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INHIBITORY ACTIVITY OF *CASSIA FISTULA* SYNTHESIZED SELENIUM NANOPARTICLES AGAINST MOULDS OF GROUNDNUT

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ABSTRACT

Mold infestation in oil seeds like groundnuts results in significant quantitative and qualitative losses, typically managed with chemical treatments posing risks to humans, animals, and the environment. Prioritizing effective biological controls with presumed lower risks, this study explored selenium biosynthesized nanoparticles as antifungal agents against *Aspergillus flavus* and *Penicillium* sp. in groundnuts. Standard methods identified the spoilage molds, while *Cassia fistula* leaf extract facilitated the biosynthesis of selenium nanoparticles. Biosynthesized Selenium Nanoparticles (CFSNPs) were characterized using UV-visible spectroscopy, Transmission Electron Microscope (TEM), Fourier Transform Infra-Red (FTIR) spectrometry, and Energy Dispersive X-ray (EDX). The stabilized CFSNPs exhibited a brownish color with a UV-visible absorption peak at 450 nm, indicating SeNP formation. TEM micrographs revealed spherical, cubic, rod, and irregularly shaped CFSNPs with clusters, while EDX spectra showed selenium (Se) as the predominant element (80.76% by weight). FTIR peaks at 4400, 3173.00, 2361.44, 1594.00, 1373.00, 1027.29, and 674.23 cm-1 confirmed characteristic vibrations. Antifungal assays demonstrated CFSNPs' efficacy, inhibiting fungal mycelial growth by 75.1% and 95.12% against *A. flavus*, and 71.5% and 86.25% against *Penicillium* species at concentrations of 0.75 mg/ml and 1.0 mg/ml, respectively. In comparison, Cassia leaf extract exhibited lower inhibitory activity against *A. flavus* (20.8%, 35.6%) and *Penicillium* spp. (23.1%, 40.6%) at similar concentrations. The antifungal properties of CFSNPs suggest potential applications in preserving agro-produce, thereby extending the shelf life of oil seed crops.

Keywords: Fungi, Inhibit, Oilseeds, Nanoparticle, Spoilage

INTRODUCTION

A major challenge facing the availability of healthy food produced worldwide is losses due to spoilage caused by organisms such as bacteria, viruses, insects, nematodes, and molds (Célia, Silva, and Susana, 2018). In warmer climates, grains are easily infected with spoilage and toxigenic molds. *A. flavus* and *Penicillium species* are important pathogenic fungi that contaminate food during storage (Amare and Keller, 2014). When molds infect food commodities, they can cause rotting, shrinking, discoloration, loss in germination capacity, and toxification, especially in oilseeds (Law *et al*., 2017). One such oil seed crop is groundnut (*Arachis hypogaea* L.), an important food crop especially in developing countries (Law *et al*., 2017). It is one of the most important oil-producing seed crops in the world and is popularly grown by farmers in various parts of the world. Groundnut seeds are sources of minerals such as niacin, calcium, phosphorus, magnesium, zinc, and potassium (Oso and Ashafa, 2021). Groundnut kernels are consumed as raw, roasted, or boiled kernels and are used as raw materials such as soil cakes and fertilizer (Law *et al*., 2017). These uses of the groundnut make it an excellent cash crop for domestic markets as well as foreign trade in several countries (Oso, and Ashafa, 2021). This significant crop is highly susceptible to infection by pathogenic fungi and consumed by a lot of people without knowledge of the fungal pathogens associated with them which can be detrimental to their health. To protect this economically important crop from spoilage by molds, control strategies such as treatment with fungicides or fumigants have been employed (Verheecke *et al*., 2014). Although these have been found effective, negative effects of these methods include the generation of resistant strains and the presence of fungicide residues which affect food safety and cause environmental pollution (Amare and Keller, 2014). Therefore, the development of biological controls with lower risk to humans and the environment is of priority. To provide eco-friendly and safer approaches for the control of undesired microflora in food commodities, the use of natural substances has increased. Nanoparticles are promising antimicrobial agents and their activity has been extensively studied (Amare and Keller, 2014). They have high surface area, and physical and chemical properties that allow them to attack microorganisms via different mechanisms. The use of plants in the synthesis of nanoparticles, also known as "green synthesis," is a rapidly growing field of research due to its eco-friendliness and cost-effectiveness. This process utilizes various plant extracts, and parts to reduce and stabilize metal ions into nanoparticles (Danazumi *et al*., 2024, FSAI, 2018). This study, therefore, seeks to assess the inhibitory efficacy of selenium nanoparticles synthesized using *Cassia fistula* leaves against molds isolated from groundnut samples under storage.

MATERIALS AND METHODS

Infested groundnut samples were collected from two markets in Ilorin, Nigeria in sterile zip-lock nylon and taken to the microbiology laboratory for further analysis. The sample was stored in a desiccator which contained desiccant to avoid absorbing moisture until analysis (Maryam, 2021).

Collection of *Cassia fistula* leaf

Fresh leaves were obtained from the plant Garden of the University of Ilorin, Kwara State Nigeria.

Isolation of Fungi from Maize Sample

Purchased groundnuts were surface sterilized and rinsed with distilled water to remove ethanol on the surface (Chukwudi *et al.*, 2022). Samples were dried and blended with a dry mill kitchen blender. One gram of each sample was added to 9 ml of sterile distilled water, shaken using an orbital shaker, and serially diluted. Aliquots 0.1mL were plated on Potato Dextrose Agar (PDA) and incubated at 28 °C for 7 days (Menza and Muturi, 2018). Fungal isolates were sub-cultured on PDA and enumerated using the method of Mezzomo *et al*. (2022)

Purification and Characterization of Fungi Isolates

Visible fungal growth was sub-cultured onto PDA plates to obtain pure cultures for identification. Pure fungal cultures were then stored in McCartney bottles and placed in the cooling incubator at 4°C to prevent further growth. Isolated Fungi were further identified according to the procedure described by Pyrzynska and Sentkowska (2022). A small amount of fungal mycelium was teased from a fungal colony, and microscopic identification of fungi was determined based on morphological and growth features using a Leica DM500 Digital Microscope (Leica Microsystems, Switzerland) in consultation with relevant identification manuals (Pyrzynska and Sentkowska 2022).

Sample Preparation and Aqueous extraction of *Cassia fistula* Leaf

Cassia fistula leaves were rinsed in distilled water and air dried at room temperature of 37^{o}C in the laboratory for 2 weeks. Afterward, dried product was blended and sieved using a mesh to obtain fine powder. The preparation was done according to the method of Pyrzynska and Sentkowska (2022). *Cassia* leaves powder (1.5 g) was measured and added to sterile distilled (100 ml) water in a conical flask. It was mixed and soaked for 30 minutes. The solution was filtered and centrifuged for 20 minutes at 4000 rpm. The extract was poured carefully into a conical flask and the colloids were discarded.

Biosynthesis and Characterization of Selenium Nanoparticle

The synthesis was carried out using the method of Pyrzynska and Sentkowska (2022). Sodium selenite (10 Mm) was prepared and homogenized by adding 10 ml of *Cassia fistula* leaf extract to 10 mM of sodium selenite and incubated at 36^{o}C for seven days. This was to allow for the bioreduction of sodium selenite to selenium nanoparticles, after which it was observed for color change. Biosynthesized selenium nanoparticles were characterized using UV visible Spectroscopy, Transmission Electron Microgram (TEM) Fourier Transform infrared spectroscopy (FTIR), and Energy Dispersive X-ray Analysis (EDX). Fourier-transform infrared spectroscopy was performed to determine the biomolecules responsible for reducing, capping, and stabilizing CFSNPs. Dried selenium nanoparticles were used for FTIR analysis (Singh and Mijakovic, 2022). Scanning electron microscopy was done to ascertain, the compositional information of the SeNPs (Choudhary and Choudhary, 2017). Energy Dispersive X-ray (EDX) microanalysis was used to determine the prevalent element in the selenium nanoparticles (Scimeca *et al.*, 2018).

Antifungal Activity of Biosynthesized Selenium Nanoparticles

The antifungal activities of Sodium Selenite, *Cassia fistula* leaves extract, and biosynthesized SeNPs (CFSNPs) were evaluated using the mycelial growth inhibition test method (Alagesan *et al.*, 2019). This was done by incorporating 0.75mg/ml and 1.0mg/ml of biosynthesized SeNPs, *Cassia fistula* leaf extract, and Sodium selenite separately into prepared Potato dextrose agar (PDA). After solidification and cooling, the plates were inoculated with a 6 mm Agar plug of a 72-hour-old culture of *Aspergillus flavus and Penicillium species.* The control was set up without the incorporation of CFSNPs, *Cassia fistula* leaf extract, and Sodium selenite. Plates were incubated at 25 ± 2^{o}C. Growth in plates was measured and the percentage growth inhibition was calculated using the formula below:

Percentage inhibition =

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

Where C = colony diameter (mm) of the control; T = colony diameter (mm) of the test plate;

RESULTS AND DISCUSSION

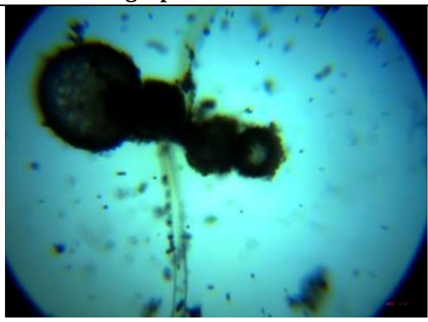
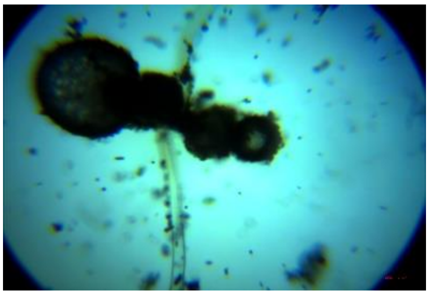
Morphological and cultural characteristics of the fungal isolates.

Morphological and cultural characteristics of isolated molds are shown in Tables 1 and 2. Abe *et al*. (2020) and Abdel-Sater *et al*. (2017) successfully isolated and characterized *Aspergillus flavus and Penicillium* spp from groundnut samples. Chein *et al.* (2019) also isolated *Aspergillus* and *Penicillium* species from groundnut kernels. In their study, *Aspergillus flavus* (38), and *Penicillium citrinum* were identified.

Table 1: Morphological characteristics of fungal isolates

Isolates	Cultural Characteristics	Microscopic Morphological Characteristics
Aspergillus flavus	Velvelty colonies	Conidiosphores are borne laterally on hyphae, non-septate, and numerous with sterigmate.
Penicillium spp	Large white colonies almost cover the whole surface.	Non-septate branched hyphal enlarged at the apex to form conidiophores. They produce brownish-black ceridia in chains.

Table 2: Cultural characteristics of the fungal isolates

No.	Growth on Potato Dextrose Agar Plate	Photomicrograph	Probable fungi
1.			*Aspergillus flavus*
2.			*Penicillium* specie.

Aqueous Extraction and Biosynthesis of Selenium Nanoparticles (SeNPs)

Plant extracts are considered safe, environmentally friendly, and non-toxic, hence their diverse applications in nanotechnology. In recent decades green plant-based nanoparticle synthesis has become an alternative to physical and chemical approaches (Salem *et al*., 2022). In the biosynthesis of selenium nanoparticles using an aqueous extract of *Cassia fistula* Leaves, there was a slight noticeable change of color to brown after seven days of incubation (Plate 1). Several authors have reported variations in the colour of SeNPs, like red (Pyrzynska and Sentkowska, 2022) and green (Alagesan and Venugopal, 2019) attributing this to the excitation of the surface plasmon resonance of selenium nanoparticles (SeNPs). Salem *et al*., (2022) also confirmed the synthesis of Se-NPs by a color change from yellow to a reddish color. The color change shows that the aqueous extract of *Cassia fistula* Leave extract could reduce the ions in the selenium element to Se-NPs. Thus, acting as a reducing and stabilizing agent.

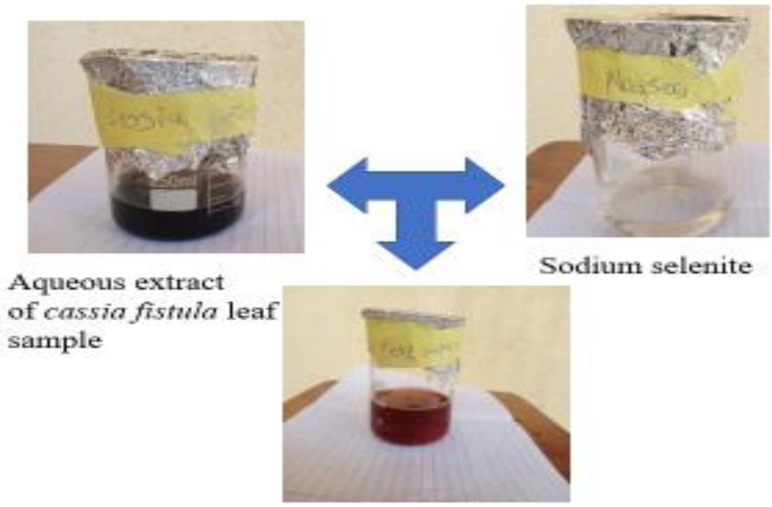


Plate 1: Aqueous extraction of *Cassia fistula* and Sodium selenite

Characterization of Biosynthesized Selenium

In this study, Selenium nanoparticles displayed an absorption peak at 450 nm which is possibly related to surface plasmon vibrations. Biosynthesized selenium nanoparticles have been reported to have varying absorption peaks. Puri and Patil, (2022) recorded the absorption peak of SeNPs at 350 nm. Hassanien *et al.* (2019) also reported an absorption maximum at 399 nm. Abirami *et al.* (2022) reported an absorption peak of 450 nm which confirmed the absorption peak of the bio-synthesized selenium nanoparticles in this study. Abirami *et al.* (2022) and Lin and Wang (2005) concluded that the semi-conductivity property of selenium nanoparticles may be responsible for their shift in absorption spectral features (Figure 1). Ashraf *et a*l. (2021) also carried out biosynthesis of copper oxide using *Cassia fistula* nanoparticles, and the resultant particles showed a broad peak at 320 nm, under different reaction conditions. Synthesis of Selenium nanoparticles using orange peel extract by Salem *et al.* (2022) revealed a significant peak at 295 nm

Fourier Transform Infrared Spectrum Analysis (FTIR)

FT-IR analysis can detect excitations of chemical bonds, and functional groups present on the surface of nanoparticles. In this study, FT-IR spectroscopic research was carried out to validate and determine the function of *Cassia leaf* extract in the biosynthesis of Se-NPs'. The interaction of the capping agent from the *Cassia leaf* extract with Selenium is shown by the wave numbers at 3173.00 cm−1, 2361.44 cm−1, 1594.00 cm−1, 1373.00 cm−1, 1027.27.29 cm−1, and 674.23 cm−1 (Figure 2). The peak of 3173.00 corresponds to the stretching vibration of the O-H compound of alcohol, the peak 2361.00 cm−1 shows carboxylic acids stretching O-H, the peak 1594.00 Amides C=O stretch, the peak 1373.00 cm−1 correspond to Nitro compounds and NO_2 stretch, the peak 1027.29 cm−1 correspond to Alkyl and Aryl halides and C-F stretch and the peak 674.23 cm−1 correspond to Alkyl and Aryl halides and C-Br stretch. This signifies that alcohol, carbonyl groups, Amides, Nitro groups, Alkyl, and aryl groups were necessary for the bio-synthesis of selenium nanoparticles (Pouri et *al*., 2018). Variations in peaks suggest that the organic constituents in the *Cassia leaf* extract were involved in the formation of Selenium nanoparticles through the reduction process (Salem *et al*., 2022)

Transmission Electron Microscopy (TEM)

The Transmission electron microscopic images are shown in Figure 3. The biosynthesized selenium nanoparticles are cubic, rod, and irregular in shape this was confirmed by SEM at 20nm. Similarly, Verma and Maheshwari (2018) and Ullah *et al*. (2021) reported that selenium nanoparticles were rod-shaped. Salem *et a*l. (2022) also synthesized Se-NPs from orange peel and affirmed their polydisperse and spherical nature with sizes ranging from 16–95 nm

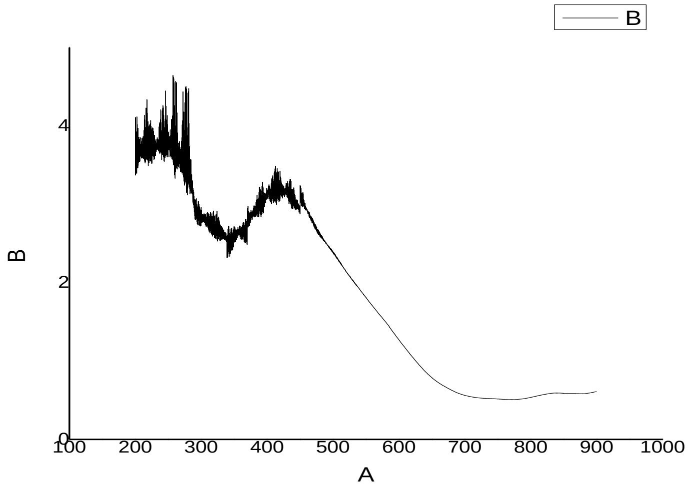


Figure 1: UV Vis Spectrum of Biosynthesized SeNPs (CFSNPs)

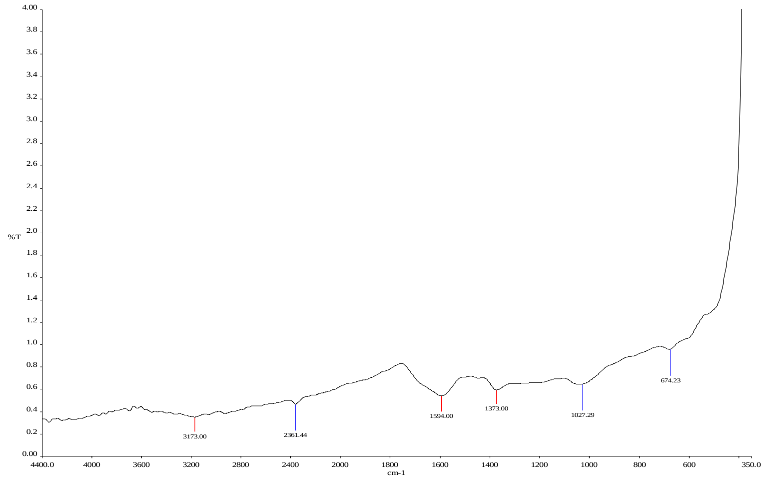


Figure 2: FTIR spectrum of the biosynthesized selenium nanoparticles

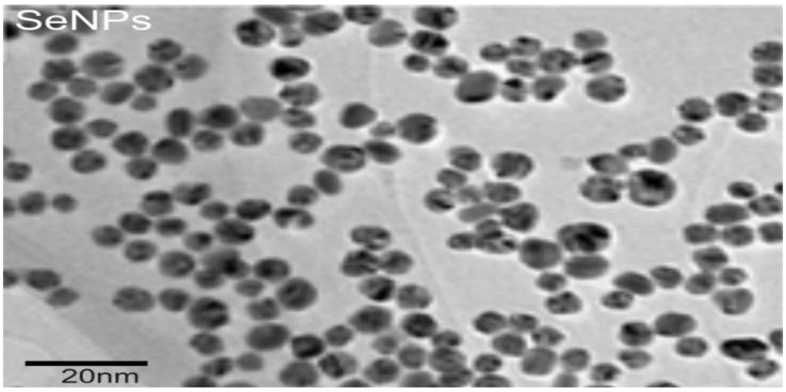


Figure 3: Scanning Electron microscopy of the biosynthesized (CFSNPs)

The EDX spectroscopy analysis of selenium nanoparticles shown in Figure 4 showed that elemental selenium (80.76%) is the most prominent element present in the CFSNPs. It showed a strong signal for selenium at 0.5 KeV. In the characterization of biosynthesized nanoparticles, EDX analysis showed weak signals for carbon and oxygen at 15.24 and 4.00 % respectively and also revealed the presence of defined elements associated with selenium such as oxygen, and carbon. These results agree with that of Salen *et al.* (2022) and Cui *et al.* (2018)

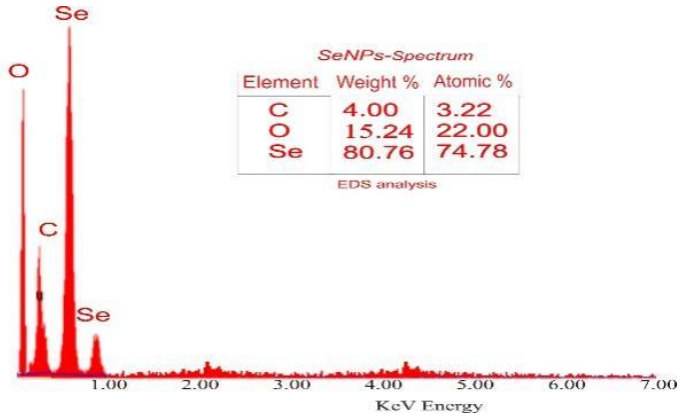


Figure 4: EDX spectrum of *Cassia fistula* biosynthesized selenium nanoparticles

Anti-fungal activities of biosynthesized Selenium nanoparticles

The biosynthesized CFSNPs exhibited excellent fungal mycelial growth inhibition of 75.1% and 91.12% against *Aspergillus flavus* and 71.5 %, and 86.25 %, against *Penicillium* species at concentrations of 0.75 mg/ml and 1.0 mg/ml respectively (Figure 5). On the other hand, aqueous leaf extract of *Cassia fistula* and sodium selenite inhibited the growth of *Aspergillus flavus* (20.8%, 35.6%), and *Penicillium spp* (23.1%, 40.6%) at concentrations of 0.75 mg/ml and 1.0 mg/ml respectively as shown in Figure 5. Similarly, Iqbal *et al*. (2022) and Shahverdi e*t al.* (2010) reported the antifungal properties of Selenium nanoparticles against *Penicillium spp* and *Aspergillus flavus.*

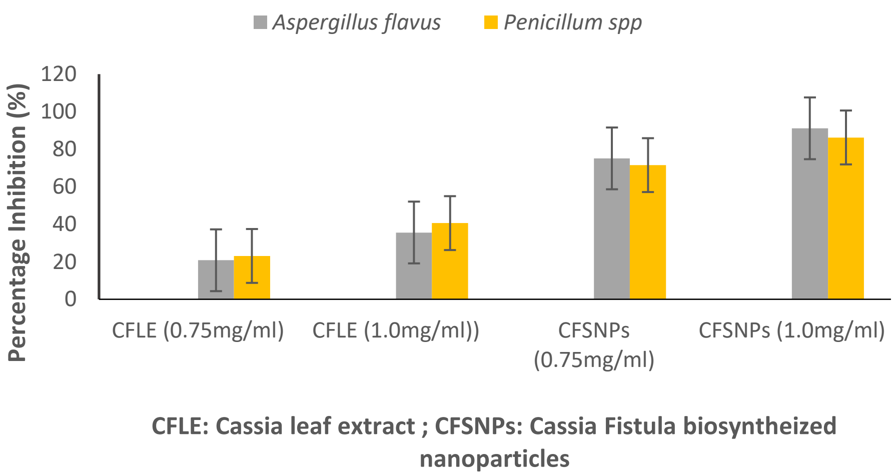


Figure 5: Percentage of mycelia inhibitory activities of *Cassia* leaf extract (CFLE) and biosynthesized nanoparticles (CFSNPs)

CONCLUSION

Based on the findings presented in this study, biosynthesized selenium nanoparticles (CFSNPs) using Cassia fistula leaf extract demonstrated significant potential as antifungal agents against *Aspergillus flavus* and *Penicillium* species in groundnuts. The nanoparticles exhibited inhibitory effects on fungal growth, compared to inhibitory activities of both Cassia leaf extract and sodium selenite. This suggests that CFSNPs could serve as a control for fungal infestations in oil seeds, thereby mitigating both quantitative and qualitative losses during storage. Hence integrating biosynthesized nanoparticles into agricultural practices holds promise for sustainable food production and security.

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