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ANTIMYCOTIC ACTIVITY OF *NIGELLA SATIVA* OIL - GOLD NANOCOMPOSITES AGAINST FUNGAL ISOLATES FROM STORED MAIZE

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ABSTRACT

Fungi contamination in grains is a serious health menace that reduces the quality and nutritional content of stored food grains. Hence, in this study, the antimycotic activity of *Nigella sativa* (NS) oil-gold nanocomposite against fungi isolated from stored maize was evaluated. The fungal species were isolated from stored maize and identified using standard procedure. Gold nanoparticles (AuNPs) were biosynthesized from an aqueous extract of *Piper guineense* leaf and characterized by the Tyndall effect, UV-visible spectra, zeta sizer, transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR). Gas chromatography-mass spectrometry (GC-MS) analysis of NS oil identified 8 chemical compounds, with Cyclododecyne being the most abundant (39.49 %). The obtained AuNPs were conjugated with NS oil to form nanocomposites. *Piper guineense* contains important phytochemicals that are capable of reducing chloroauric acid (HAuCl4) into AuNPs. The dispersion of light through the colloidal AuNPs solution was confirmed by the Tyndall effect. The AuNPs were spherical in shape and well-dispersed with average sizes ranging from 30 to 120 nm. The UV-visible spectrum showed a peak of 530nm while the FTIR result revealed the presence of different chemical groups in AuNPs. The formulated *Nigella sativa* oil nanocomposite III exhibited the highest anti-fungal activity, minimum inhibitory concentrations (MIC), and minimum fungicidal concentrations (MFC) against fungal isolates from both fields and stored maize. This outcome indicated that *Nigella sativa* oil nanocomposites could serve as an effective protectant against fungi infection in maize.

Keywords: *Nigella sativa* oil, nanocomposite, fungal isolates, maize, gold nanoparticles.

INTRODUCTION

Cereals are essential foods predominantly consumed by low-income rural populations in many parts of the world (Perron et al. 2021). In the agricultural sector, maize is considered one of the most significant universal cereals in addition to wheat and rice. Maize belongs to the family Poaceae. It is one of the major cereals used in the production of animal feed (Gitu, 2006; Erenstein *et al.*, 2021). However, its fungal infection and subsequent contamination with mycotoxins either from the field or storage facilities present serious health implications to humans and animals (Agriopoulou 2021; Habschied *et al.*, 2021).

Fungi are capable of adapting to any kind of environment and their growth is associated with high humidity or moderate environmental temperature (Cruz-Luna et al. 2021). The damages caused by these microorganisms are estimated to be approximately between 20 to 40%

in Africa (Adégbola *et al.,* 2011). The most common seed-borne fungi of maize included Aspergillus, Fusarium, Penicillium, and Rhizopus, associated with heavy damage to grains rendering them unfit for human consumption (Majumder et al. 2013; Nešić *et al*., 2021). Chemicals have often been utilized in controlling fungi infection of crops. Nevertheless, its usage has increased environmental pollution, fungi resistance and caused serious health implications. The use of nanomaterial is an alternative and friendly method of controlling fungi in plants. This is achieved due to their physiochemical properties, size, structural stability, and target affinity, improving their ability to penetrate cell membranes (Kumar *et al.,* 2013; Ajitha *et al.,* 2015).

Since nanoparticles are unlikely to develop resistance to disease-causing organisms, they are usually endorsed in medicine for the delivery of drugs to the target area (Brandelli *et al.,* 2012; Kaur *et al.,* 2018). Gold is a multivalent metal known to be stable against oxidation and degradation in living systems. In clinical therapeutics, AuNPs are preferred for their low toxicity and biocompatibility (Rahimi *et al.,* 2019). These characteristics may help nanoparticles to effectively diminish reactive oxygen species (Torres *et al.,* 2018).

The control of seed-borne fungi affecting stored food relies largely on synthetic chemicals, some of which are non-biodegradable with known residual toxicity (Pathak and Zaidi 2013; Akpomie *et al.,* 2021). Hence, the utilization of plant materials in the biosynthesis of nanoparticles since they are eco-friendly and contain beneficial metabolites in the treatment of disease **(**Abd-Rabou *et al.,* 2017**)**. *Piper guineenses* is a spicy plant that belongs to the family *Piperaceae*. It is commonly grown in West Africa and used in folk medicine for treating various diseases. It is reported to be therapeutic against oral thrush, skin rashes, and vaginosis (Alagbe *et al.,* 2021; Mgbeahuruike *et al.,* 2019). Nigella sativa (black seed) is a flowering plant of the Ranunculaceae family. During the ancient civilization, it was used as food additives and in phytomedicine for the treatment of diabetes and fever (Brandelli 2012). This research studies the antimycotic activity *of Nigella sativa* oil nanocomposite from gold nanoparticle (AuNPs) against fungi isolate from stored maize.

MATERIAL AND METHODS

Collection and identification of plant material

The use of plant materials in this study complies with relevant institutional, national, and international guidelines and legislation. The leaves of Piper guineenses were obtained from the Federal University of Technology (FUT), Bosso campus Minna, Niger state, and identified at the Biological Sciences Department Federal University of Technology Minna, Niger State. The voucher number is FUT/PLB/PIP/025. The grains for the study (field and stored maize) were obtained with permission from Maizube farm, Minna, Niger state. Nigella sativa oil used for this study was purchased from the central market in Minna, Nigeria.

Sample preparation

The leaves were harvested from the plant and washed. The leaves were air-dried for about three weeks at room temperature (±26ºC) to prevent the destruction of the thermo-liable component of the plant. The dried leaves were blended into a powder. About 10g of the powdered sample was weighed into 100 ml of sterile distilled water in an Erlenmeyer flask and then boiled for 5 min. This was filtered with Whatman No.1 filter paper to obtain the filtrate.

Determination of Phytochemical composition

Qualitative phytochemical components of *P. guineenses* leaf were determined by the method described by AOAC (1999).

Gas Chromatography-Mass Spectrometry (GC-MS) of *Nigella sativa* oil

The GC-MS analysis of Nigella sativa oil was carried out on an Agilent 19091S Gas chromatograph (GC) by the method described by Paranthaman et al. (2012).

Biosynthesis of gold nanoparticles

The biosynthesis of AuNPs was carried out according to the method described by Sing et al. (2016). About 0.5 ml of *Piper guineense* leaf filtrate was measured and mixed with 9.5 ml of 1 mM chloroauric acid ($HAuCl_4$) for the reduction of Au^{3+} ions.

Characterization of gold nanoparticles

UV-Vis Spectrophotometric

The bio-reduction of gold ions in aqueous solution was monitored using a UV-Vis spectrum measured at 300nm to 800nm using a quartz cuvette. The mixture was measured at room temperature and time within the range of 0–30min (Patil *et al.,* 2017).

Zeta sizer analysis

The particle size and distribution of the AuNP were measured using dynamic light scatting (DLS) equipment (Malvern Zeta sizer). Malvern zeta sizer equipped with 10mV He-Ne laser (633nm) and operated at an angle of 90^0.

Transmission electron microscopy (TEM)

TEM analysis was carried out for the determination of the morphology, shape, and size of AuNPs with HITACHI h-800, working at 200 kV.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR Spectroscopy of the synthesized AuNPs solution was determined by the method of Afshan *et al.,* (2020).

Conjugation of Gold Nanoparticle

The conjugation of the gold nanoparticle was carried out in polyethylene glycol and the composite formed was as described below: All the formulations were made and left for 24 hours to enable digestion of the mixture (Figure 1).

- Free/unconjugated 1: Standard drug (Fluconazole)
- Free/unconjugated 2: *Nigella sativa* oil
- Conjugate 1: *Nigella sativa* oil/Polyethylene glycol
- Conjugate 2: Biosynthesized gold nanoparticle/Polyethylene glycol
- Conjugate 3: Biosynthesized gold nanoparticle/*Nigella sativa* oil

Conjugate 4: Biosynthesized gold nanoparticle/*Nigella sativa* oil/polyethylene glycol.

Antifungal activity

Nutrient agar preparation

All the media used were prepared according to the manufacturer's guide. Sabouraud dextrose agar (SDA) was used for the isolation of fungi. Twenty-eight grams (28 g) of the sabouraud nutrient agar was dissolved in one-liter sterile distilled water and then autoclaved at 121^0C for 15 minutes.

Isolation Procedure

Fungi in the samples were isolated by inoculating samples in an already prepared Petri dish of Sabouraud dextrose agar using the pour plate technique. The plates were incubated at 25^0C for 3-5 days. After incubation, the plates were examined for colonies that appeared different in their cultural characteristics. These colonies were collected with a sterile wire loop and streaked on an already prepared SDA agar to obtain pure cultures. Each pure culture was then subcultured into agar slants in bijou bottles and kept as stock culture.

Identification of fungal Isolates

The fungi colonies were identified based on their daily cultural colony characteristics on SDA and microscopic characteristics after performing a slide culture technique and using of fungi atlas. The fungal morphology was studied macroscopically by observing the colony features (color, shape, size, and hyphae), and microscopically by a binocular microscope at ×40 objective lens using 1 % lactophenol blue stain as described by Gaddeyya *et al.,* (2012).

Antifungal activity of gold nanocomposite of N. sativa against fungi isolates from stored and field maize

The antifungal screening was carried out following the method described by Doddanna et al. (2008b). The activity of the nanoparticle was evaluated on the fungi strains isolated from maize with the liquid nanocomposite.

Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentrations

Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentrations (MFC) were determined by the methods described by Yehouenou et al. (2012) and Gnonlonfin et al. [30], on only the nanocomposite that was active using the microdilution method.

Statistical analysis

The data obtained were subjected to one way analysis of variance (ANOVA) and the significant differences among groups were determined by Duncan multiple test p-value less than 0.05 was considered significant. Values are represented as mean ± standard error of mean. Data from the antibacterial activities were compared with their respective controls and differences at $p < 0.05$ were considered significant

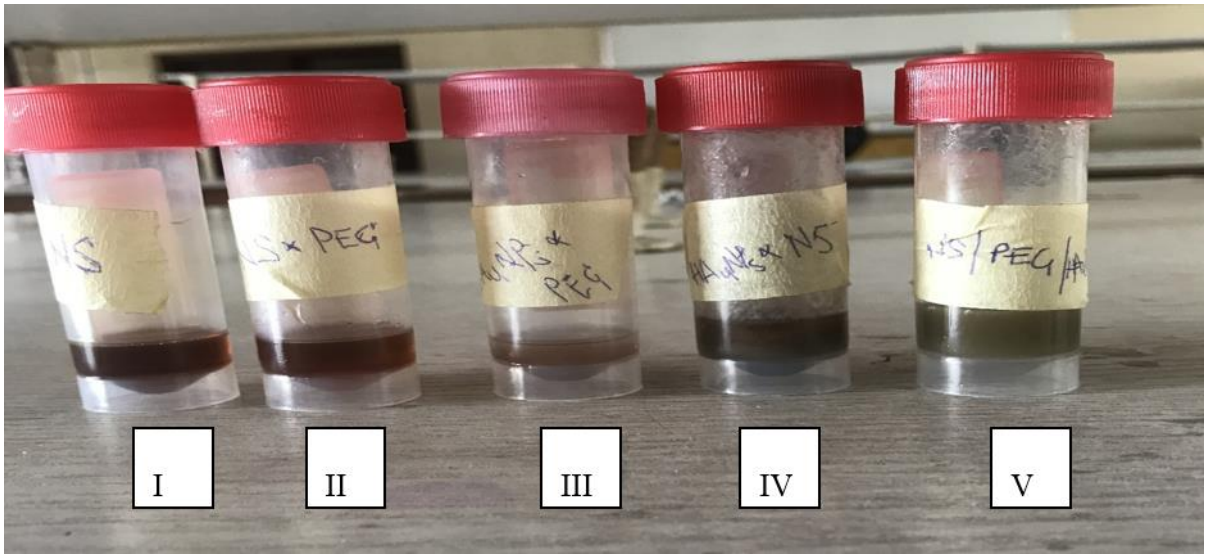

Figure 1. Functionalization of gold nanoparticle

I- *Nigella sativa* oil; II- *Nigella sativa* oil/Polyethylene glycol; III- Biosynthesized gold nanoparticle/Polyethylene glycol; IV- Biosynthesized gold nanoparticle/*Nigella sativa* oil; V- Biosynthesized gold nanoparticle/*Nigella sativa* oil/polyethylene glycol

RESULT

Phytochemical constituents of *Piper guineenses* leaf

The qualitative phytochemical analysis of *Piper guineenses* leaf revealed the presence of the following phytochemicals; alkaloids, flavonoids, tannins, anthraquinones, phenols, terpenoids, glycosides, saponins, steroids, and phlobatanins as shown in Table 1.

Table 1: Phytochemical constituent of aqueous extract of *Piper guineenses*

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Tannins	+
Anthraquinones	+
Phenols	+
Terpenoids	+
Glycosides	+
Saponins	+
Phlobatanins	+
Steroids	+

Keys: (+) Present

Gas chromatography-mass spectrometry (GC-MS) of *Nigella sativa* oil

The GC-MS spectral of the *N. sativa* oil is presented in Table 2. The chemical compositions are revealed in varying proportions. 5-Tetradecyne had the lowest amount with 1.25% followed by α-thujene with (1.44%), thymoquinone (3.41%), p-cymene (5.81%), 2-dodecylcyclobutanone (13.54%), n-hexadecanoic acid (15.58), 9,12-octadecadienoic (19.48%), while cyclododecane had the highest amount with 39.49%. The GC-MS spectra of the N. sativa oil are presented in Table 2. The chemical compositions are present in varying proportions. 5-Tetradecyne had the lowest amount with 1.25% followed by α-thujene with (1.44%), thymoquinone (3.41%), p-cymene (5.81%), 2-dodecylcyclobutanone (13.54%), n-hexadecanoic acid (15.58), 9,12-octadecadienoic (19.48%), while cyclododecane had the highest amount with 39.49%.

Table 2: Chemical composition of *Nigella sativa* oil

Compound	Percentage (%)
5-Tetradecyne	1.25
α-thujene	1.44
Thymoquinone	3.41
p-cymene	5.81
2-Dodecylcyclobutanone	13.54
n-Hexadecanoic acid	15.58
9,12-Octadecadienoic	19.48
Cyclododecane	39.49

Biosynthesis of Gold nanoparticle using aqueous leaf extract of *Piper guineenses*

As shown in Figure 2, visible color change was observed during the biosynthesis of AuNPs. Tube (a) contains a yellow color solution of $HAuCl_4$ while tube (b), contains the solution of chloroauric acid that changed from yellow to ruby red color after the reduction of $HAuCl_4$ ions to AuNPs in the presence of *Piper guineenses* leaf.

Characterization of gold nanoparticles

UV-vis Absorption spectral of gold nanoparticle

The UV-Vis spectra of $HAuCl_4$ and AuNPs synthesized from aqueous leaf extract of Piper guineenses are displayed in Figure 4. The absorption band of the colloidal solution of AuNPs displayed a broadened absorption peak at 530nm compared to the absorption peak for $HAuCl_4$ observed around 310 nm.

Particle size of gold nanoparticles

The average particle size of the synthesized AuNPs is illustrated in Figure 5. The result shows that the synthesized AuNPs have particle sizes between the range of 30 nm to 120 nm with a maximum absorption peak of 78 nm. The gold nanoparticle synthesized occupies 80 percent of the entire volume of the sample analyzed based on the intensity percentage.

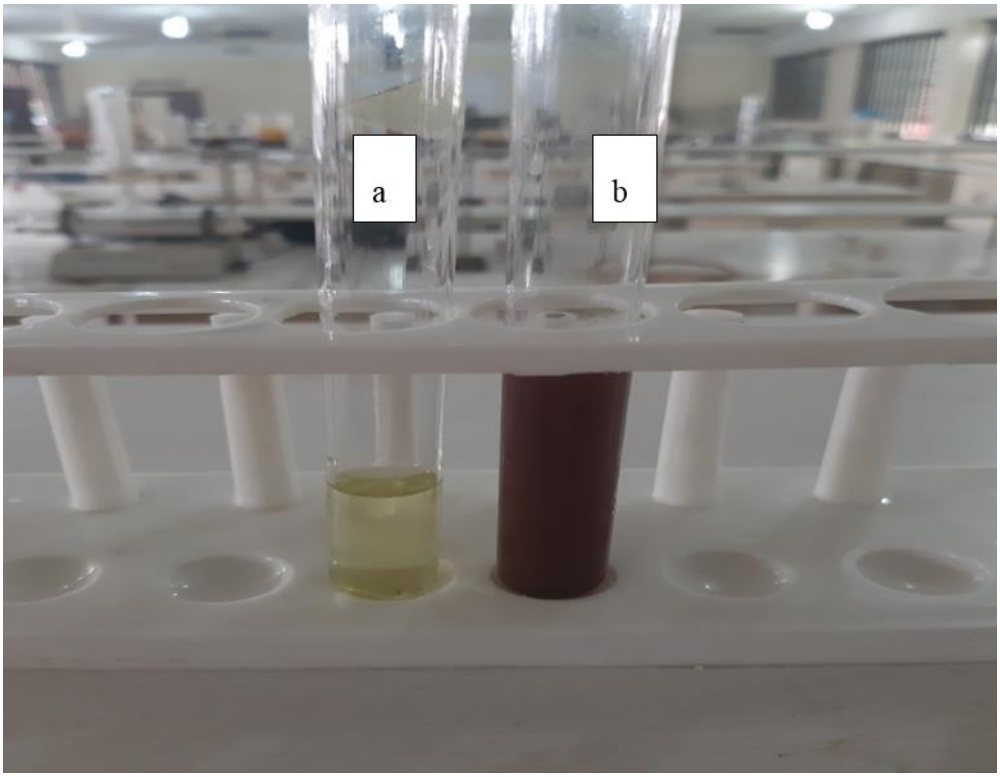

Figure 2: **Manifestation of color change that occurred during biosynthesis of gold nanoparticle.** (a) $HAuCl_4$; (b) AuNPs

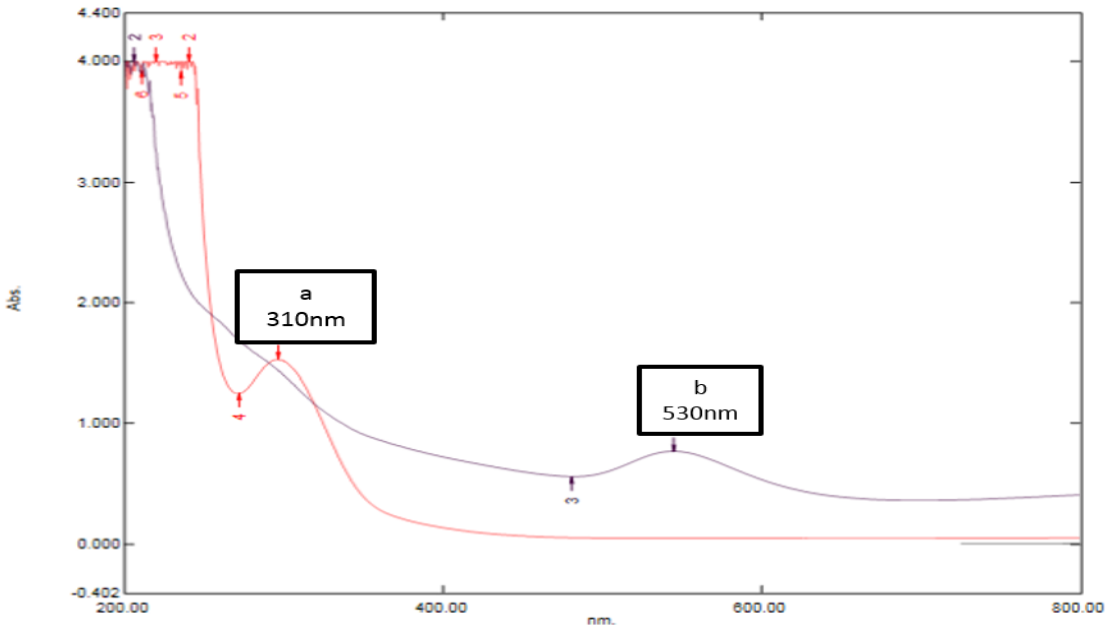

Figure 4: Absorption spectra of $HAuCl_4$ (a) and AuNPs (b) before and after reduction with *Piper guineenses*

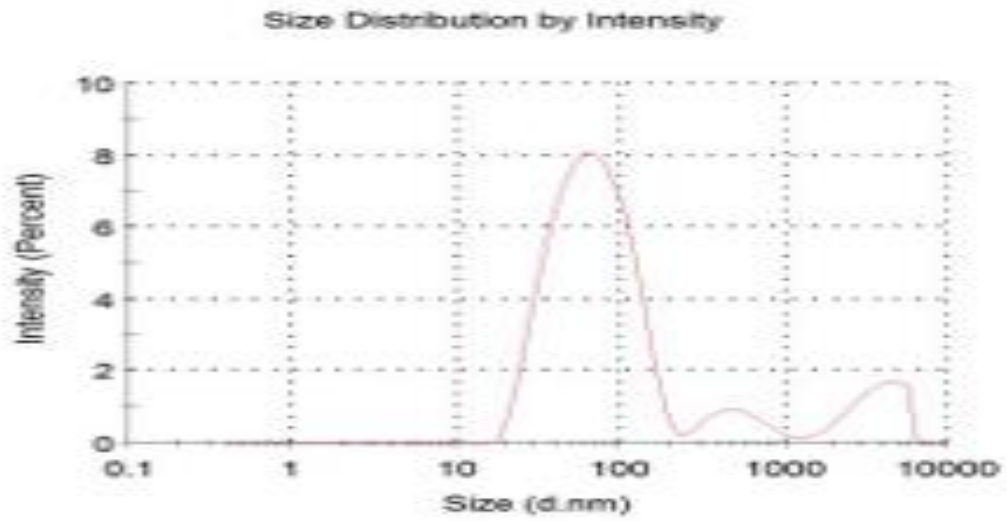

Figure 5 Particle size of AuNPs synthesized from aqueous leaf extract of *Piper guineenses*

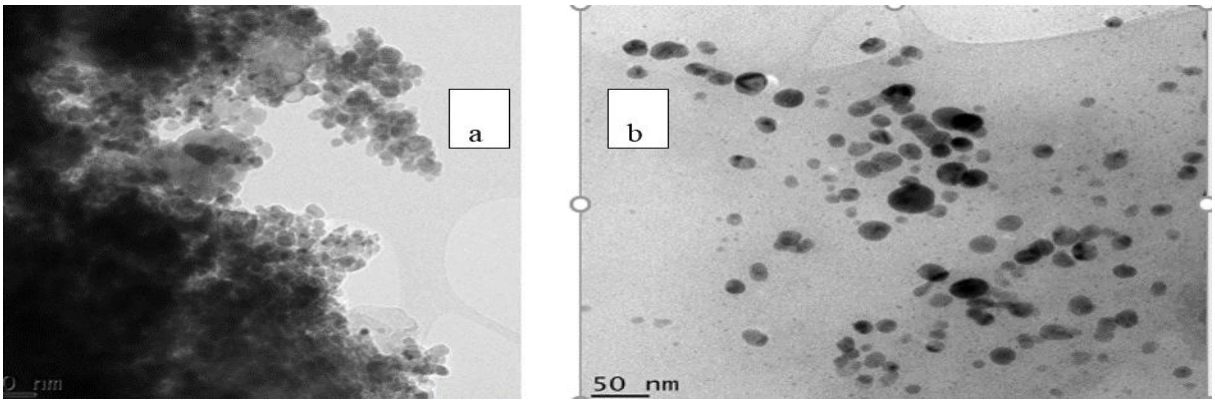

Figure 6: TEM micrograph of AuNPs synthesized from aqueous leaf extract of *Piper guineenses*

Transmission electron microscopy (TEM)

The TEM images of the synthesized AuNPs are displayed in Figure 6. The images revealed particles with roughly spherical shapes. These particles were loosely bound and exhibited various sizes at 50 nm magnification.

Fourier transform infrared (FTIR) spectra of the biosynthesized gold nanoparticle

The FTIR spectra of AuNPs in Figure 7, indicated the presence of the different chemical groups in the AuNPs. The FTIR spectral identified functional groups with the peak representative of 3425.03 cm−1, 2876.69 cm−1, 1466.60 cm−1, 1342.90 cm−1, 1279.90 cm−1, 1241.82 cm−1, 946.93 cm−1, 841.29 cm−1 and 645.83 cm−1. The strong peak at 3425.03 cm−1 corresponds to O–H (alcohol and phenols) stretch, while 2876.69 cm−1 corresponds to C-H stretching (alkanes). Peaks ranging between 1466.60 cm−1 to 1342.90 cm−1 correspond to C-C stretch (aromatics) and C-N (nitro compounds).

Antifungal activity

Identification of fungi isolate from store and field maize sample

The morphological and colonial identification of fungi isolated from stored and field maize samples are displayed in Table 3. The fungal pathogens (Figure 8), isolated from the post-harvest deterioration of maize seeds in the field were; *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* species, *Aspergillus fumigatus*, and *Trichophyton mentagrophytes* while the identified fungal pathogens isolated in storage maize were; *Aspergillus niger*, Aspergillus flavus, *Fusarium* species, *Aspergillus fumigatus, Cladosporium* species, *Acremonium* species, and *Microsporum audouinii*.

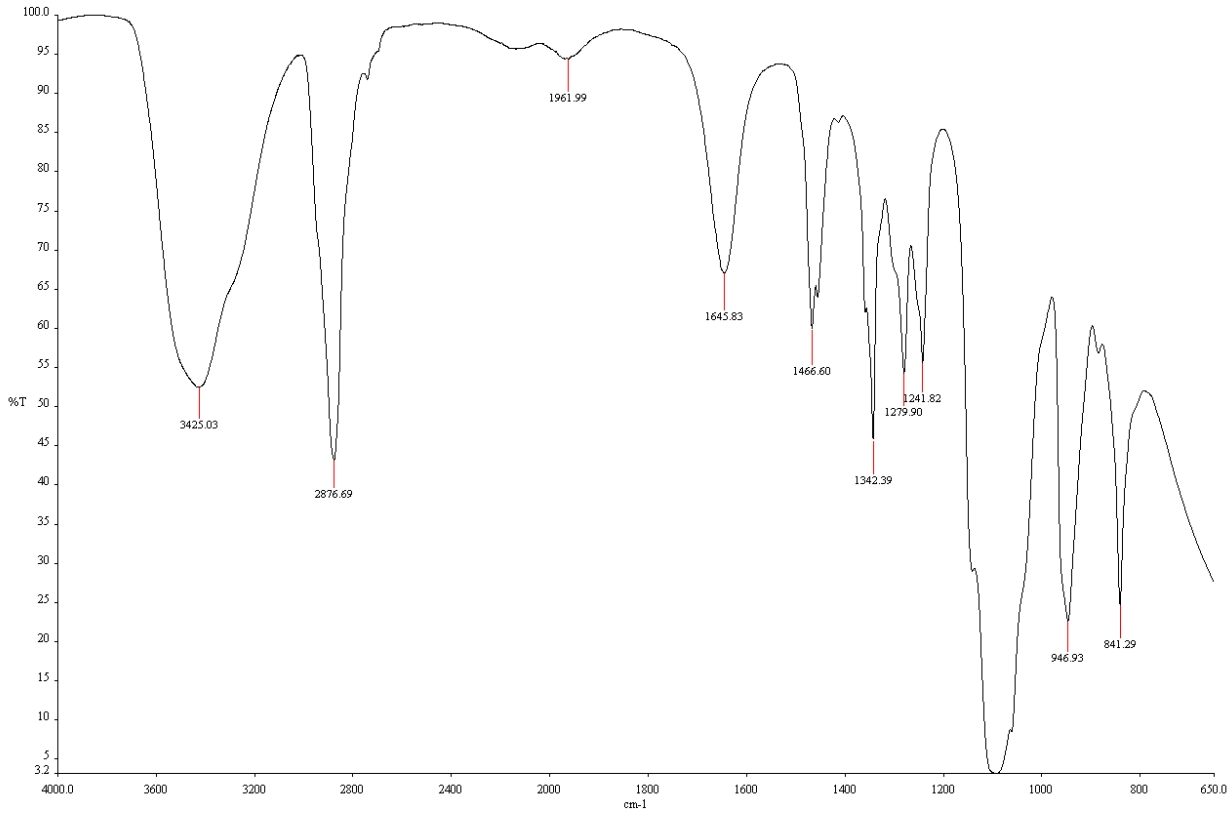

Figure 7: FTIR spectra of gold nanoparticle synthesized from aqueous leaf extract of *Piper guineenses*

Table 3: Characteristic of fungi isolates from store and field maize sample

Organisms	Field	Store	Colonial features on SDA	Morphological features on Microscope
Aspergillus niger	+	+	The initial growth is white, becoming black as it grows older	Hyphae are septate. Conidiophores formed terminate in a swollen vesicle
Aspergillus flavus	+	+	The initial growth is white, becoming pale yellow as it grows older	Vesicle are globule and phialides are produced directly from the vesicle surface. Conidiophores terminating in swollen vesicle
Fusarium species	+	+	White colony like cotton but less aerial mycelium growth	Septated hyphae. Rod like and slightly bent
Aspergillus fumigatus	+	+	The initial growth is white, becoming grey green as it grows older	Conidial heads are short, columnar and uniseriate. Conidiophores stipes are smooth- walled and vesicles are subglobose in shape
Trichophyton mentagrophytes	+	-	Dark reddish-brown color with a suede-like surface texture	Numerous single-celled microconidia formed which are hyaline, smooth-walled and spherical in shape
Cladosporium species	-	+	Colonies are slow growing, suede-like and blackish-brown in color	Conidia are produced in branched chains, smooth, one to four celled and have a distinct dark hilum
Acremonium species	-	+	The colonies are slow growing, compact and moist at first, becoming powdery and suede-like	Hyphae are fine. Conidia are one-celled and hyaline
Microsporum audouinii	-	+	Colonies are flat, spreading and pinkish-brown in color	Produces thick-walled intercalary chlamydospores

SDA: Sabourand Dextose Agar

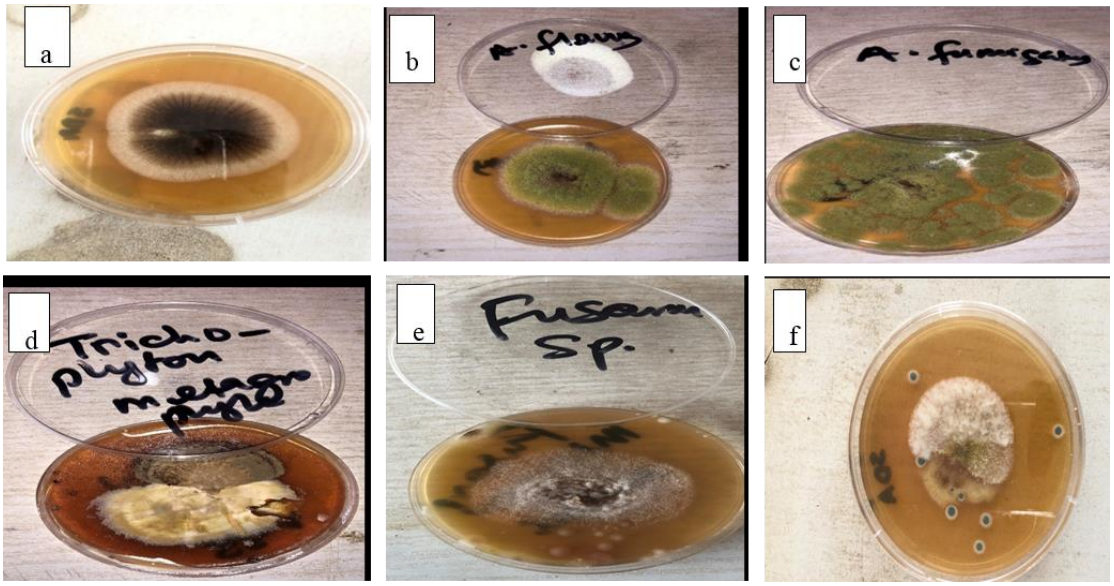

Figure 8: Fungi isolates identified from stored and field maize samples.
a-*Aspergillus niger*. b-*Aspergillus flavus*. c-*Aspergillus fumigatus*. d-*Trichophyton entagraphytes*. e-*Fusarium species*. f-*Microsporium auduinii.*

Antifungal activity of gold nanocomposite of N. sativa against fungi isolates from stored and field maize

The antifungal activity of nanocomposite formulation of *N. sativa* oil by mycelial growth inhibition against fungi isolates from stored maize and field maize are presented in Tables 4 and 5 respectively. The unconjugated NS oil and all other composite produced growth inhibition of *Aspergillus niger, Aspergillus flavus, Fusarium* species, *Aspergillus fumigatus, Trichophyton metagrophytes, Cladosporium* species, *Acremonium* species, *Cladosporium* species, and *Microsporium auduinii* isolated from the stored maize (Table 4). However, the nanocomposite III shows the highest significant ($p<0.05$) mycelial growth inhibition against all the fungi isolates when compared with the standard drug (Fluconazole), unconjugated Nigella sativa oil, and nanocomposites II and I (Table 4). Similarly, trends of mycelial growth inhibition were observed for fungi isolated from field maize; *Aspergillus niger, Aspergillus flavus, Fusarium* species, *Aspergillus fumigatus, Trichophyton metagrophytes,* and *Cladosporium* species (Table 5). The nanocomposite III also showed the highest significant ($p<0.05$) mycelial growth inhibition against all the fungi isolates identified from field maize when compared with the standard drug (Fluconazole), unconjugated *Nigella sativa* oil and nanocomposites II and I (Table 5).

Minimum Inhibitory Concentrations and Minimum Fungicidal Concentrations of nanocomposite III

Since the formulated *N. sativa* oil nanocomposite III significantly suppressed the mycelial growth of fungi, it indicated that the formulations are capable of inhibiting isolated pathogens. Thus, selected for MIC and MFC against individual fungal isolates from stored and field maize (Table 6). The MIC range of the *N. sativa* oil nanocomposite III is between 6.25 – 25.00µg/mL, while that of MFC is between 25.00 -50.00µg/mL. *Aspergillus flavus* and *Cladosporium* species were the most sensitive to the nanocomposite III treatment, having MIC and MFC of 6.25 and 25 µg/mL respectively. The inhibitory activity of the nanocomposites may be attributed to the ability of membrane

Table 4: Antifungal activity of nanocomposites as determined by mycelial growth inhibition tests of stored maize (mm)

Fungi	Control	NS	I	II	III
Aspergillus niger	9.75±0.25^{a}	10.50±0.50^{ab}	11.25±0.25^{b}	14.50±0.50^{c}	23.00±1.00^{d}
Aspergillus flavus	8.50±0.50^{a}	11.00±0.00^{b}	11.50±0.50^{b}	13.50±0.50^{c}	25.50±0.50^{d}
Fusarium species	9.25±0.75^{a}	10.50±0.50^{b}	12.50±2.50^{c}	15.00±0.00^{d}	24.50±2.50^{d}
Aspergillus fumigatus	9.25±0.25^{a}	10.75±0.75^{b}	9.75±0.75^{a}	14.50±0.50^{c}	25.25±1.25^{d}
Trichophyton metagrophytes	8.50±0.50^{a}	9.50±0.50^{ab}	10.50±0.50^{b}	13.75±0.25^{c}	24.00±1.00^{d}
Cladosporium species	10.50±0.50^{a}	10.75±0.25^{a}	11.00±1.00^{a}	16.00±1.00^{b}	24.50±0.50^{c}
Acremonium species	9.75±0.25^{a}	10.00±0.00^{a}	10.50±0.50^{a}	12.00±1.00^{b}	24.50±2.50^{c}
Microsporium auduinii	9.50±0.50^{a}	10.00±0.00^{a}	10.75±0.75^{a}	13.25±0.25^{b}	25.25±1.50^{c}

Data are presented in Mean ± Standard error of three replicate determinations. Data with different superscript alphabet across a row are significantly different ($p< 0.05$). **Key**: Control: Fluconazole; NS: *Nigella sativa* oil; I: *Nigella sativa* Oil-Polyethylene glycol; II: Biosynthesized gold Nanoparticle-Polyethylene glycol; III: Biosynthesized gold Nanoparticle-*Nigella sativa* oil-polyethylene glycol

Table 5: Antifungal activity of nanocomposites as determined by mycelial growth inhibition tests of field maize (mm)

Fungi	Control	NS	I	II	III
Aspergillus niger	10.64±0.84^{a}	10.56±0.53^{a}	11.45±0.54^{b}	14.55±0.45^{c}	24.35±0.47^{d}
Aspergillus flavus	8.35±0.45^{a}	11.56±0.43^{b}	11.34±0.03^{b}	13.35±0.53^{c}	25.03±0.22^{d}
Fusarium species	10.55±0.05^{a}	11.32±0.43^{b}	10.42±0.25^{a}	15.56±0.90^{c}	22.26±0.90^{d}
Aspergillus fumigatus	9.42±0.57^{a}	10.98±0.90^{b}	9.90±0.74^{a}	14.83±0.26^{c}	24.04±0.64^{d}
Trichophyton metagrophytes	8.24±0.43^{a}	10.03±0.26^{b}	10.24±0.43^{b}	14.45±0.09^{c}	23.24±0.52^{d}
Cladosporium species	10.07±0.74^{a}	11.53±0.09^{b}	10.43±0.03^{a}	15.03±0.25^{c}	25.24±0.76^{d}

Data are presented in Mean ± Standard error of three replicate determinations. Data with different superscript alphabet across a row are significantly different ($p < 0.05$). Control: Fluconazole; NS: *Nigella sativa* oil; I: *Nigella sativa* Oil-Polyethylene glycol; II: Biosynthesized gold Nanoparticle-Polyethylene glycol; III: Biosynthesized gold Nanoparticle-*Nigella sativa* oil-polyethylene glycol.

Table 6: Minimum Inhibitory Concentrations and Minimum Fungicidal Concentrations of nanocomposite III against the fungi isolates from stored and field maize

Fungi	Stored Sample		Field Sample	
	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	MFC (µg/mL)
Aspergillus niger	12.50	50.00	12.50	50.00
Aspergillus flavus	6.25	25.00	6.25	25.00
Fusarium species	12.50	25.00	6.25	25.00
Aspergillus fumigatus	25.00	50.00	25.00	50.00
Trichophyton entagrophytes	12.50	50.00	12.5	50.00
Cladosporium species	6.25	25.00	6.25	25.00
Acremonium species	25.00	50.00	-	-
Microsporum audouinii	25.00	25.00	-	-

MIC= Minimum Inhibitory Concentrations, MFC: Minimum Fungicidal Concentrations

DISCUSSION

Plants are natural sources of bioactive compounds that can act as antioxidants or as antimicrobial agents in a biological system. Chemical compounds confirmed in *Piper guineenses* extract are known to be good stabilizing agents, possess antioxidant properties; abilities to donate electrons in a chemical reaction, and also to bind to nanoparticles during synthesis to prevent their excessive growth (Sivaraman et al. 2009; Shittu and Ihebunna 2017). Terpenoids may target and destroy the microbial cell membrane leading to the loss of their intracellular constituents (Erguden 2021). Tannins are reported to be able to suppress protein synthesis in microorganisms, hindering the enzymes from digesting nutrients (Echo *et al*., 2012).

Some essential oils can penetrate microbial cells due to the lipopolysaccharides content of the cell membrane to inhibit their growth. This may give easy access for N. sativa oils to function and exert their effect on fungi. The outcome may be dependent on the chemical constituents and their concentration in the oil (Ahn *et al.,* 2020). The exposure of plasma membrane to p-cymene and thymoquinone has been reported to have a detrimental effect on the integrity of the fungi cells thereby reducing their cellular generated energy (Zhang *et al.,* 2017; Epand *et al.,* 2010). Hexadecanoic acid targets DNA gyrase and hinders its function in nucleic acid synthesis leading to the death of the microorganism (Sanabria-Ríos *et al.,* 2020).

The color change to ruby red observed during the synthesis of AuNPs is an indication of the formation of stable AuNPs (Nagalingam et al. 2018). This color change is associated with the excitation of surface plasmon vibrations in the AuNPs ((Huang *et al.,* 2007; Shittu *et al.,* 2016). The biosynthesis of nanoparticles from plant products is an environmentally friendly method that does not release toxic chemicals. The phytochemical contents in plants are reported to have bio-reductive properties and thus, shown in their ability to transfer electrons to Au (III) to form AuNPs ((Nune et al., 2009). Phytochemicals have been known to stabilize and improve the development of nanoparticles (Jadhav et al. 2016). The UV-visible spectra demonstrate the integrity of a colloidal solution, as well as the stability and quality of synthesized nanoparticles (Mourdikoudis *et al.,* 2018). The different sizes and distribution of vibrational energies of the AuNPs promote the widening of the absorption peak observed (Afshan *et al.,* 2020). The maximum absorption peak of UV-visible light is dependent on the particle sizes and concentration of nanoparticles Goh et al., (Goh *et al.,* 2014). AuNPs are known to display absorption bands

within the range of 500–600 nm on account of surface plasmon resonance (Parida *et al.,* 2021).

The AuNPs occupied 80 percent of the entire volume of the sample analyzed based on the intensity percentage. The interaction of the reducing agents with the gold ions and the adsorption processes of stabilizing agents on the AuNPs affect the variation in AuNPs sizes. Hence, the observed wide range of AuNPs sizes (Khodashenas and Ghorbani 2019). The size of nanoparticles determines the extent to which they will interact with receptors in a living system and since small-sized particles have a larger surface area, they will deliver drugs faster compared to larger nanoparticles (Barar 2015).

The dispersed nature of the AuNPs could be attributed to the method of synthesis and their interactions with the stabilizer which is suspected to induce an effective collision of the particle preventing agglomeration of the AuNPs (Haruta 2004; Zhao *et al.,* 2017). The phytochemical coating of AuNPs shielded the particles from forming clusters, leading to a discrete form of the particles as observed in this study (Nune *et al.*, 2009). Shapes, sizes, and surface areas of nanoparticles play an important role in cellular uptake and also facilitate chemical processes (Zhao *et al.,* 2017).

Peaks from 1279.90 cm−1 correspond to C-N stretching (aromatic amines). The functional groups identified in the AuNPs suggest the presence of chemical constituents with the particles. The hydroxyls, amines, and other groups observed in this spectrum were obtained from the phytochemicals in the extract and adsorbed on the surfaces of the AuNPs. These bioactive compounds function as strong capping mediators and influence the reduction of Au ions (Afshan et al. 2020; Santhoshkuma *et al.,* 2017). Hence, the presence of phenolics, alcohols, alkanes, and alkene compounds is considered to be responsible for the reduction and stabilization of AuNPs (Ahmada *et al.,* 2016).

Some of the fungi isolated from the maize are those frequently associated with humid regions. Their presence in the grains is linked to poor storage facilities, contaminations from high moistness in which the grains are exposed or the methods of their preservation (Onyeze *et al.,* 2013; Ramsdam *et al.,* 2021). During the process of packing the grains for storage, several types of fungi may remain attached to maize seeds triggering deterioration and leading to a reduction of the nutritive value or simply remaining viable to infect germinating seedlings (Tsedaley 2016; Akwaji *et al.,* 2016). The surrounding temperature, storage time, or high moisture content may be a contributing factor in the high number of species of fungi isolates identified from the stored maize (Giorni *et al.,* 2009).

The formulated nanocomposite significantly suppressed the mycelial growth of fungi, indicating that the formulations are capable of inhibiting isolated pathogens. Several plant oils exhibit antifungal activity against a variety of fungi including seed-borne (Ramsdam *et al.,* 2021). Alkaloids, polyphenols, and glycosides are some of the compounds present in *Piper guineense*, that can enhance the effects of *N. sativa* nanocomposite on the growth of fungi by triggering destruction to the integrity of the cell membrane, leading to leakage of its cellular components, inhibition of protein synthesis or enzymes involved in DNA synthesis of the fungi (Echo *et al.,* 2012; Pinho *et al.,* 2016; Daniel *et al.,* 2021). The high quantity of some chemical compounds in *N. sativa* oil may have contributed to the antifungal activity observed in this study. 9,12-octadecadienoic have been reported to possess antimicrobial activity (Wei *et al.,* 2011). The hydrophobicity of cyclododecane may disturb the fungi cell membrane, and allow the penetrability of the volatile compound, leading to leakage of the cell content and ultimate cell death (*et al.,* 2016). The antifungal activity may be due to the synergistic effect of the AuNPs, extract, and *N. sativa* oil. Green synthesized nanoparticles are known to abate microbial infestation (Kaur *et al.,* 2018; Duhan *et al.,* 2017).

The inhibitory activity of the nanocomposites may be attributed to the ability of membrane proteins in the fungi to aid the uptake of nanoparticles into the cells. The sizes and shapes of nanoparticles influence their ability to target and absorb into specific cells effectively with smaller-sized particles having a greater surface area to deliver drugs (Shittu *et al.,* l. 2022; Arvizo *et al.,* 2010). The inhibitory action of the nanoparticles in the organisms may involve the inability of DNA synthesis, resulting in the non-expression of ribosomal subunit proteins, and enzymes crucial for the production of cellular energy (Kim *et al.,*

2012; Botteon *et al.,* 2021). AuNPs may have affected the role of the cellular enzymes leading to the degradation of proteases in fungi and the interruption of some biochemical processes (Pathak *et al.,* 2013). The larger surface areas of the smaller-sized AuNPs may have contributed to the membrane permeability of the nanocomposites and the ultimate cell death of the fungi (Rotimi *et al.,* 2019).

CONCLUSION

Piper guineenses and *N. sativa oil* contains important bioactive components that are capable of reducing $HAuCl_4$ into AuNPs which are absorbed in 530nm, with spherical-shaped particles size of 30-120nm confirmed with the dispersion of light by the Tyndall effect. FTIR results revealed the functional groups present in the bioactive compounds which facilitated the bio-reduction of gold into corresponding nanoparticles. The formulated nanocomposite III exhibited the highest anti-fungal activity with MIC and MFC of 6.25 and 25.00µg/mL against the fungal isolates from both fields and stored maize This result demonstrates that N. sativa oil nanocomposite could serve as a potential strategy for the management of fungal infection of stored maize.

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CONTRIBUTIONS

OKS conceived and designed the research. JTO performed research, analyzed data and wrote the first draft. TYG interpreted data, revised and edited final manuscript: All authors read and approved the final manuscript.

CONFLICT OF INTEREST

There exists no conflict of interest among authors.

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