

## Antifungal Activity of Chitosan Nano-Encapsulated *Cinnamomum zeylanicum* Bark Essential Oil Against Mycotoxigenic Fungi from maize and Sorghum

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### ABSTRACT

Grain contamination by mycotoxins-producing fungi threatens food safety and quality, decreasing agricultural productivity and increasing economic losses. This study evaluated the antifungal efficacies of both free Cinnamon Bark Essential Oil (CBEO) and its Nano formulation with chitosan nanoparticles (CBEO-NP), against five Mycotoxigenic fungi isolated from maize and sorghum, collected across various locations within Niger State, Nigeria. The CBEO was extracted using soxhlet extraction method with n-hexane as extracting solvent and characterized by GC-MS analysis. Chitosan nanoparticles were synthesized via ionic gelation method and the characterization of the nanoparticles was done using Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Ultraviolet-visible Spectrophotometry (UV-Vis), and Fourier Transform Infrared Spectroscopy (FTIR) analyses. The antifungal bioassay was carried out at varying concentrations of 25, 50, 75, and 100 % (v/v) for CBEO and 40, 80, 120, 160 and 200 mg/ml for CBEO-NP using agar well diffusion method. The GC-MS profiling of CBEO revealed 22 bioactive constituents, with cinnamaldehyde (36.56 %) as the dominant compound. Chitosan nanoparticles synthesized, exhibited an average size of 45.61 nm. The results for the antifungal bioassays showed susceptibility of all the tested fungi at the highest concentrations of 100% (v/v) for CBEO and 200 mg/ml for CBEO-NP with *Aspergillus niger* (21.0 ± 1.0 and 25.0 ± 1.0) mm, *Aspergillus fumigatus* (18.33 ± 1.53 and 21.0 ± 1.0) mm, *Aspergillus flavus* (18.0 ± 1.00 and 21.67 ± 0.58) mm, *Penicillium spp.* (22.68 ± 1.53 and 24.0 ± 1.00) mm and *Fusarium spp.* (21.68 ± 0.58 and 22.33 ± 2.08) mm respectively. Both CBEO and CBEO-NPs showed significant inhibitory properties against the mycotoxigenic fungi with an enhanced efficacy observed in its nano-formulation. These findings thus, underscore the importance of CBEO-based nano-formulation as a promising natural alternative for combating fungal contamination in grains and improving food safety.

**Keywords:** *Cinnamomum zeylanicum*, Chitosan nanoparticles, Maize, Sorghum, Antifungal activity.

## INTRODUCTION

Mycotoxigenic fungi, particularly species of *Aspergillus*, *Fusarium*, and *Penicillium*, are major contaminants of cereals in tropical regions and a persistent cause of food quality deterioration, economic loss, and public health concerns (Luo *et al.*, 2021). Their ability to produce mycotoxins - toxic secondary metabolites (e.g., aflatoxins, ochratoxins, and deoxynivalenol), contaminate grains causing serious toxicity to both humans and animals upon consumption (Misihairabgwi *et al.*, 2019). Grain contamination by mycotoxins not only threatens human and animal health, but also leads to significant economic losses by reducing crop yields, impacting trade, and increasing food insecurity worldwide (Meneely *et al.*, 2022). They are significant contributors to the spoilage and bio-deterioration of around one-fourth of global food crops, further exacerbating food security challenges (Chatterjee *et al.*, 2023).

Maize and sorghum are among Nigeria's most important staple cereals, cultivated extensively by smallholder farmers across multiple agro-ecological zones. Maize occupies roughly 6.5 million hectares (Onumah *et al.*, 2021), making it the country's leading cereal by area and production, and serves as the predominant source of calories for many households. About 80 percent of maize grain goes directly into human diets or animal feed, while the remaining 20 percent enters industrial processing, yielding various food and non-food products (Onumah *et al.*, 2021). On a per-capita basis, Nigerians consume nearly 35 kg of maize each year, which accounts for around 10 percent of daily caloric intake and similarly about 10 percent of the value generated through agro-industrial channels (Wossen *et al.*, 2017).

Sorghum, valued for its tolerance to harsh environmental conditions, ranks as the world's fifth most cultivated cereal and Africa's second (Kazungu *et al.*, 2023). In Nigeria, sorghum production reached an estimated 58.7 million tons in 2023 (Bakari *et al.*, 2023). Annual revenues from sorghum-derived products such as grains for food and feed, sweet stems for fodder, fiber for brooms, and substrates for bioethanol and alcoholic beverages exceeded US\$130 million (Bollam *et al.*, 2021). Despite the economic and nutritional importance of these two important grains, both maize and sorghum are highly susceptible to contamination by toxigenic moulds that proliferate under favorable environmental conditions (Bhunja, 2018).

Synthetic fungicides have been used as an essential tool in managing fungal pathogens in cereals and controlling mycotoxins contamination in food items. Over the past decade, azole fungicides have been shown to be effective in controlling infections of *Fusarium spp.* in wheat and in reducing deoxynivalenol (DON) contamination (Yoshida *et al.*, 2012). However, their efficacies have been increasingly compromised by the emergence of fungicide resistance. Resistance in *Fusarium graminearum* and other *Fusarium* species has been increasingly documented, which causes Fusarium head blight (FHB) in barley and wheat, stalk rot and Gibberella ear rot in maize, and one of the utmost liable

for economic setback in the agriculture and food industry worldwide (Dweba *et al.*, 2017). Given the rising incidence of resistant fungal strains and the limitations of synthetic antifungal agents, recent research has focused on eco-friendly biocontrol strategies. Reviews by Almeida *et al.* (2024) and Carrascál-Hernández *et al.* (2022) highlight essential oils (EOs), nano-delivery systems, and composite coatings as promising alternatives.

Cinnamon bark essential oil (CBEO) is recognized for its potent antimicrobial activity, mainly attributed to cinnamaldehyde, eugenol, and linalool (Mutlu-Ingok *et al.*, 2020). Essential oil from CBEO are classified as generally recognized as safe (GRAS), by the U.S. Food and Drug Administration (2016), making them suitable for food application. However, challenges such as volatility, hydrophobicity, and sensitivity to environmental factors reduced their stability and bioavailability (Pavoni *et al.*, 2020).

Nano-encapsulation with biodegradable carriers like chitosan offers a promising strategy to enhance essential oil performance by improving stability, solubility, and controlled release (Jamil *et al.*, 2016). This study evaluates the antifungal efficacy of free CBEO and nano-encapsulated CBEO against mycotoxigenic fungi isolated from maize and sorghum in Minna, Nigeria.

## **MATERIALS AND METHODS**

### **Plant Collection and Identification**

The dried *Cinnamomum zeylanicum* bark was purchased from Kure market, Minna, Niger, State, Nigeria (Latitude 9°33'N to 9°45'N, Longitude 6°32'E to 6°42'E) in the month of December 2024. The plant was identified at the department of Plant Biology, Federal University of Technology, Minna and assigned the voucher number, FUT/PLB/LAU/002. *Cinnamomum zeylanicum* bark was pulverized into fine powder and stored in an airtight container.

### **Sample Collection**

A total of 24 samples of maize and sorghum were randomly collected across four major markets in Minna metropolis (Kure, Gwari, Tunga, and Farm Centre) and transported aseptically to the laboratory for analysis.

### **Chemicals and Reagents**

The chemicals and reagents used in this study were of analytical grade. The chemicals and reagents employed in this study include distilled water, Sabouraud dextrose agar (SDA), lactophenol cotton blue, chloramphenicol, ethanol, normal saline, sodium hypochlorite, n-hexane, chitosan powder, acetic acid, sodium tripolyphosphate (TPP), tween 80, sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO), azoxystrobin/difenoconazole, among others.

### **Isolation and Identification of Fungi**

Fungi were isolated using the pour plate method. One gram of each sample was homogenized in 9 mL sterile normal saline ( $10^{-1}$  g/mL) and serially diluted five-fold. From the third dilution, 1 mL was plated onto 90 mm Petri dishes, mixed with ~15 mL

Sabouraud Dextrose Agar (SDA), and incubated at 28 °C for 5–7 days. Colonies were counted between days 3–5 and fungal load expressed as CFU/g (Sahab *et al.*, 2014). Distinct colonies were sub-cultured on SDA to obtain pure isolates, which were maintained on SDA slants for subsequent analyses (Samson *et al.*, 2014). Identification was based on both macroscopic and microscopic features using standard taxonomic keys (Leslie & Summerell, 2006; Pitt & Hocking, 2009; Samson *et al.*, 2014).

### **Extraction and Characterization of Cinnamon Bark Essential Oil**

Powdered *Cinnamomum zeylanicum* bark (150 g) was extracted using Soxhlet apparatus with n-hexane for 6 hours at 69°C. Solvent was removed via rotary evaporation and extracts were stored at 4°C (AOAC, 1990). The CBEO components were analyzed using a Varian 3800/4000 GC–MS system with BP5 capillary column under standard conditions. Identification was based on mass spectral comparison with NIST libraries (Adams, 2007).

### **Preparation and Characterization of Chitosan Nanoparticles**

Chitosan nanoparticles were prepared following ionic gelation. About 1% (w/v) chitosan solution was obtained by dissolving 1 g of chitosan (90% DD) in 100 mL of 1% acetic acid under magnetic stirring at 37 °C until completely dissolved. Subsequently, 5 mL of 0.5% (w/v) TPP solution was added dropwise at 0.2 mL/min under continuous stirring, and the pH was adjusted to 8 using 1 M NaOH. The mixture was stirred for an additional 1 hour at room temperature and centrifuged at 20,000 × g for 30 minutes at 4 °C to collect the nanoparticles. The synthesized chitosan nanoparticles (CNPs) were characterized using standard analytical techniques to determine their physicochemical properties (Farid *et al.*, 2024).

### **Encapsulation of Cinnamon Bark Essential Oil (CBEO)**

Encapsulation of CBEO in CNPs was carried out using modified methods of Hosseini *et al.* (2013); Farid *et al.* (2024). Three grams of synthesized CNPs were re-suspended in 30 mL of 1% (v/v) acetic acid and homogenized by stirring. Tween 80 (0.2% v/v) was added as an emulsifying agent and mixed thoroughly. Subsequently, 15 mL of CBEO was added dropwise into the CNP suspension under continuous stirring at room temperature and further stirred for 1 h to ensure encapsulation. The CBEO-loaded nanoparticles were collected by centrifugation at 20,000 × g for 30 min, washed with deionized water, and stored at 4 °C until analysis.

### **Evaluation of the Antifungal Efficacies of Antifungal Agents**

The antifungal activities of CBEO, CBEO-NPs, unloaded chitosan nanoparticle (negative control) and azoxystrobin/difenoconazole fungicide (positive control), were observed using a modified method of Masiello *et al.* (2019). Agar well diffusion method was carried out by adding the various concentrations prepared into the 5mm wells. The plates were incubated at 28°C for 5 days. The antifungal activities were evaluated by measuring the diameter (mm) of inhibition zone.

### **Data Analyses**

The data obtained from the research were statistically expressed as mean  $\pm$  standard deviation for three replicates. Analysis of variance was determined using One-Way Analysis of Variance (ANOVA) to determine the presence of significant difference among the groups. Acceptable value of ( $p < 0.05$ ) was considered to be statistically significant. The Statistical Package for the Social Sciences (IBM SPSS) software version 23 was used for these analyses. Graph pad prism software version 9.5.1.733 was also used to represent some of the data in the form of bar charts.

### **RESULTS**

Table 1 shows both the macroscopic and microscopic characteristics of the fungal isolates. Based on the morphological features, five fungal isolates from maize and sorghum samples were identified as belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium*. The isolates were further characterized as *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Penicillium spp.* and *Fusarium spp.*, based on colony morphology, pigmentation and microscopic features.

**Table 1: Morphological Identification of Fungal Isolates from Maize and Sorghum Samples**

Isolate code	Colony colour	Texture	Edge shape	Colony diameter (mm)	Microscopic characteristics	Fungal isolate
MA1	Brownsih-black	Powdery to granular	Irregular	45	Conidiophores long and smooth; vesicles globose; conidia black, rough-walled, in radiating chains	<i>Aspergillus niger</i>
MA2	Yellow-green	Velvety to floccose	Entire	40	Conidiophores rough; vesicles subglobose; phialides biseriate; conidia rough, spherical	<i>Aspergillus flavus</i>
SA3	Blue-green to grey-green	Powdery	Entire	35	Conidiophores short, smooth; vesicles columnar; conidia round, finely roughened	<i>Aspergillus fumigatus</i>
SP1	Bluish-green	Velvety	Lobate	30	Branched conidiophores forming brush-like penicilli; conidia globose, smooth	<i>Penicillium spp.</i>
SF1	Pink to violet	Cottony	Irregular	40	eptate hyphae; sickle-shaped macroconidia with 3–5 septa; microconidia oval to cylindrical	<i>Fusarium spp.</i>

Table 2 presents the chemical composition of cinnamon bark essential oil (CBEO), characterized by 22 components. Among the volatile compounds present, cinnamaldehyde,(E) was the main compound of the phytocomplex (36.56%) followed by cinnamaldehyde,dimethylacetal (11.87%) and other compounds whose percentage values ranged from 0.63 to 6.43%.

**Table 2: Bioactive Compounds Detected in CBEO Using GC-MS Analysis**

Peak #	Compound detected	Retention time (min)	Peak Area %	Molecular Formula	Molecular Weight
1	beta-Phellandrene	9.98	1.20	C <sub>10</sub> H <sub>16</sub>	136.23
2	Linalool	10.58	2.57	C <sub>10</sub> H <sub>18</sub> O	154.25
3	alpha-Terpeneol	14.75	0.63	C <sub>10</sub> H <sub>18</sub> O	154.25
4	Cinnamaldehyde, (E)	15.26	36.56	C <sub>9</sub> H <sub>8</sub> O	132.16
5	Cinnamaldehyde,dimethylacetal	17.08	11.87	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	180.24
6	Eugenol	17.50	4.60	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164.21

7	Copaene	20.00	2.09	C <sub>15</sub> H <sub>24</sub>	204.35
8	β-caryophyllene	20.75	1.46	C <sub>15</sub> H <sub>24</sub>	204.35
9	Acetate,cinnamyl ester	21.75	1.17	C <sub>11</sub> H <sub>12</sub> O <sub>2</sub>	176.21
10	Caryophyllenyl alcohol	23.00	3.36	C <sub>15</sub> H <sub>26</sub> O	222.38
11	Caryophylleneoxide	24.43	1.23	C <sub>15</sub> H <sub>24</sub> O	220.35
12	Tetradecanal	25.50	1.19	C <sub>14</sub> H <sub>28</sub> O	212.37
13	BenzylBenzoate	26.00	3.51	C <sub>14</sub> H <sub>12</sub> O <sub>2</sub>	212.25
14	n-Hexadecanoic acid	27.70	2.48	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.42
15	Hexadecanoic acid, methyl ester	30.00	2.33	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45
16	alpha.-Phellandrene,dimer	31.13	4.74	C <sub>20</sub> H <sub>32</sub>	272.47
17	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	32.41	4.84	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292.46
18	Octadecenoic acid, methyl ester	34.25	3.57	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.51
19	9-Octadecenoic acid, methyl ester	35.07	6.43	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.49
20	9,12-Octadecadienoic acid methyl ester	38.71	5.26	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.48
21	Eicosanoic acid, methyl ester	40.00	5.75	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	326.56
22	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	43.47	1.15	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356.54

Figure 1 shows the particle size distribution of the synthesized chitosan nanoparticles (CNPs) at a relatively nano-range size of approximately 45.61nm, with Polydispersity index of ~0.24 using dynamic light scattering (DLS) technique.

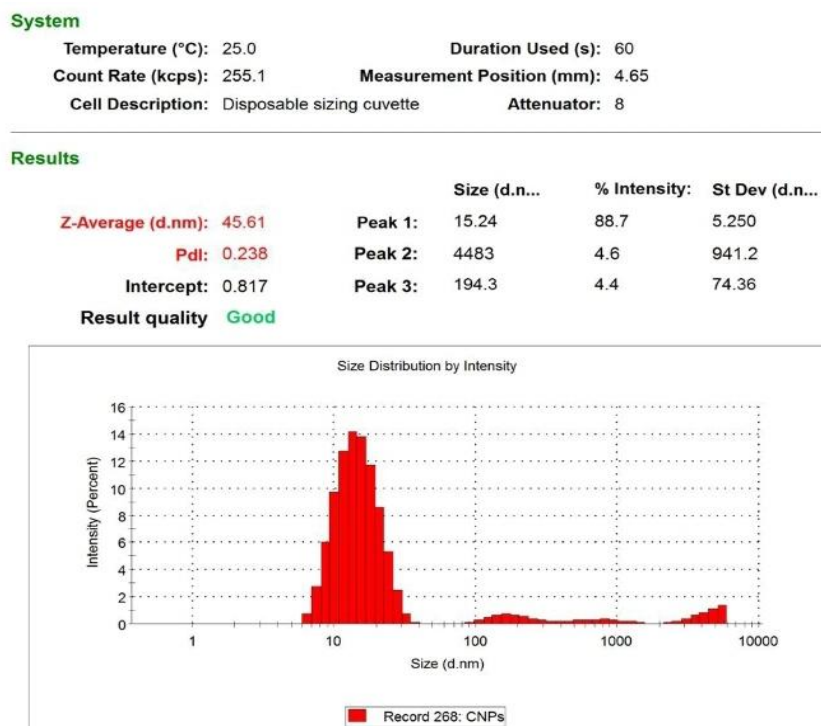


Figure 1: Dynamic light scattering (DLS) based analysis of average particle size and the poly-dispersity index, PDI for chitosan nanoparticles (CNPs).

Figure 2 shows the morphology characterization of the CNPs using Scanning Electron Microscopy technique. The chitosan nanoparticles (CNPs) exhibited a spherical with rough surface texture due to agglomeration.

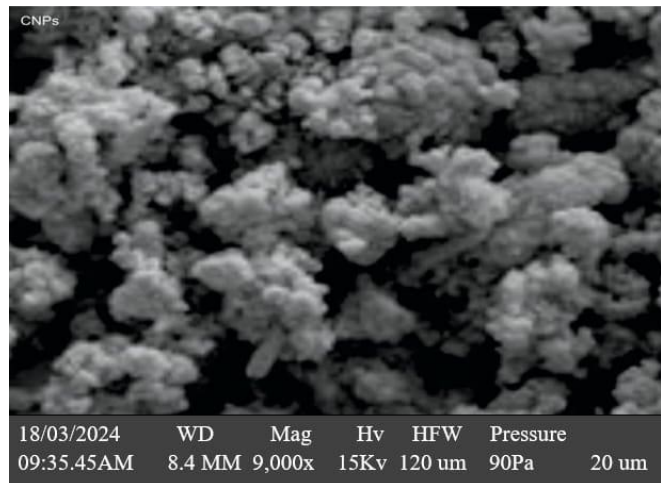


Figure 2: Scanning Electron Microscope (SEM) of CNPs at about 9000x magnification  
Figure 3 shows the UV–VIS spectrum of the synthesized chitosan nanoparticles at a distinct absorption peak of approximately 279.5 nm. The well-defined nature of the peak, with no significant broadening, suggests that the nanoparticles are uniformly dispersed and possess low polydispersity index.

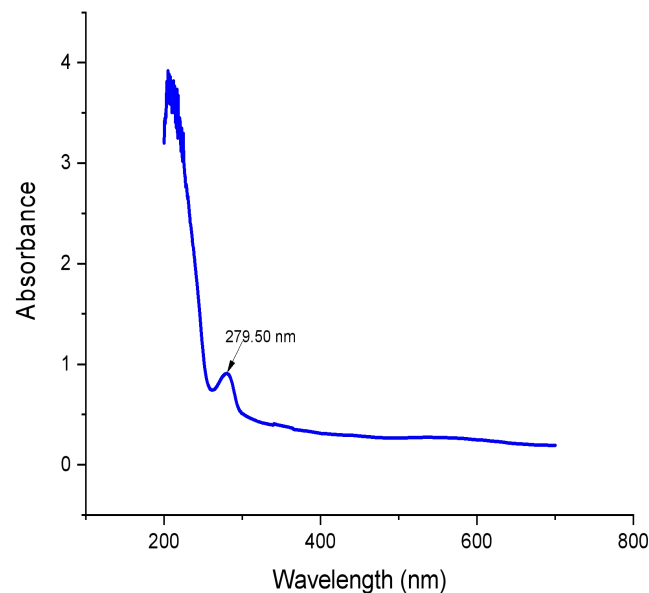


Figure 3: UV- VIS Spectrophotometric Analysis of CNPs

Figure 4 shows the FTIR spectrum of the synthesized CBEO-NPs composite. The FTIR spectrum displayed distinct absorption bands confirming effective encapsulation of the

essential oil. The broad O–H stretching band at  $3454.07\text{ cm}^{-1}$ , originating from chitosan and phenolic oil components, indicates strong hydrogen bonding between both materials. The band at  $3067.11\text{ cm}^{-1}$  corresponds to aromatic/aliphatic C–H stretching from cinnamaldehyde and other volatile constituents, while the small peak at  $2352.26\text{ cm}^{-1}$  reflects methylene C–H stretching of aliphatic chains. A sharp C=O stretching band at  $1700.65\text{ cm}^{-1}$  confirms the presence of cinnamaldehyde in the nanoparticles. The band at  $1449.33\text{ cm}^{-1}$  (C=C or CH<sub>2</sub> bending) and peaks at  $1191.35$ ,  $1072.34$ , and  $1007.43\text{ cm}^{-1}$  (C–O and C–N stretching) indicate contributions from both chitosan and the essential oil, signifying strong intermolecular interactions. The peak at  $850.98\text{ cm}^{-1}$  (aromatic C–H bending) verifies the presence of substituted aromatic rings, while the low band at  $582.18\text{ cm}^{-1}$  suggests M–O interactions typical of composite structures. Overall, these spectral features confirm the coexistence of functional groups from both components and the successful incorporation of CBEO into the chitosan nanoparticle matrix.

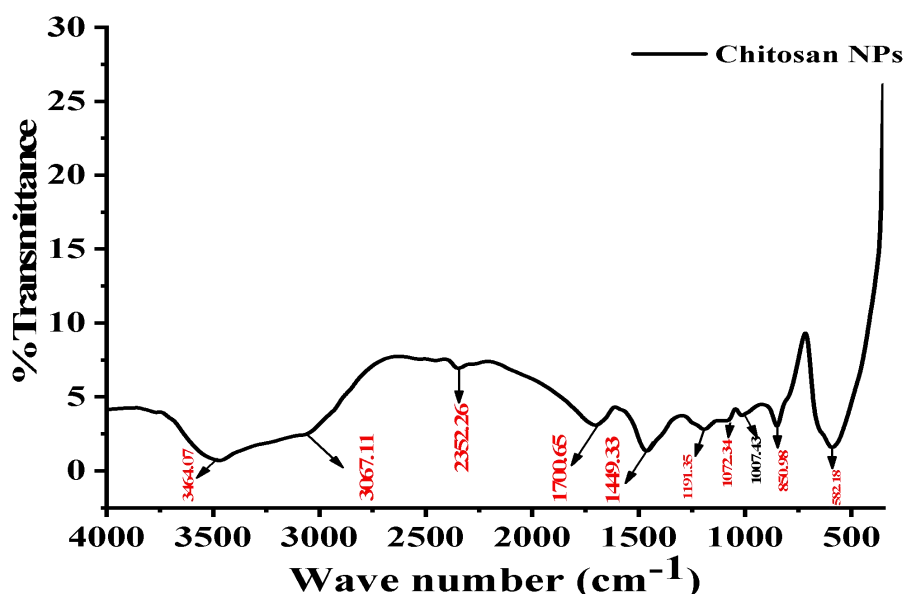


Figure 4: FTIR spectrum of CBEO-NPs showing characteristics absorption bands.

Table 3 Presents the inhibitory effects of CBEO, CBEO-NPs and controls (control a and control b) with each value reported as mean  $\pm$  SD ( $n = 3$ ). Free CBEO showed no antifungal activity at 25% and 50%. At 75%, activity was observed only against *A. niger* ( $16.0 \pm 1.0\text{ mm}$ ) and *Penicillium spp.* ( $17.6 \pm 0.58\text{ mm}$ ). At 100%, all fungi were inhibited, with zones ranging from  $18.0 \pm 1.0\text{ mm}$  (*A. flavus*) to  $22.68 \pm 1.53\text{ mm}$  (*Penicillium spp.*). CBEO-NPs exhibited no inhibition at 40–120 mg/mL. At 160 mg/mL, four fungi were susceptible: *A. niger* ( $21.67 \pm 0.58\text{ mm}$ ), *A. fumigatus* ( $19.33 \pm 0.58\text{ mm}$ ), *Penicillium spp.* ( $19.0 \pm 1.0\text{ mm}$ ), and *Fusarium spp.* ( $16.67 \pm 0.58\text{ mm}$ ), with *A. flavus* showing no activity. At 200 mg/mL, CBEO-NPs inhibited all species, producing zones from  $21.67 \pm 0.58\text{ mm}$  (*A. flavus*) to  $25.0 \pm 1.0\text{ mm}$  (*A. niger*). The positive control fungicide (100%) produced the highest inhibition across all fungi ( $23.0$ – $34.3\text{ mm}$ ). In contrast, unloaded

chitosan nanoparticles showed only minimal inhibition at their two highest concentrations.

**Table 3: Zones of Inhibition (mm) of Antifungal Agents against Mycotoxigenic Fungi**

Mycotoxigenic Fungi	CBE0 (25%)	CBE0 (50%)	CBE0 (75%)	CBE0 (100%)	CBE0-NP (40 mg/ml)	CBE0-NP (80 mg/ml)	CBE0-NP (120 mg/ml)	CBE0-NP (160 mg/ml)	CBE0-NP (200 mg/ml)	Control a (100%)	Control b (160mg/ml)
<i>Aspergillus niger</i>	NA	NA	16.0 ± 1.0	21.0 ± 1.0	NA	NA	NA	21.67 ± 0.58	25.0 ± 1.0	34.3 ± 0.33	1.00 ± 0.33
<i>Aspergillus fumigatus</i>	NA	NA	NA	18.33 ± 1.53	NA	NA	NA	19.33 ± 0.58	22.50 ± 1.0	24.0 ± 0.58	1.00 ± 0.00
<i>Aspergillus flavus</i>	NA	NA	NA	18.0 ± 1.00	NA	NA	NA	NA	21.67 ± 0.58	25.3 ± 0.33	1.27 ± 0.33
<i>Penicillium spp.</i>	NA	NA	17.67 ± 0.58	22.68 ± 1.53	NA	NA	NA	19.0 ± 1.00	24.0 ± 1.00	24.0 ± 0.58	1.33 ± 0.33
<i>Fusarium spp.</i>	NA	NA	NA	21.68 ± 0.58	NA	NA	NA	16.67 ± 0.58	22.33 ± 2.08	23.0 ± 0.58	1.33 ± 0.33

Values are presented as mean ± SD (n = 3).

**Key:** NA: No Activity, CBE0: Cinnamon Bark Essential Oil and CBE0-NP: Cinnamon Essential Oil-Chitosan Nanoparticles, Control a: Azoxystrobin/difneconazole fungicide and control b: Unloaded Chitosan Nanoparticle

Figure 5 shows the comparative inhibitory effects among the tested antifungal agents used at their highest concentrations of 100% (control a), 200 mg/ml (CBE0-NP) and 100% (CBE0). The azoxystrobin/difneconazole fungicide showed the highest inhibitory effects across all the tested fungi, because of its broad-spectrum inhibitory properties, followed by the nano-formulation as it enhanced the oil stability and solubility and lastly the free CBE0, to a lesser degree in comparison to the two due to its volatility and poor dispersion in aqueous media.

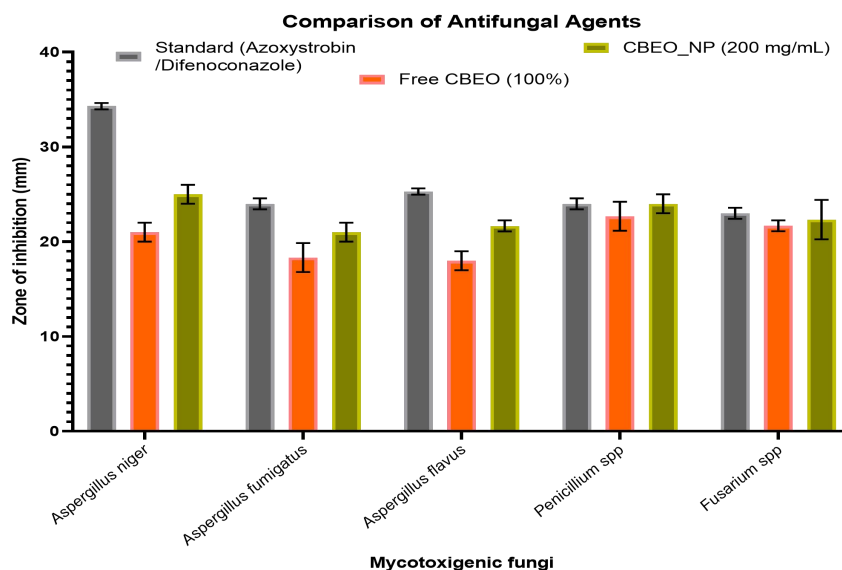


Figure 5: Comparison of Antifungal Activities among the Antifungal Agents

## DISCUSSION

The present study investigated the occurrence of mycotoxigenic fungi in maize and sorghum, the bioactive composition of cinnamon bark essential oil (CBEO), and the antifungal efficacy of CBEO-loaded chitosan nanoparticles (CBEO-NPs). Morphological analysis of fungal isolates revealed the presence of *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Penicillium spp.*, and *Fusarium spp.*, which are commonly associated with cereal grain contamination. Colony and microscopic characteristics were consistent with previous reports (Klich, 2002; Samson *et al.*, 2014; Frisvad *et al.*, 2019), and the predominance of *Aspergillus* species reflects their adaptability to low moisture conditions typical of improperly stored grains. These fungi are well-known producers of mycotoxins such as aflatoxins, fumonisins, and ochratoxins, highlighting potential food safety risks (Ezekiel *et al.*, 2014).

GC-MS analysis of CBEO identified 22 bioactive compounds, with cinnamaldehyde (36.56%) as the major constituent, alongside eugenol, caryophyllene, and other sesquiterpenes. These compounds contribute to the antimicrobial properties of CBEO, acting through disruption of fungal cell walls and inhibition of enzymatic activity (Zhang *et al.*, 2017).

Characterization of chitosan nanoparticles (CNPs) revealed an average particle size of 45.61 nm with low polydispersity (PDI 0.24), predominantly spherical morphology, and slight surface aggregation, confirming suitability for encapsulation and antimicrobial delivery (Ha *et al.*, 2019). UV–VIS and FTIR analyses further revealed successful synthesis and encapsulation, with absorption peaks indicative of intermolecular hydrogen bonding and electrostatic interactions between CBEO and the chitosan matrix, preserving the structural integrity of key bioactive compounds (Soni *et al.*, 2021).

*In vitro* antifungal testing showed that free CBEO inhibited all fungal isolates at 100% concentration, with inhibition zones ranges between  $18.00 \pm 1.00$ – $22.68 \pm 1.53$  mm. CBEO-NPs demonstrated enhanced activity at higher concentrations (160–200 mg/mL), achieving zones of  $21.67 \pm 0.58$ – $25.0 \pm 1.0$  mm, consistent with the sustained release behavior of nanoencapsulated formulations (Sarrafzadeh *et al.*, 2021). While the synthetic fungicide azoxystrobin/difenoconazole exhibited the highest inhibition ( $34.3 \pm 0.33$  mm). CBEO-NPs offer advantages in biodegradability, safety, and controlled antifungal delivery.

Overall, the study demonstrates the prevalence of mycotoxigenic fungi in staple grains, the potential of CBEO as a natural antifungal agent, and the enhancement of its efficacy through chitosan nanoparticle encapsulation, supporting the application of nano-formulated essential oils in food preservation and mycotoxin management.

## **CONCLUSION**

The study demonstrated that maize and sorghum purchased in Kure market Minna are significantly contaminated with mycotoxigenic fungi, predominantly *Aspergillus*, *Penicillium*, and *Fusarium* species, highlighting the potential risk of mycotoxin exposure to consumers. GC-MS analysis identified cinnamaldehyde as the major bioactive component of cinnamon bark essential oil (CBEO). Encapsulation of CBEO within chitosan nanoparticles (CBEO-NPs) improved its stability, bioavailability, and controlled release, resulting in enhanced antifungal efficacy against the isolated fungal strains, although synthetic fungicides displayed higher inhibition. These findings indicate that CBEO-NPs represent a promising, safe, and biodegradable alternative for managing fungal contamination in staple grains.

## **CONFLICT OF INTEREST**

The authors declared no conflicts of interest

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