ANTIFUNGAL ACTIVITY OF BIOSYNTHESIZED SELENIUM NANOPARTICLES ON POSTHARVEST SPOILAGE FUNGI OF SHELLED MELON

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Abstract: Mold infestation in oil seeds like melon leads to significant losses. Traditional chemical treatments for these molds harm humans, animals, and the environment. Biological control methods, such as selenium biosynthesized nanoparticles (SeNPs), offer a safer alternative. This study investigated the antifungal properties of SeNPs synthesized using Cassia fistula leaf extract against *Aspergillus niger* and *Penicillium* species in melon seeds. The spoilage molds were identified using standard techniques, and the SeNPs were characterized through UV-visible spectroscopy, TEM, FTIR, and EDX. The biosynthesized Cassia fistula Selenium Nanoparticles (CFSNPs) showed a brownish color and a UV-visible absorption peak at 450 nm, indicating the successful formation of SeNPs. TEM analysis revealed various shapes of CFSNPs, while EDX confirmed selenium as the dominant element. FTIR spectra showed the presence of different functional groups, suggesting successful synthesis. The CFSNPs exhibited significant antifungal activity, inhibiting mycelial growth by 72.1% and 92.12% against *A. niger*, and by 73.5% and 82.25% against *Penicillium* species at concentrations of 0.75 mg/ml and 1.0 mg/ml, respectively. In contrast, Cassia leaf extract alone showed lower inhibition rates. The enhanced antifungal activity of CFSNPs highlights their potential for preventing spoilage in agro-produce, thereby extending the shelf life of oil seed crops.

Keywords: Fungi, Inhibit, Oilseeds, Nanoparticle, Spoilage,

Introduction

Major constraints facing productivity and availability of healthy food produce worldwide are losses due to spoilage caused by bacteria, viruses, insects, nematodes, and molds (Celia *et al*., 2018). In warmer climates, grains are easily infected with spoilage and toxigenic molds. *P*athogenic fungi can contaminate food during storage (Amare and Keller, 2014) causing discoloration, rotting, shrinking, seed necrosis, loss in germination capacity, and toxification, especially in oilseeds (Law *et al*., 2017). One such oil seed crop is melon (*Colocynthis citrullus* L.), an important food crop especially in developing countries (Law *et al*., 2017). *Colocynthis citrullus*, seeds are a popularly consumed oil seed crop in West Africa particularly Nigeria. The seeds, are consumed in various forms as a condiment in local soup and are commonly referred to as Egusi (Bankole and Joda, 2004). They contain 11% starch and soluble sugars, about 53% oil, and 28% protein (60% in the defatted meal) (Kamel *et al*., 2022). One major challenge of these significant seeds is that they deteriorate quickly in storage due to fungal activities (Bankole and Joda, 2004). Fungi such as *Aspergillus* and *Penicillium* species are the major cause of spoilage of grains and seeds, second only to insects as spoilage organisms. These fungi are widely distributed storage fungi of melon seeds, causing decreased nutritive value seed discolorations, high free fatty acid and peroxide values, and decreased seed germination, resulting in the production of toxic metabolites including aflatoxin (Kamel *et al*., 2022). Some of the isolated fungi are known producers of mycotoxins. 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 effective biological controls with presumed lower risk to humans and enhance safe environment. Nanoparticles are promising antimicrobial agents and their activity has been extensively studied (Amare and Keller, 2014). 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-friendly and cost-effective nature that utilizes various plant extracts, such as leaves, roots, and flowers, to reduce and stabilize metal ions into nanoparticles (FSAI, 2018). This study, therefore, seeks to determine the inhibitory efficacy of selenium nanoparticles synthesized using *Cassia fistula* leaves against molds isolated from melon samples under storage.

Material and Methods

Collection of Materials

Fresh *Cassia fistula* leaves were obtained from the Botanical Garden of the University of Ilorin, Kwara State Nigeria. Infested melon samples were collected from two markets in Ilorin, Nigeria in sterile zip-lock nylon and stored in a desiccator which contained desiccant to avoid absorbing moisture until analysis (Maryam, 2021).

Isolation and Characterization of Fungi from Sample

Isolation of Fungi from melon samples was carried out according to the method of Chukwudi *et al.* (2022). The fungal isolates were sub-cultured on PDA and enumerated using the method of Mezzomo *et al*. (2022) and Menza and Mutur (2018). Isolated Fungi were identified according to the procedure described by Pyrzynska and Sentkowska (2022).

Sample Preparation and Aqueous extraction of Cassia fistula Leaf

Cassia fistula leaves were prepared according to the methods of Pyrzynska and Sentkowska (2022). *Cassia* leaves powder (1.5 g) was measured and added to 100 ml of sterile distilled water in a conical flask and the solution was 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 Nanoparticles

Synthesis was carried out using the methods of Ihum *et al*. (2019) and Pyrzynska and Sentkowska (2022). 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 & Mijakovic, 2022). Scanning electron microscopy was done to ascertain, the morphological and 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 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, and *Cassia fistula* leaf extract, separately into prepared Potato dextrose agar (PDA). After solidification and cooling, the plates were inoculated with an Agar plug 6 mm of 72-hour-old culture of *Aspergillus niger and Penicillium species.* The control experiment was set up without the incorporation of CFSNPs, and *Cassia fistula* leaf extract. All plates were incubated at 25 ^{C} ± 2^{o}C. The 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; (Kamaruzzaman, *et al*., 2021).

Results and Discussion

Morphological and cultural characteristics of isolated mold are shown in Table 1. *Aspergillus niger* and *Penicillium* spp were isolated from shelled melon. Abe *et al*. (2020) and Abdel-Sater *et al*. (2017) successfully isolated and characterized *Aspergillus niger* and *Penicillium* spp from melon samples.

Table 1: Morphological Characteristics of The Fungal Isolates

Isolates	Cultural Characteristics	Growth on Agar Plate
Aspergillus niger	Colonies appear black due to the dense production of conidia. Textures are often powdery with a pale yellow or light brown coloration.	
Penicillium spp	Characteristic small, low, and heavily Sporulating, blue or green colonies	

Biosynthesis of selenium nanoparticles

Table 2 shows the biosynthesis of selenium nanoparticles. In the biosynthesis of selenium nanoparticles using aqueous extract of *Cassia fistula* Leaves, there was a slightly noticeable change of color to brown after seven days of incubation. Several authors have reported variations in the color 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).

Characterization of biosynthesized selenium nanoparticles.

Selenium nanoparticle 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) observed the absorption peak of SeNPs at 350 nm (Figure 1). The FTIR spectroscopic studies showed the presence of peaks at 3173.00, 2361.44, 1594.00, 1373.00, 1027.27.29, and 674.23 (Figure 1). The result signified that alcohol, carbonyl groups, Amides, Nitro groups, Alkyl, and Aryl groups were important in the bio-synthesis of selenium nanoparticles (Pouri et *al*., 2018). The Transmission electron microscopic images are shown in Figure 2. The biosynthesized selenium nanoparticles are spherical, 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. The EDX spectroscopy analysis of selenium nanoparticles shown in Figure 2 shows that elemental selenium (80.76%) is the most prominent element present in the CFSNPs.

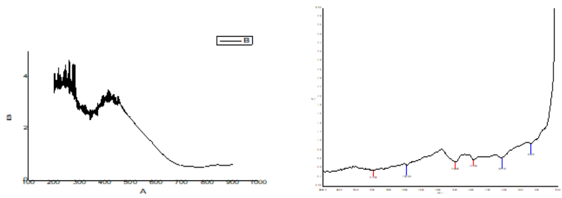

Figure 1: UV- Vis and FTIR Spectrum of Biosynthesized SeNPs (CFSNPs)

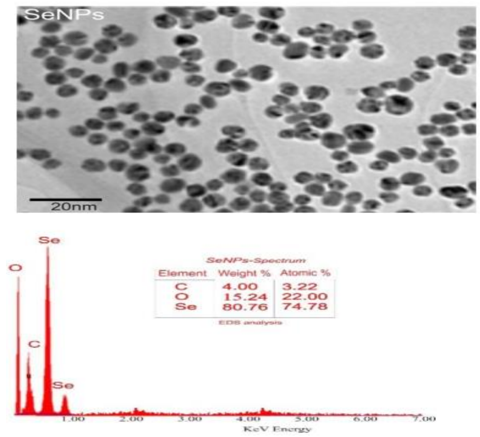

Figure 2: Scanning Electron microscopy of the biosynthesized (CFSNPs) and EDX Spectroscopy

Anti-fungal activities of biosynthesized Selenium nanoparticles.

The biosynthesized CFSNPs exhibited excellent fungal mycelial growth inhibition of 75.1% and 91.12% against *Aspergillus niger* and 71.5%, and 86.25%, against *Penicillium* species at concentrations of 0.75mg/ml and 1.0mg/ml respectively (Table 3). On the other hand, aqueous leaf extract of *Cassia fistula* and sodium selenite inhibited the growth of *Aspergillus niger* (20.8%, 35.6%), and *Penicillium spp* (23.1%, 40.6%) at concentrations of 0.75mg/ml and 1.0 mg/ml respectively as shown in Table 3. Similarly, Iqbal *et al*., (2022) and Shahverdi *et al.* (2010) reported antifungal properties of Selenium nanoparticles against *Penicillium spp* and *Aspergillus niger.*

Table 2: Percentage of mycelia inhibitory activities of *cassia leaf* extract (CFLE) and biosynthesized nanoparticles (CFSNPS)

Test Isolate	CFLE (0.75mg/ml)	CFLExtract (1.0mg/ml))	CFSNPs (0.75mg/ml)	CFSNPs (1.0mg/ml)
Aspergillus niger	20.8%	35.6%	72.1%	92.12%
Penicillum spp	23.1%	40.6%	73.5%	82.25%

Conclusion

This study successfully identified *Aspergillus niger* and *Penicillium* species as significant spoilage molds affecting melon seeds. Selenium nanoparticles (CFSNPs) were biosynthesized using Cassia fistula leaves. Synthesized CFSNPs demonstrated substantial antifungal activity, against the growth of these molds. The results indicate that CFSNPs could be a suitable alternative to traditional chemical fungicides, as they offer a safe and environmentally friendly solution to protect economically important crops such as melon seeds. Incorporating CFSNPs into agricultural practices could help enhance food safety, and extend the shelf life of oil seeds.

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