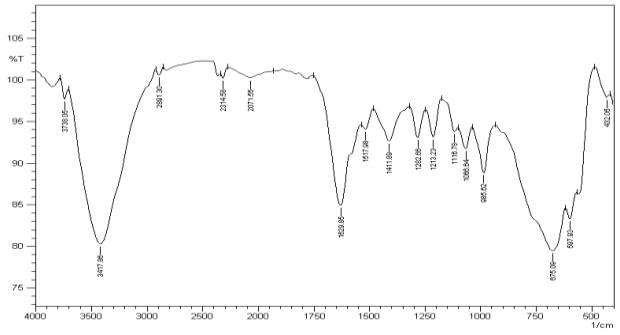

Figure 3e. Fourier transform infrared spectroscopy of AgNPs synthesized using *Mangifera indica* L. cv. Langra leaf extract

Scanning electron microscopy (SEM) and EDX analysis

Scanning electron microscopy analysis was done to identify the shape and size of the formed silver nanoparticles. Moreover, EDX analysis was also performed to indicate whether or not the silver nanoparticles were present in the sample.

SEM and EDX analysis of Mangifera indica L. cv. Fajri synthesized AgNPs

The SEM analysis was done to study the morphology of the formed silver nanoparticles. The size of the formed silver nanoparticles was measured with the help of ImageJ software. The nanoparticles were uniformly distributed and were present in the form of clusters in some places (**Figure 4a**). The shape of the nanoparticles was hexagonal and spherical. The sizes of cv. Fajri-mediated silver nanoparticles were in the range of 12-99 nm. Moreover, the EDX analysis was performed to observe the presence of silver nanoparticles in the sample. In the EDX analysis, two peaks were observed which showed that AgNPs were present in the sample along with some oxygen atoms (**Figure 4b**).

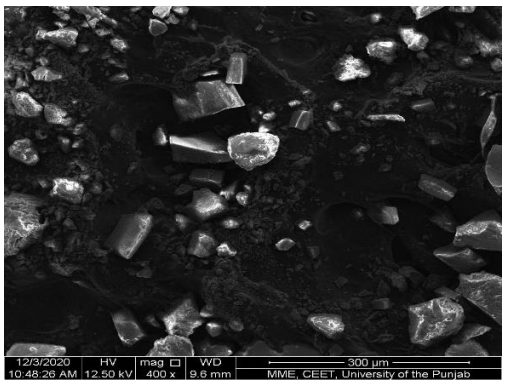

Figure 4a. Scanning electron microscopy analysis of *Mangifera indica* L. cv. Fajri mediated AgNPs

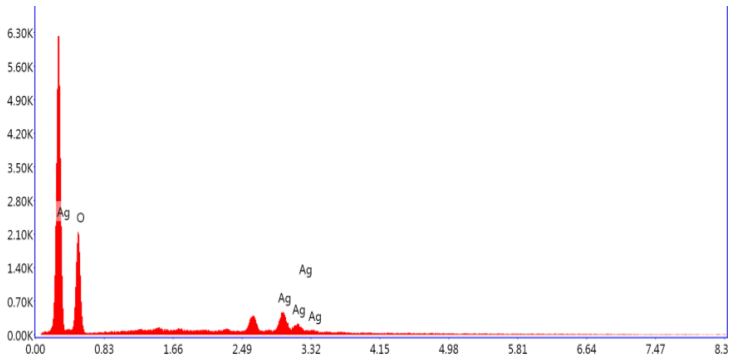

Figure 4b. EDX analysis of *Mangifera indica* L. cv. Fajri mediated AgNPs

SEM and EDX analysis of Mangifera indica L. cv. Malta synthesized AgNPs

The SEM analysis of AgNPs formed by the leaf extract of cv. Malta showed that the nanoparticles were irregular in shape (**Figure 4c**). The shape of the nanoparticles was triangular, hexagonal, and somewhat spherical. The size of the nanoparticles was measured using ImageJ software which was 15-137 nm. The EDX analysis was also performed to observe the presence of AgNPs in the sample. The EDX graph showed different peaks indicating the presence of silver nanoparticles (**Figure 4d**).

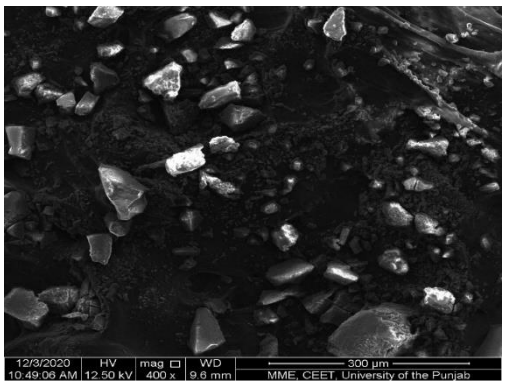

Figure 4c. Scanning electron microscopy analysis of *Mangifera indica* L. cv. Malta mediated AgNPs

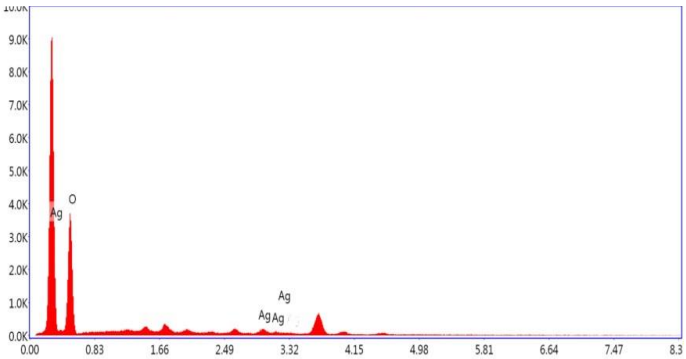

Figure 4d. EDX analysis of *Mangifera indica* L. cv. Malta mediated AgNPs

SEM and EDX analysis of Mangifera indica L. cv. Sinduri synthesized AgNPs

The silver nanoparticles formed by the leaf extract of cv. Sinduri were also analyzed by SEM (**Figure 4e**). The analysis showed that nanoparticles were uniformly distributed having a size of about 16-235 nm. The results showed that the shape of nanoparticles was spherical. The EDX analysis was also performed which showed different peaks. In the energy dispersive X-ray analysis, the presence of AgNPs was confirmed in the sample (**Figure 4f**).

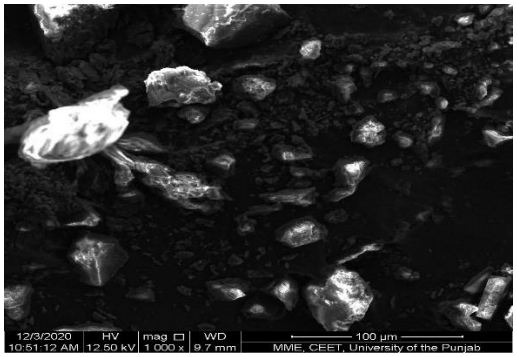

Figure 4e. Scanning electron microscopy analysis of *Mangifera indica* L. cv. Sinduri mediated AgNPs

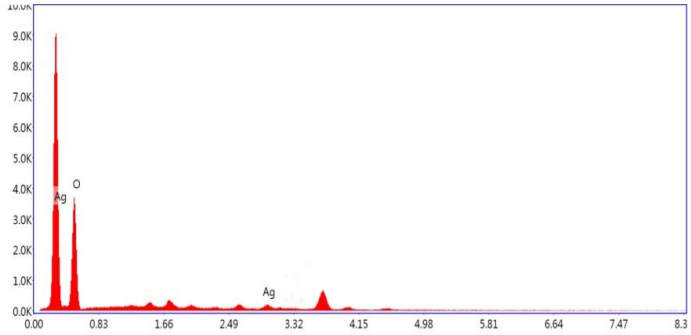

Figure 4f. EDX analysis of *Mangifera indica* L. cv. Sinduri mediated AgNPs

SEM and EDX analysis of Mangifera indica L. cv. Sufaid Chaunsa synthesized AgNPs

The SEM analysis showed the morphology of the AgNPs formed using the leaf extract of cv. Sufaid Chaunsa (**Figure 4g**). The SEM analysis showed that nanoparticles were present in the form of clusters and were uniformly arranged. However, the shape of nanoparticles was found to be hexagonal. The size of the nanoparticles was measured using the ImageJ software which was 15-130 nm. The EDX analysis was also performed which indicated the presence of AgNPs in the sample (**Figure 4h**).

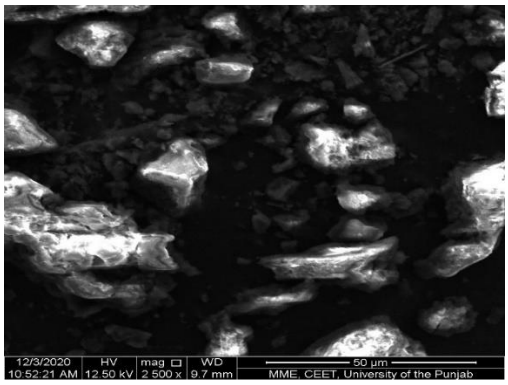

Figure 4g: Scanning electron microscopy analysis of *Mangifera indica* L. cv. Sufaid Chaunsa mediated AgNPs

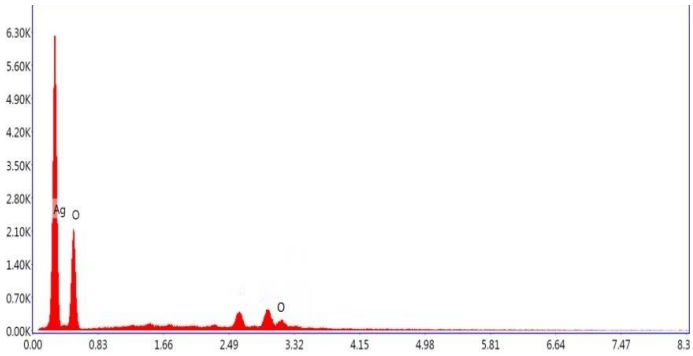

Figure 4h: EDX analysis of *Mangifera indica* L. cv. Sufaid Chaunsa mediated AgNPs

SEM and EDX analysis of Mangifera indica L. cv. Langra synthesized AgNPs

The AgNPs formed using the leaf extract of *Mangifera indica* L. cv. Langra was also analyzed using SEM and found that they were arranged uniformly, but were in dispersed form (**Figure 4i**). The size of the formed nanoparticles was 13-136.7 nm and the shape of the nanoparticles was observed to be triangular and somewhat spherical. The EDX analysis was also performed to observe if AgNPs were present in the sample. The energy dispersive X-ray analysis confirmed the presence of the silver nanoparticles in the sample (**Figure 4j**). The antifungal activity of different varieties of *Mangifera indica* L. was observed at varying concentrations of AgNPs such as 20, 40, 60, 80 and 100 mg/L.

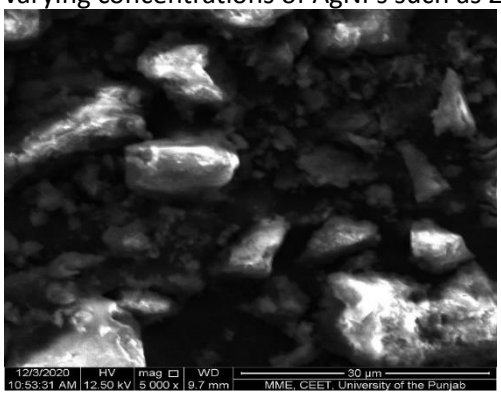

Figure 4i. Scanning electron microscopy analysis of *Mangifera indica* L. cv. Langra mediated AgNPs

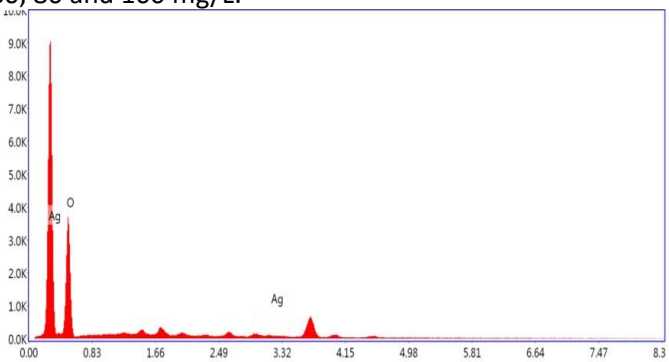

Figure 4j. EDX analysis of *Mangifera indica* L. cv. Langra mediated AgNPs

Antifungal activity of *Mangifera indica* L. synthesized AgNPs against *Aspergillus niger* Tiegh.

Antifungal activity of Mangifera indica L. cv. Fajri synthesized AgNPs

The inhibition effect of AgNPs synthesized by *Mangifera indica* L. cv. Fajri leaf extract was recorded at all concentrations of AgNPs in the MEA media (**Figure 5a**). However, the inhibition rate of fungal growth was highest in 100 mg/L of AgNPs being 79.8%. The inhibition rate of fungal growth was also observed by treating it with silver nitrate reagent which showed 13.9% inhibition rate of fungal growth.

Antifungal activity of Mangifera indica L. cv. Malta synthesized AgNPs

The cv. Malta-synthesized AgNPs showed the highest inhibition rate of 78.3% at 100 mg/L of AgNPs (**Figure 5b**). It showed a 15.6% inhibition rate at 20 mg/L and 22.2% inhibition rate of fungal growth at 40 mg/L of AgNPs. Similarly, at 60 and 80 mg/L it showed an inhibition rate of 42.8% and 58.8%, respectively, against *Aspergillus niger* Tiegh.

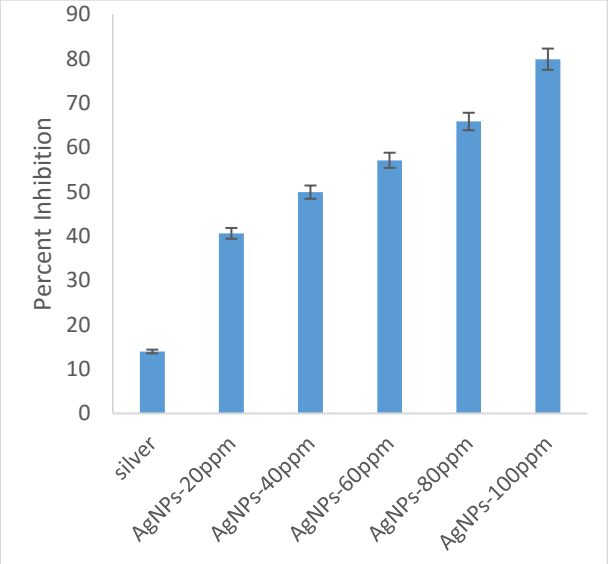

Figure 5a. Effect of different concentrations of *Mangifera indica* L. cv. Fajri mediated synthesized silver nanoparticles on the growth of *Aspergillus niger* Tiegh.

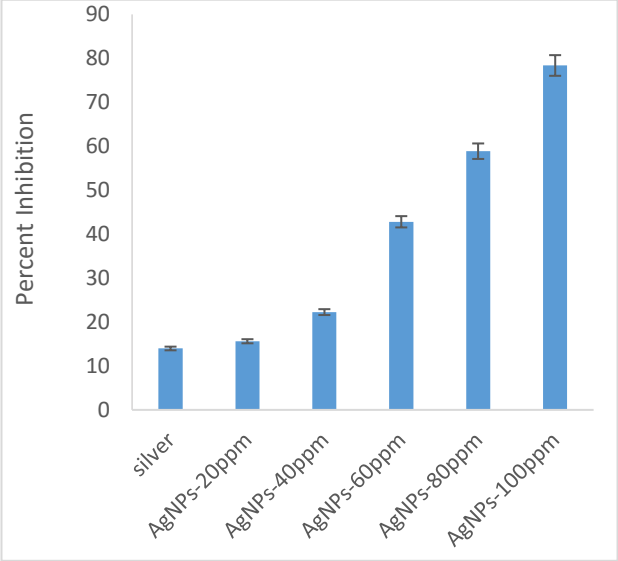

Figure 5b. Effect of different concentrations of *Mangifera indica* L. cv. Malta mediated synthesized silver nanoparticles on the growth of *Aspergillus niger* Tiegh.

Antifungal activity of Mangifera indica L. cv. Sinduri synthesized AgNPs

The Petri plates containing MEA media were treated with different concentrations (20, 40, 60, 80, and 100 mg/L) of AgNPs solution (**Figure 5c**). Cultivar Sinduri synthesized AgNPs showed an inhibition rate of 36.0% at 20 mg/L. Similarly, at concentrations of 40, 60, and 80 mg/L, it showed 45.2%, 46.2%, and 59.4% inhibition rates, respectively. Like cvs. Fajri and Malta, cv. Sinduri also showed the highest inhibition rate of 76.8% at 100 mg/L of AgNPs.

Antifungal activity of Mangifera indica L. cv. Sufaid Chaunsa

An inhibition rate of 38.0% was shown by cv. Sufaid Chuansa synthesized AgNPs against *Aspergillus niger* at 20 mg/L of AgNPs (**Figure 5d**). The inhibition rate was observed as 72% at 100 mg/L of AgNPs. However, at lower levels, i.e., 40, 60, and 80 mg/L, the inhibition rates of 49.6%, 60.4%, and 67.3% were observed, respectively. Like all the above varieties of *Mangifera indica* L., cv. Sufaid Chaunsa also showed the highest inhibition rate of fungal growth at 100 mg/L.

Antifungal activity of Mangifera indica L. cv. Langra synthesized AgNPs

The highest inhibition rate (74.4%) was recorded by cv. Langra at 100 mg/L of AgNPs., whereas at the lowest level (20 mg/L), the inhibition rate was recorded to be 15.6% (**Figure 5e**). Similarly, the inhibition rates of 33.5%, 55.8%, and 57.1% against *Aspergillus niger* were recorded at 40, 60, and 80 mg/L, respectively. The inhibition rate by treating it with silver nitrate reagent was also observed which was 13.9% (**Figure 5e**).

Effect of leaf extract of different varieties of Mangifera indica L. synthesized AgNPs on the growth of Aspergillus niger Tiegh.

All the varieties of *Mangifera indica* L. showed the highest inhibition rate of fungal growth at 100 mg/L of AgNPs (**Figure 6**). Of all five mango varieties, cv. Fajri showed the highest inhibition rate (79.8%) against *Aspergillus niger*.

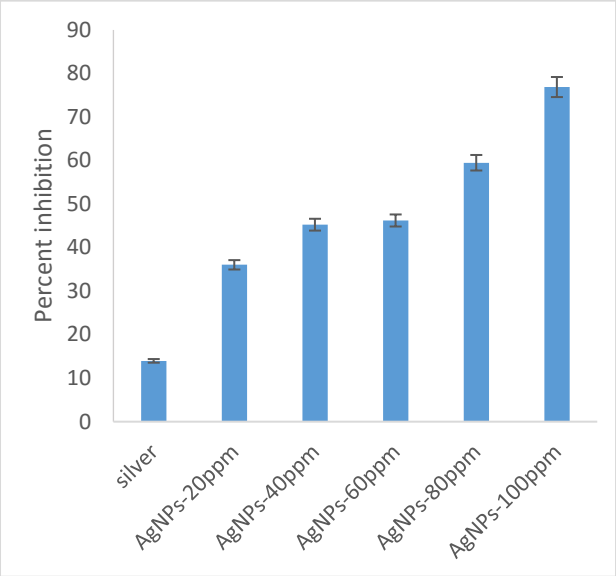

Figure 5c. Effect of different concentrations of *Mangifera indica* L. cv. Sinduri mediated synthesized silver nanoparticles on the growth of *Aspergillus niger* Tiegh.

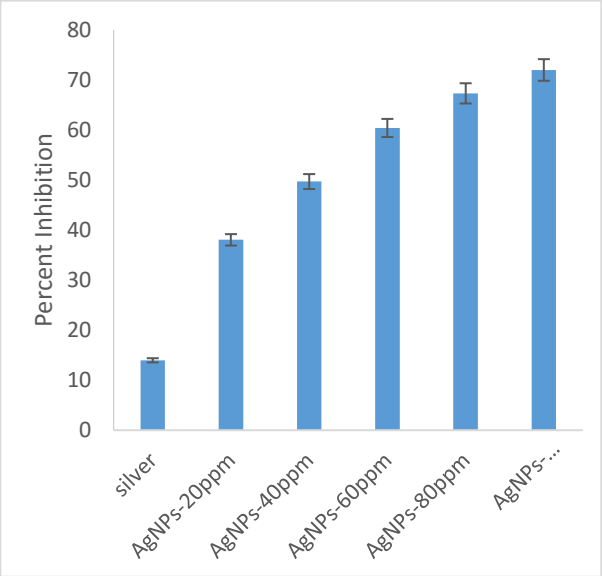

Figure 5d. Effect of different concentrations of *Mangifera indica* L. cv. Sufaid Chaunsa mediated synthesized silver nanoparticles on the growth of *Aspergillus niger* Tiegh.

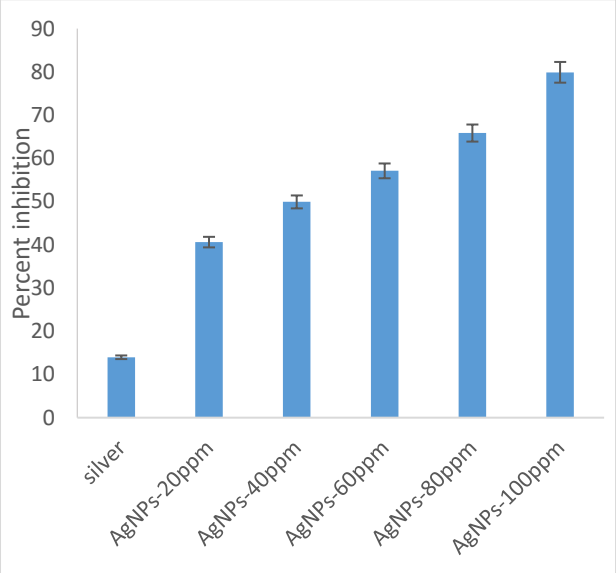

Figure 5e. Effect of different concentrations of *Mangifera indica* L. cv. Langra mediated synthesized silver nanoparticles on the growth of *Aspergillus niger* Tiegh.

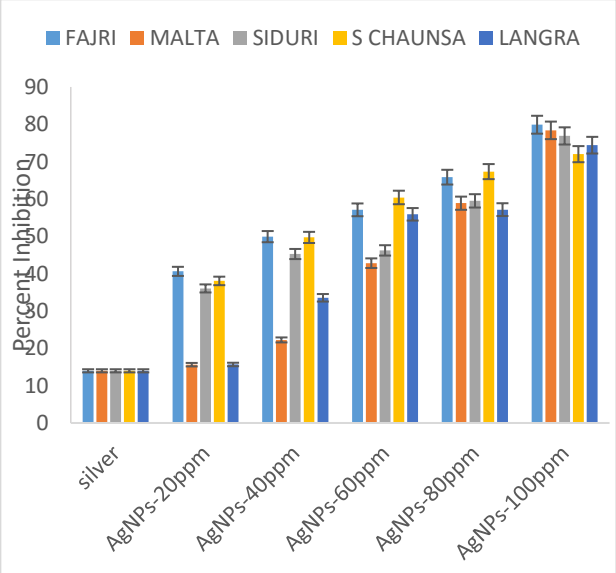

Figure 6. Effect of different concentrations of *Mangifera indica* mediated synthesized silver nanoparticles on the growth of *Aspergillus niger* Tiegh.

Discussion

Although silver nanoparticles have many uses, they play an important role as antifungal and antibacterial agents. Nowadays green synthesis of silver nanoparticles has become very common, because it is a very convenient method for the synthesis of AgNPs, being cost-effective and eco-friendly (Hashem et al., 2022).

After the biosynthesis of silver nanoparticles, the nanoparticles were characterized with the help of different techniques such as UV-Vis spectroscopy, FTIR, and SEM. In UV-Vis spectroscopy, AgNPs synthesized by different varieties of *Mangifera indica* L. showed different absorbance peaks. Cultivar Fajri showed the highest peak at 448 nm. Similarly, AgNPs synthesized by the leaf extract of cv. Malta showed the same peak at 448 nm. AgNPs synthesized by cvs. Sinduri, Sufaid Chaunsa, and Langra showed peaks at 452, 454, and 446 nm, respectively. Other scientists also reported that silver nanoparticles show resonance peaks within 400-460 nm (Sarsar et al., 2013). Ahmed et al. (2016) reported that silver nanoparticles showed a surface plasmon resonance band in the range of 448-460 nm. In another study, the color change of the extract after the reaction was ascribed to surface plasmon resonance and it was

also reported that the silver nanoparticles mainly showed the surface plasmon resonance band in the range of 446-448 nm (Banerjee et al., 2014).

Biosynthesized nanoparticles were also characterized using SEM. The AgNPs formed by different mango varieties had different shapes and sizes. The SEM analysis showed that silver nanoparticles synthesized by the leaf extract of cv. Fajri has a size range between 12 and 99 nm. Similarly, other four varieties, i.e., Malta, Sinduri, Sufaid Chaunsa, and Langra had size ranges of 15-137 nm, 16-235 nm, 15-130 nm, and 13-136.7 nm, respectively. In another similar study, the size of the silver nanoparticles was documented in the range of 60-70 nm (Maria et al., 2015). Using a different plant source such as *Aloe vera* the size of AgNPs was recorded within the range of 287.5-293.2 nm, but the average size of an individual nanoparticle was recorded up to 70 nm (Medda et al., 2015). In another study, the shape of silver nanoparticles was documented to be spherical (Sathishkumar et al., 2012). In contrast, in another study, the shape of the nanoparticles was reported to be triangular, cubical, and spherical, and all distributed uniformly. It was also observed that in some places they were present in the form of clusters (Nethradevi et al., 2012). All the varieties showed best results at 100 mg/L of AgNPs, whereas a minimal effect was recorded at 20 mg/L of AgNPs.

Silver nanoparticles are also believed to play an effective role as antifungal. For example, in the current study, it was found that varying levels of AgNPs had considerable antifungal effect. In another study, silver nanoparticles at varying concentrations such as 10, 25, 50, and 100 mg/L played an effective role in counteracting *Monosporascus cannonballus* (a thermophilic fungus), and it was observed that 100 mg/L of silver nanoparticles had a great inhibitory effect of almost 90% against the fungal pathogen as compared to the other concentrations tested (Kim et al., 2012). Hashem et al. (2022), reported that the biosynthesized AgNPs at 500 µg/mL showed a remarkable antifungal activity against four most commonly occurring *Aspergillus* species viz. *A. niger*, *A. terreus*, *A. flavus*, and *A. fumigatus.* The inhibition zones of the four species were reported to be 16, 20, 26, and 19 mm, respectively. Similarly, AgNPs (2000 mg/L) biosynthesized using the leaf extract of *Azadirachta indica* displayed complete inhibition against the most common fungi *Sclerotinia sclerotiorum* and *Colletotrichum falcatum* and 68% to 80% inhibition against *Colletotrichum gloeosporioides* and *Rhizoctonia solani,* respectively (Singh et al. 2024). Some scientists are also of the view that silver nanoparticles (AgNPs) developed through biological means could be effectively exploited for biological and medical applications (Gibala et al., 2021; Restrepo Burgos et al., 2023).

Conclusion

From the present study, it was concluded that the biosynthesis of AgNPs from the leaf extracts of different varieties of *Mangifera indica* proved to be beneficial. The biosynthesis of AgNPs was confirmed by different characterization techniques including UV-Vis spectroscopy, FTIR, and SEM. The biosynthesized AgNPs so developed exhibited considerable antifungal activity against *Aspergillus niger*. The AgNPs formed using the mango cultivar cv. Fajri proved to have the best antifungal activities with an inhibition rate of 79.8% at the concentration of 100 mg/L against *Aspergillus niger*. Researchers can further harness the potential of *Mangifera indica* as a sustainable and effective source for synthesizing AgNPs with diverse applications in biomedicine, agriculture, and environmental remediation.

Author(s), Editor(s) and Publisher's declarations

Conflict of interest
The authors declare no conflict of interest.

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Contribution of authors
Conceptualization and designing the study: SA; Conduction of experiment: AM.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent

All authors have critically read this manuscript and agreed to publish in IJAaEB.

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It is declared that we the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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