Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XXIV, Issue 1. 2025, pag. 33 - 44

FOOD AND ENVIRONMENT SAFETY

EVALUATING THE POTENCY OF *NIGELLA SATIVA*-MEDIATED SILVER NANOPARTICLES AGAINST AFLATOXIGENIC FUNGI IN PLANTING SOIL

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Received 14 October 2024, accepted 31 March 2025

Abstract: *This study investigates the antifungal properties of silver nanoparticles (AgNPs) biosynthesized using Nigella sativa (black seed) extract against aflatoxigenic fungal species isolated from soil samples. The synthesis of AgNPs was confirmed through UV-Vis spectroscopy, and their functional groups were characterized using Fourier transform infrared spectroscopy (FTIR). The antifungal activity of the AgNPs was assessed against several aflatoxigenic fungi, including Aspergillus neoniger, Aspergillus niger, terreus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus, and Aspergillus nidulans, employing the agar well diffusion method. Results demonstrated that the AgNPs exhibited significant inhibitory effects on the growth of all tested fungal strains, including both aflatoxigenic and non-aflatoxigenic species, with a pronounced fungicidal effect. In comparison, fungal isolates were also evaluated against conventional antifungal agents, showing varied inhibition zones. This study highlights the potential of black seed extract-mediated AgNPs as a promising, eco-friendly, and efficient alternative to traditional antifungal treatments.*

Keywords: *Food safety, Fungi, Nanotechnology, Silver Nanoparticles (AgNPs), Aflatoxins*

1. Introduction

Fungal contamination, particularly by aflatoxigenic species of *Aspergillus*, presents a significant challenge to agricultural productivity, food safety, and public health worldwide. Among the most concerning fungal contaminants are *Aspergillus flavus* and *Aspergillus parasiticus*, which are capable of producing aflatoxins. These highly toxic and carcinogenic mycotoxins pose severe health risks to humans and animals [1, 2]. Aflatoxins can contaminate crops such as maize, peanuts, and grains under warm and humid conditions, often during storage or post-harvest processing [3]. Consumption of foods contaminated with these mycotoxins has been linked to acute poisoning, liver damage, immunosuppression, and an increased risk of cancer [1]. As a result, the presence of aflatoxins in soil and agricultural products impacts public health and imposes substantial economic losses, undermining food security and agricultural sustainability [4]. Antifungal treatments, such as synthetic fungicides, have been widely used to control fungal growth and aflatoxin production. However, these approaches often come with significant drawbacks, including environmental pollution, high costs, and the development of fungal resistance [4]. Moreover, the excessive use of chemical antifungals can accumulate toxic residues in the environment and on crops, further exacerbating food safety concerns [5]. These challenges drive the urgent need to develop safer, more effective, and sustainable antifungal strategies. A promising alternative is the use of nanotechnology, particularly silver nanoparticles (AgNPs), which have shown remarkable antimicrobial properties, including antifungal activity against a range of fungal species, including aflatoxigenic fungi. AgNPs, typically ranging from 1 to

DOI: https://doi.org/10.4316/fens.2025.004

100 nanometers in size, possess unique physicochemical properties that differentiate them from bulk silver, such as increased surface area, enhanced reactivity, and the ability to penetrate microbial cell walls [6]. Their small size and large surface area allow AgNPs to interact effectively with fungal cells, disrupting cellular functions and inhibiting growth [7]. In addition, AgNPs are less prone to inducing resistance in microorganisms compared to traditional antifungal agents, making them a highly attractive option for long-term use [8]. The antimicrobial potential of AgNPs has spurred interest in their application for controlling fungal diseases in agriculture [9]. Several studies have reported the effectiveness of AgNPs against various fungal pathogens, including those responsible for aflatoxin production [10, 11]. Furthermore, AgNPs synthesized through green methods, using plant extracts, offer additional advantages such as eco-friendliness and biocompatibility. Green synthesis of AgNPs avoids the use of toxic chemicals, reducing environmental and health risks associated with traditional chemical synthesis methods [12].

Among the plants used in green synthesis, *Nigella sativa* (black seed) has emerged as a promising source due to its rich bioactive compounds and its ability to act as a reducing agent in nanoparticle synthesis. *Nigella sativa* has a long history of use in traditional medicine for treating a variety of ailments, including microbial infections, due to its antimicrobial, anti-inflammatory, and antioxidant properties [13, 14]. The bioactive compounds in black seed, such as thymoquinone, have been shown to possess potent antimicrobial activities, making it an ideal candidate for the biosynthesis of AgNPs [15].

The green synthesis of AgNPs using *Nigella sativa* extracts not only offers an eco-friendly and cost-effective approach but also enhances the biocompatibility and stability of the nanoparticles, which are essential for their application in environmental and agricultural contexts [11].

In recent years, studies have demonstrated the efficacy of black seed-synthesized AgNPs in inhibiting the growth of various fungal species, including *Aspergillus* spp. [16]. However, there is limited research on the potential of *Nigella sativa*-derived AgNPs for controlling aflatoxigenic fungi specifically, especially those isolated from soil samples. Soil-borne fungi, such as *Aspergillus flavus* and *Aspergillus parasiticus*, play a critical role in aflatoxin contamination of crops. Therefore, targeting these fungi in their natural habitat is essential for developing effective mitigation strategies.

This study aims to explore the inhibitory effects of silver nanoparticles biosynthesized using *Nigella sativa* extract on aflatoxigenic fungi isolated from soil samples. By synthesizing AgNPs through a green method, we not only reduce the environmental impact associated with nanoparticle production but also enhance the effectiveness of these nanoparticles in controlling fungal growth and aflatoxin production. We hypothesize that *Nigella sativa*-synthesized AgNPs will exhibit strong antifungal activity against aflatoxigenic fungi, thus offering a sustainable and environmentally friendly alternative to traditional antifungal treatments. This research is expected to contribute to the development of novel, eco-friendly antifungal agents that can be used for soil decontamination and crop protection, ultimately enhancing food safety and agricultural sustainability. Through this approach, we aim to provide a solution to the pressing problem of aflatoxin contamination, highlighting the potential of green-synthesized AgNPs as a potent and sustainable strategy for mitigating fungal pathogens in agricultural settings.

2. Materials and methods

2.1. Collection of samples

Soil samples of 20 g each from five farming locations in the Federal University of Agriculture Abeokuta and its Environs were collected by digging the earth's surface to 12 cm deep. Soil samples below 12 cm were obtained and aseptically transported to the laboratory.

200 g of black seeds were purchased from a local store in Abeokuta, Nigeria. They were packaged in a sterile zip-lock bag, labelled, and transported to the laboratory for immediate analysis.

2.2. Formulation of methyl red desiccated coconut agar

The method [17] was used in the preparation of (MRDCA). 100 g of desiccated coconut was soaked in 500 mL of 100 °C distilled water for 30 minutes (pH 4.77), blended aseptically for 5 minutes and filtered using cheesecloth. 6 g of agar was added to the mixture and 0.04 g of methyl red was also added.

The media was then sterilized at 121 °C for 15 minutes, cooled and poured uniformly (20 mL) into sterile Petri dishes (8.5 cm) while vigorously stirring with a sterile hockey stick. Care was taken to avoid trapping bubbles in the media.

2.3. Preparation of media

All media was prepared according to the manufacturer's instructions. Methyl red desiccated coconut agar, yeast extract agar and 11.7 g potato dextrose agar were weighed and suspended into a conical flask containing 300 mL of distilled water, this was swirled gently to achieve homogeneity, covered with cotton wool and aluminum foil, then placed in an autoclave for sterilization at 121 °C for 15 psi and then allowed to cool.

2.4. Isolation of microorganisms

Microorganisms were isolated by serial dilution where 1 g of the sample was added to 9 mL of sterile distilled water and properly mixed to make a stock solution, and then labeled accordingly. 1mL of each stock solution was then pipetted into test tubes containing 9 mL of distilled water and a 10-fold serial dilution up to 10^{-5} was done. 0.5 mL from the 10^{-2} and 10^{-4} dilution was withdrawn and dispensed into the sterile petri dish which has been properly labeled, 20 mL of freshly prepared potato dextrose agar cooled to 50 °C was poured into each plate, mixed thoroughly and then allowed to solidify. The plates were kept at room temperature for up to 72 hours.

2.5. Characterization and identification of fungal isolates

Distinct colonies that developed on the plates were grouped based on their cultural characteristics and then sub-cultured onto fresh potato dextrose agar plates to obtain a pure culture. The plates were kept at room temperature for up to 72 hours. The morphological characterization of fungi isolates was based on macroscopic examination: colony structure, color, texture and pigment. Identification of fungi depends largely on morphological characteristics such as the type and arrangement of spores produced, as well as the mycelia type. The isolates were assigned to tentative identity using taxonomic description. Pure colonies were identified by further mounting on slides and stained with lacto phenol cotton blue stain. The slides were then observed under the microscope (AmScope T490B) using x40 and x10 objective lenses.

2.6. Toxigenicity test of fungal isolates

The isolates were sub-cultured using sterile forceps on Methyl red desiccated coconut agar medium and incubated in a dark cupboard at room temperature for three days [17]. After incubation, the growth on the plates was exposed to ultraviolet light for 3 hours at 365 nm to observe the production of fluorescence by the isolates.

The isolates that fluorescence was considered toxigenic [21], the organisms were preserved using yeast broth for further analysis.

2.7. Extraction procedure of black seed (*Nigella Sativa)*

The black seeds were washed and rinsed to remove dirt, dust and the seeds were oven dried at 45 °C for 3 hours. The dried seeds are ground into a fine powder using a sterile grinder (this increases the surface area and facilitates better extraction of active compounds). Twenty-five grams (25 g) of black seed was added to 500 mL of distilled water in a sterile conical flask covered with cotton wool, wrapped with aluminium foil, and shaken vigorously. The mixture was then placed in a water bath at 55 °C for 25 minutes for heating and proper homogenization, the mixture was kept in a cupboard overnight for 12 hours for better soaking.

The mixture was filtered using What's man No. 1 filter paper to remove the solid particles and impurities, the filtrate was stored at 4 °C for preservation.

2.8. Preparation of 1mM concentration of silver nitrate

Making a silver nitrate solution with a concentration of 1 mM. 0.17 g of silver nitrate salt were added to a 1000 mL conical flask holding 1000 mL of distilled water to create a solution with a concentration of about 1 mM for the silver nitrate.

2.9. Synthesis of silver nanoparticles

A flask containing 20 mL of the seed extract was filled to the exact amount of 80 mL with aqueous silver nitrate. After giving it a good shake, this was kept in a dark cabinet for 12 hours. A pale yellow to dark brown color shift in the solution was noticed, indicating AgNP production. After the addition of silver nitrate and the color change, a sonicator, (sonifier SFX150) was used to ensure thorough mixing and dispersion of the extract and silver nitrate. This was done to ensure a more uniform reaction and improved synthesis of silver nanoparticles [22].

2.10. Purification of silver nanoparticles

The synthesized Silver Nanoparticles solution was centrifuged at 4,000 rpm for 15 minutes and the supernatant was discarded. the pellet was freeze-dried using a freeze dryer for about 15 hours at -45 °C and the AgNPs were stored in a clean airtight sterile container for further characterization.

2.11. Characterization of silver nanoparticles

UV-Vis Spectroscopy

UV-Vis Spectroscopy (Ultrospec 7500 UV-Vis) determines how much UV-Vis light is absorbed by the nanoparticles. The surface Plasmon resonance (SPR) peak of silver nanoparticles is a distinctive result of the collective oscillation of conduction electrons in response to light. A sample of the suspension of nanoparticles was examined at various wavelengths, usually between 300 and 700 nm. Silver nanoparticles often exhibit an SPR peak between 400 and 450 nm in length.

The SPR peak's existence and location support the size and form of the nanoparticles and show that the synthesis was effective.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (IRTracer-100) determined how much-infrared light the sample absorbs, giving details on molecular interactions and functional groups. An infrared spectrum analysis was performed on the dried material or suspension of nanoparticles. This defines the interactions between the stabilizing chemicals from the black seed extract and the nanoparticles and the functional groups on their surface.

Using this method, it was feasible to determine which functional groups in the seed extract might be linked to the biomolecules that cause the reduction of

silver ions to AgNPs and the subsequent capping of the resulting particles.
The studies were conducted when the cast sample pellet was placed within the auxiliary sample holder for Fourier transform infrared spectroscopy (FTIR). The spectra of the plant extract and the generated nanoparticles were compared to a reference to determine the functional groups that were present in each sample.

2.12. Inhibitory effects of silver nanoparticles using Agar Well Diffusion method

Using the agar well diffusion method, the antifungal activity of the produced AgNPs was assessed against the toxigenic strains of fungi. The isolates in the yeast extract broth were inoculated into petri dishes using the pour plate method, freshly prepared and sterilized potato dextrose agar was used and allowed to solidify.
After solidification, a sterile metal cork-borer was used to puncture the plates (3-4 mm) in diameter and was labeled appropriately, the holes were filled with AgNP solution using concentration (100 μg/mL).

2.13. Antifungal Drugs Used for Control (Ketoconazole and Griseofulvin)

Various concentrations of ketoconazole (5 μg/ml and 15 μg/mL) were introduced into the well and Griseofulvin concentration (25 μg/mL) was also for control studies. Under sterile conditions, all of the plates were incubated for 72 hours at room temperature. After the incubation period, each well's clear zones were noted. The areas where fungal growth has been suppressed were represented by the clear zones. With the use of a sterile measuring ruler the zone of inhibition around the wells was measured in millimeters (mm).

3. Results

A total of ten fungi were isolated from soil samples collected from the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Table 1 presents the morphological and macroscopic characteristics of the fungal isolates, including colony color, elevation, growth patterns, and the reverse side of the agar plate while plates *a.* to *f.* depict the images of the fungal isolates on the agar plates.

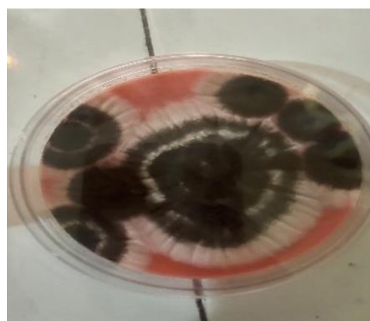
Table 1

Macroscopic characteristics of fungal isolates

ISOLATES	SURFACE	REVERSE SIDE OF THE AGAR	ELEVATION	GROWTH RATE
Aspergillus neoniger	Dark brown to black	Yellow to brown	Umbonate	Rapid
Aspergillus niger	Black	Yellow to brown	Umbonate	Rapid
Aspergillus terreus	Cinnamon Pink	Pale or bright yellow to deep brown	Umbonate	Moderate to rapid
Aspergillus flavus	Greyish green	Colourless to yellow	Umbonate	Moderate to rapid
Aspergillus fumigatus	Blue green	White to tan	Umbonate	Rapid
Penicillium chrysogenum	Dark lemon to green	Yellow white	Flat	Moderate
Aspergillus pseudocaelatus	Light to dark green	Yellowish to brownish	Umbonate	Moderate
Aspergillus parasiticus	Dark green	Brownish	Umbonate	Moderate to rapid
Trichoderma asperelloides	Light to dark green	Pale yellow to colourless	Umbonate	Moderate
Aspergillus nidulans	Light to dark green	Purplish red to brownish with age	Umbonate	Rapid

a. *Aspergillus parasiticus*

b. *Penicilliun Chrysogenum*



c. *Aspergillus neoniger*

d. *Aspergillus niger*

e. *Aspergillus parasiticus*

f. *Aspergillus fumigatus*

Fig. 1. Macroscopic Characteristics of some fungal isolates

Table 2 shows the microscopic characteristics of the fungal isolates which include types of spores, colour of spores, conidiophores and septation.

Table 3 shows the aflatoxin production capacity of the fungal isolates from soil samples. Organisms that exhibited blue or bluish-green fluorescence after being exposed to ultraviolet light at 365 nm for 3 hours were considered mycotoxin producers.

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Table 2

Microscopic characteristics of fungal isolates

ISOLATES	COLOUR OF SPORE	TYPE OF SPORE	SEPTATION	CONIDIA SHAPE
Aspergillus neoniger	Dark brown	Conidiophore	Septate	Spherical
Aspergillus niger	Black	Conidiophore	Septate	Globose to subglobose
Aspergillus terreus	Yellow - brown	Conidiophore	Septate	Globose to subglobose (spherical to slightly oval)
Aspergillus flavus	Greenish yellow	Conidiophore	Septate	Spherical to sub spherical
Aspergillus fumigatus	Blue-green	Conidiophore	Septate	Spherical
Penicillium chrysogenum	Grey-green	Conidiophore	Septate	Spherical to ellipsoidal
Aspergillus pseudocaelatus	Whitish (lacks pigmentation)	Conidiophore	Septate	Spherical to sub spherical
Aspergillus parasiticus	Pink	Conidiophore	Septate	Spherical (nearly round)
Trichoderma asperelloides	Green	Conidiophore	Septate	Oval to ellipsoidal
Aspergillus nidulans	Dark green	Conidiophore	Septate	Round to slightly elliptical

Table 3

Toxigenic and nontoxigenic fungal isolates

ISOLATES	TOXIGENICITY
Aspergillus neoniger	POSITIVE
Aspergillus niger	POSITIVE
Aspergillus terreus	POSITIVE
Aspergillus flavus	POSITIVE
Aspergillus fumigatus	POSITIVE
Penicillium chrysogenum	NEGATIVE
Aspergillus pseudocaelatus	NEGATIVE
Aspergillus parasiticus	POSITIVE
Trichoderma asperelloides	NEGATIVE
Aspergillus nidulans	POSITIVE

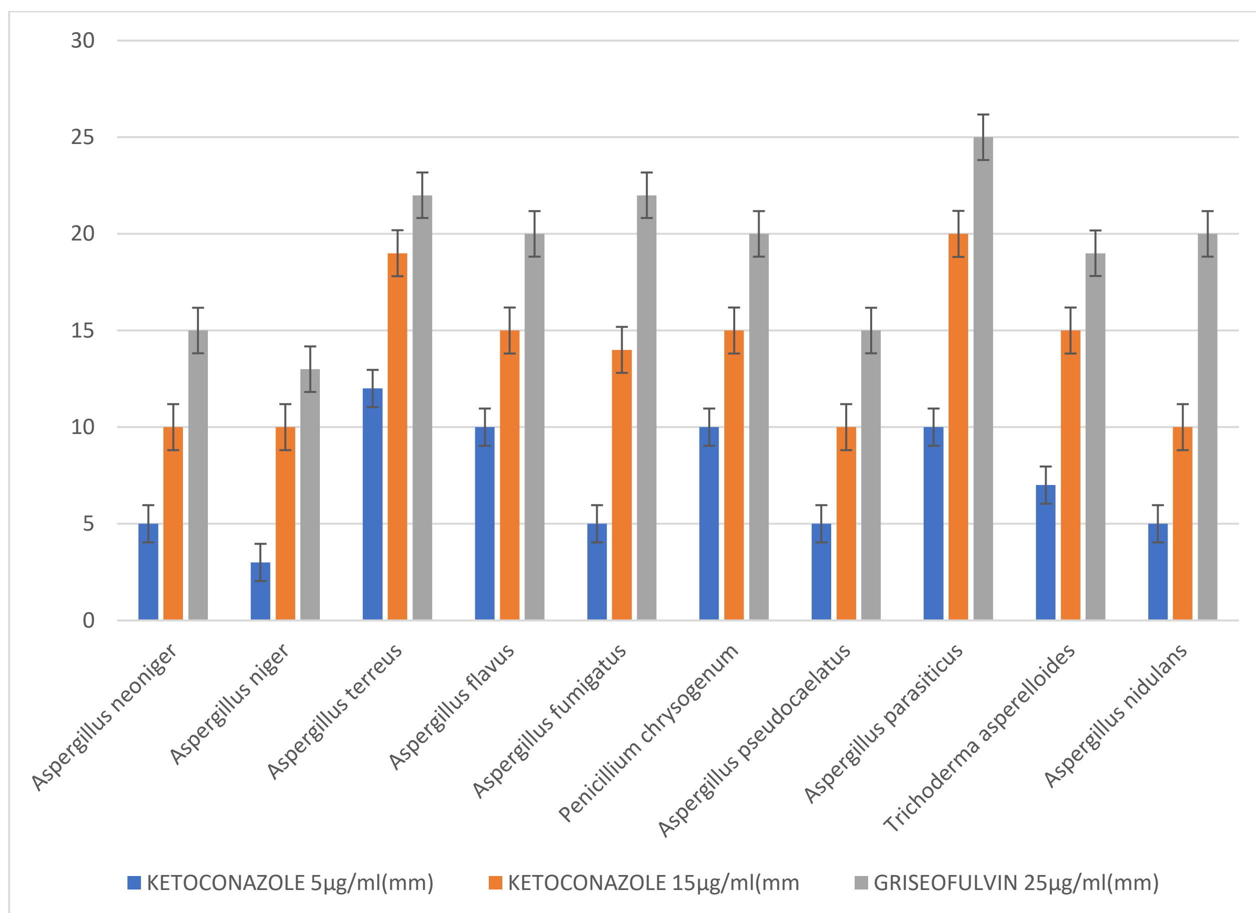

Fig. 2. Zone of inhibition of antifungal drugs (Ketoconazole and Griseofulvin)

Figure 2 shows the zone of inhibition of the growth of toxigenic and non-toxigenic fungi isolated from soil samples (mm) using Antifungal drugs (ketoconazole and Griseofulvin). Plate 1 a and b shows the formation of silver which was visually confirmed by a color change in the mixture from pale yellow to dark brown following the addition of aqueous *Nigella sativa* extract to the $AgN0_3$ solution, accompanied by the settling of a brown precipitate at the bottom, indicating successful nanoparticle synthesis.

a. Black seed extract

b. Formulated AgNPs

Fig. 3. Plate 1- Green synthesis of silver nanoparticles

Characterization of silver nanoparticles
Figure 4 illustrates the characterization of silver nanoparticles using Fourier transform infrared spectroscopy (FTIR), which identifies the functional groups responsible for inhibiting fungal isolates. The results display various peaks corresponding to the functional groups of chemical compounds present in the nanoparticles.

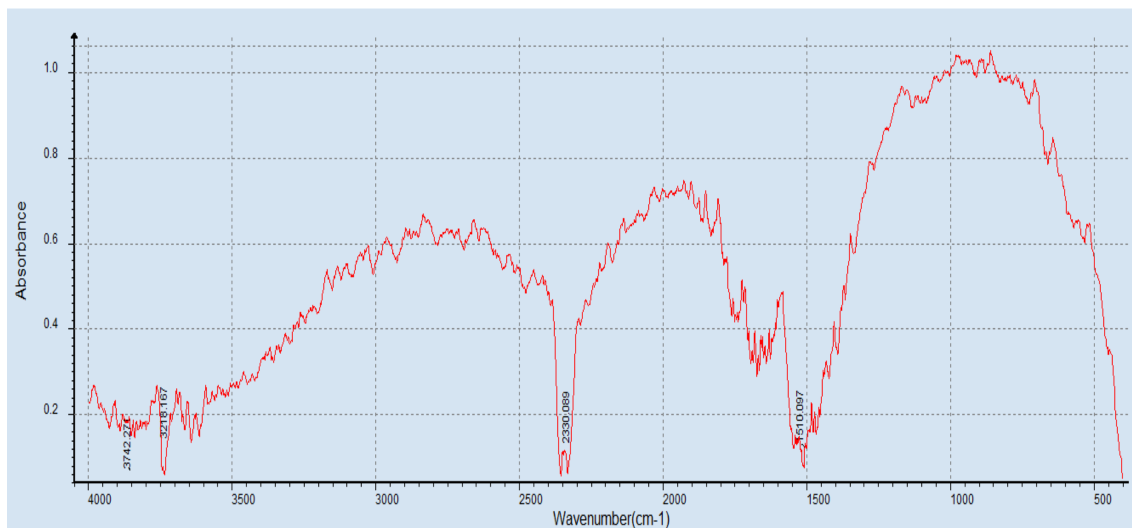

Fig. 4. Characterization of silver nanoparticles by Fourier Transform Infrared Spectroscopy (FTIR)

Figure 5 presents the characterization of silver nanoparticles through UV-Vis spectroscopy, which assesses the absorption of ultraviolet and visible light by a sample at various wavelengths. The results reveal peaks in the UV-Vis spectrum at both 340-370 nm and 400-450 nm, indicating the presence of specific organic compounds and nanoparticles that absorb light within these regions.

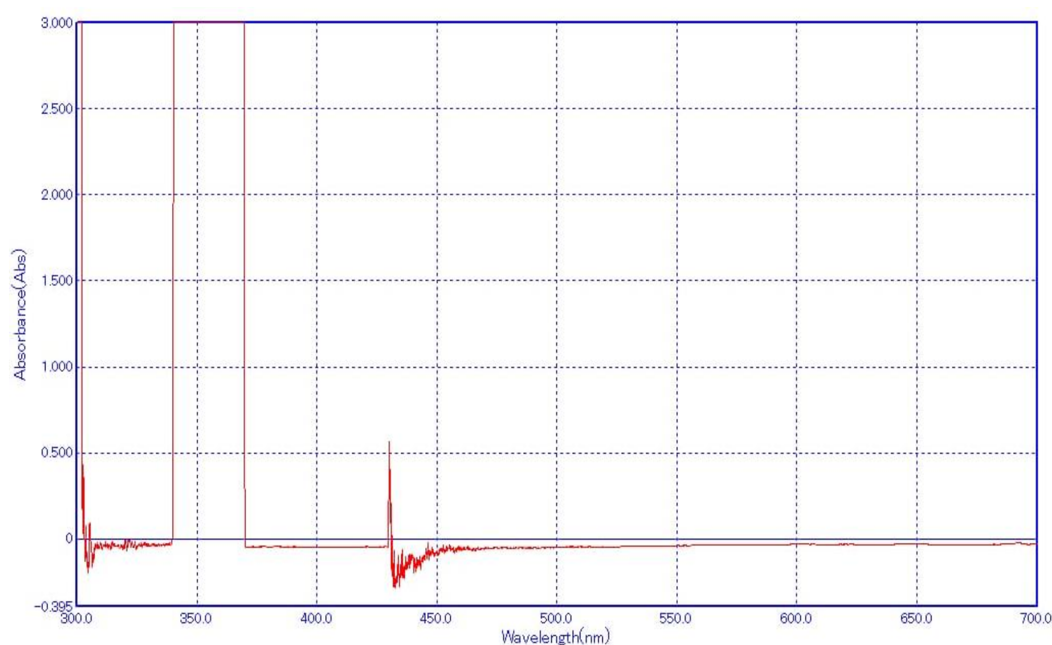

Fig. 5. Characterization of silver nanoparticles by UV-vis Spectroscopy

Table 4 shows the complete susceptibility of fungal isolates to silver nanoparticles photosynthesized from black seed.

Table 4

Treatment with silver nanoparticles photosynthesized from black seed

TREATMENT	RESULTS
100mg/L of AgNPs +*Aspergillus neoniger*	NO GROWTH
100mg/L of AgNPs +*Aspergillus niger*	NO GROWTH
100mg/L of AgNPs +*Aspergillus terreus*	NO GROWTH
100mg/L of AgNPs +*Aspergillus flavus*	NO GROWTH
100mg/L of AgNPs +*Aspergillus fumigatus*	NO GROWTH
100mg/L of AgNPs +*Penicillium chrysogenum*	NO GROWTH
100mg/L of AgNPs +*Aspergillus pseudocaelatus*	NO GROWTH
100mg/L of AgNPs +*Aspergillus parasiticus*	NO GROWTH
100mg/L of AgNPs +*Trichoderma asperelloides*	NO GROWTH
100mg/L of AgNPs +*Aspergillus nidulans*	NO GROWTH

4. Discussion

The findings from this study provide compelling evidence for the antifungal properties of silver nanoparticles (AgNPs) biosynthesized from *Nigella sativa* (black seed) extract. The successful synthesis of AgNPs, confirmed through UV-Vis spectroscopy and characterized via Fourier Transform Infrared Spectroscopy (FTIR), demonstrates a reliable and eco-friendly approach to nanoparticle production. The distinct absorption peaks observed in the UV-Vis spectra suggest effective reduction and stabilization of silver ions, while FTIR results indicate the presence of various functional groups that may enhance the antimicrobial activity of the nanoparticles.

The significant inhibitory effects of the AgNPs on a range of aflatoxigenic fungi, including *Aspergillus flavus*, *A. niger*, and *A. Fumigatus* highlight their potential as a broad-spectrum antifungal agent. The agar well diffusion method employed in this study reveals that AgNPs not only inhibit the growth of aflatoxigenic fungi but also affect non-toxigenic strains, suggesting a versatile mechanism of action. This broad-spectrum efficacy is particularly important in agricultural settings, where contamination by multiple fungal species can compromise food safety and crop yields. In comparing AgNPs to traditional antifungal agents, the study noted varied inhibition zones among the isolates treated with conventional medications. While agents like ketoconazole and griseofulvin are effective against specific fungal infections, their limitations in the spectrum and the potential for resistance development are well-documented [18]. The more consistent inhibition observed with AgNPs suggests that these nanoparticles could serve as a viable alternative or complementary treatment in managing fungal infections, especially in agricultural contexts where resistance is a growing

concern. The biogenic synthesis of AgNPs using black seed extract demonstrates a dual role as both a reducing and capping agent. The phytochemicals present in black seed extract likely enhance the stability and antimicrobial properties of the nanoparticles [19]. The mode of action of AgNPs involves the disruption of fungal cell walls and interference with essential cellular functions, leading to inhibited growth and increased susceptibility of fungal isolates [18].

The application of AgNPs synthesized from black seed extract offers an innovative solution for controlling aflatoxigenic fungi, which are notorious for the health risks associated with food contamination [20]. The eco-friendly nature of biosynthesized AgNPs aligns with sustainable agricultural practices, promoting a shift towards natural and less toxic antifungal alternatives. This is particularly significant in light of increasing consumer demand for organic and chemical-free produce [19]

Moreover, the integration of AgNPs into existing pest management strategies could enhance the overall efficacy of disease control in crops. Their ability to target a wide range of fungal pathogens may reduce reliance on broad-spectrum chemical fungicides, which often have adverse environmental effects and contribute to the decline of beneficial soil microorganisms

Further research is warranted to elucidate the full mechanism of action of AgNPs against fungal pathogens, including long-term effects on fungal populations and soil health. Studies evaluating the efficacy of AgNPs in field conditions will be crucial in assessing their practical applicability in agricultural settings.

5. Conclusion

This study highlights the potential of silver nanoparticles biosynthesized from *Nigella sativa* extract as a promising antifungal agent against aflatoxigenic fungi. The significant antifungal activity, coupled with the eco-friendly synthesis process, positions AgNPs as a valuable tool in the fight against fungal contamination in agriculture. Future research should explore the practical applications of AgNPs in field conditions and their long-term effects on soil health and microbial communities. As the challenges of fungal resistance and environmental sustainability continue to grow, the development and implementation of biogenic nanoparticles may provide a critical solution for ensuring food safety and public health.

6. Acknowledgement

The author acknowledges the input of our erudite Professor, Flora Oluwafemi, on whose back we ride when it comes to research on mycotoxins.

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