Chapter

The Potential Application of Nanoparticles on Grains during Storage: Part 2 – An Overview of Inhibition against Fungi and Mycotoxin Biosynthesis

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Abstract

Mycotoxins are secondary metabolites synthesized by filamentous fungi. They are common food contaminants that cause mycotoxicosis in humans and animals. Due to the severity of health risk pose by these mycotoxins, many countries have enacted strict measures to curb this menace. One promising measure is the use of nanoparticles. Herein, we present an overview of the application of titanium dioxide, chitosan, ultradisperse humic sapropel suspension, and carbon-based nanoparticles, a novel and innovative method of reducing mycotoxin production and the subsequent contamination of grains. All nanoparticles considered enhanced cell permeability by disrupting the membrane, resulting in the outflow of cellular materials. However, concentration, volume, type, and illumination (sunlight) influenced the fungicidal potential of NPs.

Keywords: filamentous fungi, mycotoxins, nanoparticles, fungicide, reactive oxygen species

1. Introduction

Microorganisms, including fungi, contaminate grains during storage. These fungi do not only reduce grain quality, but also produce mycotoxins which pose health risks to consumers [1, 2]. According to Kady et al. [3], *Aspergillus, Fusarium, Penicillium,* and *Rhizopus* are the most common genera in barley, wheat, maize, and sorghum. These grains serve as staple food worldwide. Nowadays, nanotechnology is advancing in many fields, namely biotechnology, analytical chemistry, agriculture, and others. However, its application in crop protection is still in its early stages [4, 5].

The biocidal activity of nanoparticles is well documented. Herein, we proposed the utilization of nanoparticles to inhibit fungal growth and the production/synthesis of mycotoxins. Therefore, the second part of this chapter aims to discuss other

promising nanoparticles (titanium dioxide nanoparticles, chitosan nanoparticles, ultradisperse humic sapropel suspension (UDHSS) nanoparticles, and carbon-based nanoparticles/nanomaterials) of interest which could be applied during grain storage. The toxicological aspects, as well as the proposed modes of application are discussed.

2. Titanium dioxide nanoparticles

Titanium dioxide (TiO_2) nanoparticles (TiO_2-NPs), or ultrafine TiO_2, are particles of TiO_2 with diameters 1–100 nm. The TiO_2-NPs activity is exciting to researchers because of its specific characteristics which include; size, shape, crystal structure, surface stability among others [6]. They are among top five NPs used in consumer items such as cosmetics, food products, paints, and medicines [7]. TiO_2 received USFDA approval hence regarded as safe. It is widely used as food colorant in candies, sweets, chewing gums, etc. Anatase (used in printing inks and photocatalysts), rutile (used in colorants and sunscreens), and brookite are the three primary forms of TiO_2-NPs [8–12]. In 1985, Matsunaga et al. [13] first documented the antimicrobial activity of TiO_2. They observed that microbial cells were dead when exposed to a TiO_2-Pt catalyst illuminated with UV light.

The biocidal activity of TiO_2 has been reported [14–19]. **Table 1** shows the fungicidal activity of TiO_2-NPs against fungi species known to contaminate grains with the mycotoxins they synthesize.

TiO_2-NPs have been widely applied as antimicrobial agents in recent years due to their unique properties such as resistance to high temperatures, low solubility, high surface area, cost-effectiveness, hydrophilicity, and strong oxidizing properties [20].

TiO_2-incorporated polyethylene (PE) film inhibited growth of *E. coli* and *S. aureus*. UV light significantly enhanced the biocidal activity within 60 minutes of illumination [20]. Several studies [21–26] have documented the biocidal efficacy of TiO_2 against *E. coli*, *S. aureus*, *P. aeruginosa,* and *P. expansum*.

The photocatalytic oxidation of surfaces coated with TiO_2 and ultraviolet A (UVA) was effective against *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecium* than the control [27]. A collaborated research [28] assessed the biocidal activity of the crude and annealed TiO_2-NPs. The results revealed that doped Ag-TiO_2 (7%) NPs killed 100%, 95%, and 96% of *P. aeruginosa*, *S. aureus*, and *E. coli,* respectively, at 40 mg/30 mL.

Assessing ecotoxicity of TiO_2-NPs against bioluminescent bacterium (*Aliivibrio fischeri*), algae (*Pseudokirchneriella subcapitata*, *Scenedesmus subspicatus,* and *Chlorella vulgaris*), protozoon (*Tetrahymena pyriformis*), water flea (*Daphnia magna*), and an aquatic macrophyte, *Lemna minor* [29] revealed these organisms showed significant behavioral and physiological changes when exposed to low TiO_2-NP concentrations (0.1 and 0.05 μg/L), thus demonstrated the ability of TiO_2-NPs to alter molecular pathways via which these organisms obtained vital nutrition for growth and synthesis of compounds (i.e., chlorophyll, etc.).

Maneerat and Hayata [26] tested the fungicidal activity of TiO_2 photocatalysts against *P. expansum* in the form of TiO_2 powder and TiO_2 coated on a plastic film. Both TiO_2-NPs suppressed the conidial germination and growth of the fungi. The quantity of TiO_2-NPs added correlated with the fungicidal activity.

Nitrogen-doped TiO_2 [TiO_2 (N)] exhibited potent biocidal activity with regards to reducing the number of surviving organisms than carbon-doped TiO_2 [TiO_2 (C)]. Therefore, TiO_2 (N) NPs can inactivate spores of *B. anthracis* (hazardous

Organism	Reference
C. albicans, *S. cerevisiae*	[31]
A. niger AS3315	[32]
F. verticillioides	[33]
A. niger spores	[34]
A. niger, *S. cerevisiae*	[35]
F. oxysporum f. sp. *lycopersici*	[36]
C. albicans ATCC 10231, *F. solani* ATCC 36031	[37]
C. albicans	[27]
C. famata	[38]
C. vini, *Hansenula anomala* CCY-138-30	[39]
Cladobotryum varium, *Trichoderma harzianum*, *Spicellum roseum*	[40]
Cladosporium cladospoiroides, *Epicoccum nigrum, F. mucor*, *Penicillium oxalicum*, *Trichoderma asperellum*, *Pestaotiopsis maculans*	[41]
Diaporthe actinidae	[25]
Erysiphe cichoracearum, *Peronophythora litchii*	[42]
Molds and yeasts (not specified)	[43]
Fusarium spp. (*equisetii*, *oxypartan*, *anthophilum, verticillioides*, *solani*)	[44, 45]
P. citrinum	[46, 47]
P. expansum	[26]
S. cerevisiae	[13, 48]

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Table 1.
Fungicidal activities of TiO_2-NPs on mycotoxins-producing fungi

microorganism) under illumination by conventional light sources such as incandescent lamps [30].

2.1 Mechanistic action of TiO_2-NPs antimicrobial activity

TiO_2-NPs are the photocatalysts used to destroy unwanted organic compounds in the air, water, soil, and, more recently, in food [21].

Photocatalysis can be defined as the catalyst-driven acceleration of a light-induced reaction [49–52]. Homogeneous and heterogeneous photocatalytic processes utilize metal complexes (transition metal complexes like iron, copper, chromium, etc.) and semiconducting materials such as TiO_2, ZnO, SnO_2, and CeO_2 as catalysts. In the presence of light and heat, metal complexes become excited and form metal ion complexes, in contrast, semiconducting materials become excited due to the combination of electronic structures which is characterized by a filled valence band, empty conduction band, and light absorption properties, resulting in the generation of reactive oxygen species (ROS) or hydroxyl radicals. These hydroxyl radicals inflict damage to microbial cells [49–51, 53–55]. The subsequent hole in the valence band could further react with H_2O in the grains or hydroxide ions adsorbed on the surface of TiO_2-NPs to generate hydroxyl radicals (OH•), with electron in the conduction band reduce O_2 to superoxide ions (O_2^-) [21]. Gogniat and Dukan [56] demonstrated that DNA was denatured by hydroxyl radicals generated via the Fenton reaction resulting in cell death.

Electron paramagnetic resonance (EPR) spectroscopy study confirmed the photoproduction of hydroxyl radicals (OH•) from different TiO_2. The efficiency of hydroxyl radical generation depends on the source/origin of TiO_2 [57].

Cells are negatively charged [58] under optimum physiological condition due to heparan sulfate proteoglycans [59]. However, disease could trigger the cells to synthesize certain compounds which cause cell surface to become positively charge. Microbial cell could act as a hole for electron transfer between organism and its components [60]. The iron cluster on cell surface, in the periplasmic space, or inside the cell (proteins (such as ferritin)), could act as a precursor for iron-catalyzed Haber-Weiss reaction, which generates additional hydroxyl radicals in the presence of H_2O_2 and the superoxide ion [61].

Different treatments (photocatalysis, water, TIO_2, UV-A) applied to elucidate the effects of lipid peroxidation on *S. cerevisiae* revealed high malondialdehyde (MDA) in TiO2 -treated subjects with 2 hours. The results demostrated that TiO_2 was sufficient to damage membrane, thus interfered with permeability of the cell which led to the leakage of vital intracellular molecules (**Figure 1**) [48]. Similarly, Draper and Hadley [62] found photocatalysis-induced cell wall damage on S. cerevisiae [48]. This may decrease intracellular enzymatic activity as well as leaking of amino acids and NH_4^+, suggesting a drastic impact on proteins [63].

Cellular respiratory enzymes lost their activity after been exposed to irradiated TiO_2 (0.5 mg/mL), and the kinetics correlated with the losses of cell viability. Furthermore, when glucose was used instead of succinate as the electron donor, similar effects were observed. From this outcome, Li et al. [78] proposed that ROS generated from an irradiated TiO_2 surface, interacted with the polyunsaturated phospholipids in *E. coli*. Moreover, cell membrane structure was perforated due to lipid peroxidation creating a hole for more TiO_2-NPs to pass into interior of the cell, thus rendering respiratory proteinsinactive and subsequent cell death.

A progressive decrease in esterase activity was observed after exposing *S. cerevisiae* to irradiated TiO_2 [63]. Other researchers documented overexpression and inhibition (expressed at lower levels, including those encoding six cbb3-type cytochrome C oxidase subunits, an electron transfer flavoprotein, and

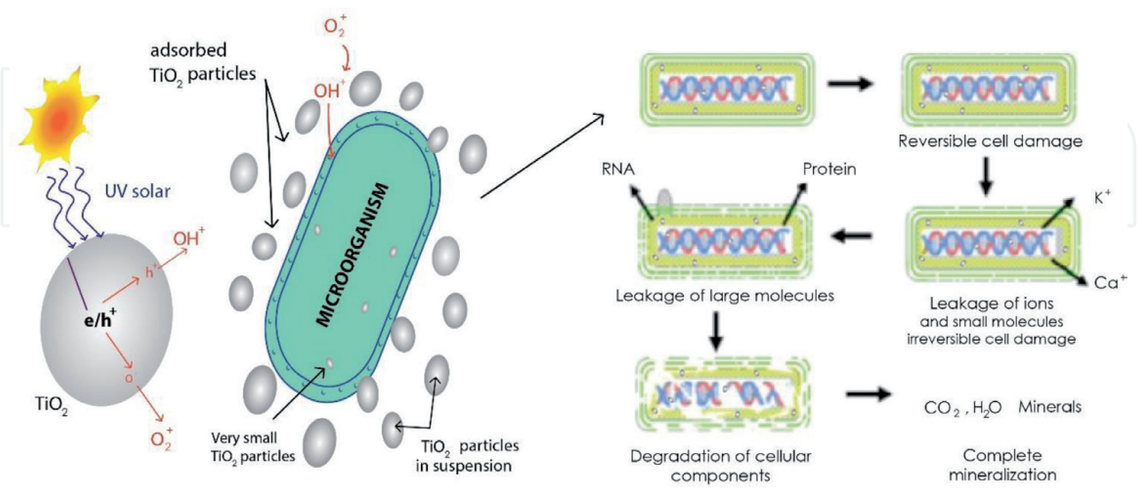

Figure 1.
Schematic illustration of the solar photocatalytic process for microbial cell inactivation in the presence of an aqueous suspension of TiO_2. Modified with permission from ref 4498160008350 [72]. Contact between the cells and TiO2-NPs affects membrane permeability; however, this is reversible. The availability of more NPs could enhance the damage to cell wall, thus allowing leakage of small molecules such as ions. Damage at this stage may be irreversible, and this accompanies cell death. Higher molecular weight components such as proteins could further be leaked followed by protrusion of the cytoplasmic membrane into the surrounding medium through degraded areas of the peptidoglycan and lysis of the cell. Intracellular components are then degraded progressively especially from the point of contact with photocatalyst, followed by complete mineralization.

two oxidoreductases) of genes associated with energy production and conversion processes. TiO_2-NPs exerted a stimulating effect on the respiratory chain and the electron transfer mechanism of the microorganism [64, 65].

Likewise, Matsunaga et al. [13] observed that incubating TiO_2/Pt NPs under metal halide lamp irradiation with *E. coli, Ch. vulgaris, L. acidophilus*, and *S. cerevisiae* inhibited cell respiration mechanisms and subsequent cell death. However, the results were not consistent as *Ch. vulgaris* had a thick cell wall mainly composed of polysaccharides and pectin hence, had comparative advantages (protection) over the other microbes.

Kubacka et al. [65] examined genome/proteome-wide expression profiles of *P. aeruginosa* PAO1 cells treated with TiO_2-based nanocomposite films. An increase and decrease in the levels of 165 and 151 transcripts were respectively reported in cells with TiO_2-coated Ethylene vinyl alcohol (EVOH) particles. Few proteins were detected at a statistically significant level ($p \leq 0.1$) in cells treated with TiO_2-coated EVOH particles compared to the control. TiO_2-UV treatment significantly suppressed (from 5.4- to 15.1-fold) the expression levels of genes essential for cell wall. However, 14 genes encoding for lipid metabolism essential for cell membrane were over-expressed (from 5.6- to 23.0-fold), unexpectedly, 2 were expressed at a lower level (from 5.5- to 7.4-fold).

In vivo and in vitro studies confirm that hydroxyl radicals inflict damage (breakage) on DNA strands. The extent of damage was minimized when dimethyl sulfoxide, catalase, or mannitol were incorporated in the reaction mixture [66]. However, the findings [66] contradicts previous studies [21, 67]. Exposing either purine or pyrimidine bases to TiO_2 and light from a 100-W Hg lamp resulted in the detection of NO_3^- and NH_4^+ ion. However, when native DNA and RNA molecules were subject to the same conditions, unknown peroxide species, along with phosphate and carbon dioxide, were detected, suggesting the breakage and mineralization of sugar-phosphate backbone of DNA and RNA molecules, respectively [68].

Kikuchi et al. [67] demonstrated the role of ROS on photocatalytic bactericidal activity. They utilized a porous polytetrafluoroethylene (PTFE) membrane in their system to physically separate the *E. coli* suspension from the TiO_2 thin. The results showed an impressive photokilling capability of the system with and without (control) PTFE - which was attributed to the generated H_2O_2. A group [69] demonstrated the stimulating effect of TiO_2-NPs on lipolytic activity in *A. niger*. The results showed that TiO_2-NPs significantly increased lipase biosynthesis (more than 1.5 times) compared to the control experiment. Treatment with TiO_2-NPs (size: 40 nm, concentration: 10 mg/L) in all culture media, enhanced lipolytic activity by 78.57% and 57.49% on the 4th and 5th day of cultivation, respectively. This finding reaffirms that smaller NPs can penetrate the cell membrane easily than bigger NPs, thus easily interact with molecular proteins, resulting in stimulating effects.

Gomes et al. [70] assessed the effects TiO_2-NMs (NM103, NM104, and NM105) and bulk TiO_2 against *Enchytraeus crypticus* with and without UV radiation. Microarray analysis revealed 10431 differentially expressed genes (DEGs) ($p < 0.01$) triggered as a result of exposure to TiO_2-NMs under no-UV. All samples under UV exposure registered an up-regulation of several transcripts, including caspase apoptosis-related cysteine peptidases, a signature of apoptosis activation, whereas under darkness the apoptotic signaling pathway was inhibited, suggesting that the oxi-radicals generated during the photoactivation of TiO_2 might substantially contribute to the apoptotic response and damage to the cell membrane. DNA damage was triggered after exposing samples to bulk/nano TiO_2 [71]. However, the findings of Gomes et al. [70] contradicted the [71] as reported that TiO_2-NMs_under no-UV impaired DNA repair, while bulk_TiO_2 under no-UV activated DNA repair mechanisms, suggesting that size of the TiO_2-NPs contributes to biocidal activity.

3. Chitosan nanoparticles

Chitin and chitosan have been widely used in the fabrication of polymer scaffolds [73]. Chitosan is a linear polysaccharide, a nontoxic biopolymer derived from the deacetylation of chitin, and used in many fields, including agriculture, medicine, and in vinification due to its biocidal potential. In agriculture, chitosan is used as biopesticide; in medicine, it is used to stop bleeding, wound healing, and as an antibacterial agent. Biodegradability, high permeability, nontoxic to humans, and cost-effectiveness are the features which make chitosan NPs unique. Chitosan and its derivatives have attracted considerable attention due to their biocidal activities [74, 75]. Several authors have reported the beneficial application of chitosan and its oligosaccharides which includes antitumor [76], neuroprotective [77], antimicrobial [78–85], and anti-inflammatory [86] agents. **Table 2** summarizes the fungicidal activities of chitosan against important agricultural microorganisms contaminating stored grains.

Fungal decay on pear fruit was suppressed by the combination of chitosan, yeast antagonist *Cryptococcus laurentii*, and $CaCl_2$. The results showed that mixture of chitosan at 0.5% and *C. laurentii* exerted greater effects compared to chitosan or *C. laurentii* alone. $CaCl_2$ showed little antifungicidal activity; however, it combination with chitosan and *C. laurentii* led to an effective and stable reduction of fungal decay [87], thus minimize or eradicate the menace of postharvest losses. Anthracnose in papaya caused by *Colletotrichum gloeosporioides* was controlled by the combination of *Burkholderia cepacia*, chitosan (0.75%) and $CaCl_2$ [88]. Postharvest blue, green, and grey molds affecting apple, oranges, and lemons were effectively controlled by mixing glycol chitosan (0.2%) with *Candida saitoana* [89–91]. Ag/chitosan-NPs showed significant antifungal activity against *A. flavus*, *A. alternata*, and *R. solani* hence could be used during grain storage [92, 93]. The synergistic effect (fungicidal activities) of hybrid copper(II) chitosan NPs to inhibit the growth of *F. graminearum*, *Verticillium dahlia* 57, and *F. solani* 169 was reported. In both cases, the NPs exerted an excellent efficacy in repressing the growth of fungi [94, 95]. Other authors reported that certain strains of *A. flavus*, *Cladosporium cladosporioides*, *P. aurantiogriseum*, and *Torulaspora delbrueckii* were resistant to chitosan at levels as high as 1% [7, 96]. The application of chitosan (0.025 and 0.05%) was effective against *Saccharomycodes ludwigii* and *Saccharomyces exiguous*. A rapid reduction in the number of yeast colonies was observed 2–4 min after application [97].

According to an earlier report, the effectiveness of the biocidal activity of chitosan depends on the molecular weight, degree of acetylation, and concentration [98, 99]. The application of NPs coated with polyethylene glycol (PEG) and natural garlic oil against *Tribolium castaneum*, a vital storage pest showed high efficiency over an extended period (8 months) due to the slow and persistent release of the active components [100]. The study highlighted the potential application of PEG-NPs as capsules to encapsulate various natural bioactive ingredients (i.e., oil from *Azadirachta indica*, extracts of *Khaya anthotheca*, alkaloid extracts of *Piper guineense* [101], etc.) for controll release and subsequent killing of microorganisms and pests during grain storage. Furthermore, [102, 103] extensively reviewed the literature on the biocidal activities of natural compounds (i.e., herbs, species, etc.) and its potential application in postharvest control.

3.1 Mechanistic action of chitosan nanoparticle antimicrobial activity

According to literature [116, 117], chitosan is composed of polycationic copolymers, with glucosamine and N-acetylglucosamine as axillary units, which contributes to its antimicrobial activity. The difference in environmental pH, pKa

Reference	Sources of chitosan (CTS)	Deacetylation (%)	Microorganisms	Concentration	Form applied
[104]	Not reported (industrially made)	71.5	*A. niger*, *A. parasiticus*	0 (control), 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/mL	Solutions
[105]	Not reported (industrially made)	75–85	*A. flavus* IMI242687, *C. cladosporioides* IMI 274019, *M. racemosus IMI 017313, P. aurantiogriseum* IMI 297953, *Byssochlamys* spp. BF, *Byssochlamys* spp. GCB, *Byssochlamys* spp. SB, *S. cerevisiae* 28, *S. cerevisiae* 3085, *S. cerevisiae* SD, *Z. bailii*906, *Z. bailii* HP, *S. exiguus* 391, *S. pombe, S. ludwigii*	0, 1, 5, 10 g/L (fungi) and 5 mL (for yeast)	Solutions
[106]	Not reported	85, 81, and 82 for low-, medium-, and high-molecular weight chitosan respectively	*A. alternata*, *B. fabae*, *F. oxysporum*, *P. digitatum, P. debrianum*, *R. solani*	250, 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 mg/L	Solutions
[97]	Not reported	79	*S. exiguus*, *S. ludwigii*, *T. delbrueckii*	0.05%, 0.005%	Solutions
[107]	Not reported	Not reported	*C. neoformans* strain *B3501*	Different concentration (0, 0.625, 1.25, 2.5, and 5 mg/mL) was employed	Biofilm
[108]	Industrially prepared chitosan	95	Psychrophilic, mesophilic, *Pseudomonad*, yeasts and molds	Not reported	Coating
[87]	Crab shell	~90	*P. expansum* (blue mold)	Various concentrations were applied for in vivo (0, 0.1, 0.5 and 1.0% (w/v)) in vitro (0, 0.001, 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0% (w/v)) experiment	Coating
[109]	Crab shell	82	*F. oxysporum f*. sp. ***radicis-lycopersici*** **1**27, *Candida krusei* VKPM Y-2594, *E. coli* ATCC 5945, *P. aureofaciens* VKPMB-7542, *E. agglomerans* VKPM B-7541, *B. subtilis* VKPM B-7540	0.1, 0.5, or 1.0 mg/mL for the fungicidal test while 0.9 mL of LMW or DDC-LMW chitosan was used for the bacteriostatic test	Solutions

Reference	Sources of chitosan (CTS)	Deacetylation (%)	Microorganisms	Concentration	Form applied
[110]	Not reported	75-85	*R. stolonifera*, *E. coli* DH5α strain	1.0 mL	Coatings and solutions
[111]	Not reported (industrially made)	100 (MMW) and 97 (LMW)	*R. oryzae* CECT 2340, *A. alternata* CECT 20560, *A. niger* CECT 2088	6 mL	Films and solutions
[112]	Not reported (industrial made)	85–89	Molds and total flora isolated from strawberries (*R. Stolonifer* and *B. cinerea*)	Final concentration before the spraying was 0.02%, w/v	
[113]	Not reported	80%	*R. solani* Kuhn, *F. oxysporum* (Schl.) *f.* sp. *Cucumernum owen, C. cucumerinum Ell.* Et Arthur, *B. cinerea Pers*., *C. orbiculare* (Berk. & Mont.) *Arx*, *P. asparagi* (sacc.a) *Bubak*, *A. Kikuchiama Tanaka*, *P. italicum Wehmer*, *Fusarium oxysporum Schl. F. Sp. Uasinfectum* (Atk.) Snyd. & Hans, *V. ctahliae Kleb., R. sclani Kuhn., B. berengeriana de Not. f.* Sp., *Piricola* (Nose) *Koganezaea et Sakuma*, *Sclerotinia sclerotiorum (Lib.) de Bary*, *Venturia nashicola Tanaka et Yamamoto*, *Gibberella zeae (Schw.) Petch* and *Phytophthora infestabs* (Mont.)	20, 30, 50, 100, and 150 mg/L	Solutions
[114]	Not reported	90	*A. niger*	0.1% or 1% (w/v)	Coatings, films, and liquid
[115]	Shrimp shell	Not reported	*A. alternata f.* sp. *lycopersici*	100–6400 μg/mL	Solutions

Table 2.
Some selected studies on fungicidal activities of chitosan NPs.

of chitosan and its derivatives creates an electric field for an electrostatic interaction between the polycationic structure and the anionic components of the cell (i.e., lipopolysaccharide and cell surface proteins), thus altering cell permeability [118–123]. High pH enhance rapid protonation, which increase the positive charge density (polycationic activity) of chitosan. A positive correlation was established between charge density and the biocidal activity of quaternized chitosan [124–127]. The inhibition potential of chitosan could be incapacitated when the charge density is reduced [120] due to changes of pH values. A similar outcome was reported by Qin et al. [128]. The antimicrobial mechanism was associated with the interaction of the negatively charged cell membranes and the cationic NH^{3+}groups of the chitosan derivative, which increase membrane permeability resulting in lysis [129] and leakage of macromolecules killing the cells. A carboxyfluorescein (CF)-loaded liposome study showed the effectiveness of lower molecular weight (LMW) chitosan on the cell membrane. The results showed that 0.75 μg/μL of LMW chitosan triggered moderate (≈7%) leakage of carboxyfluorescein found in the large unilamellar vesicles [130]. Similarly, Ing et al. [131] reported that chitosan NPs prepared from different concentrations of LMW and high molecular weight (HMW) showed efficient inhibitory activity against *C. albicans* (MIC_{LMW} = 0.25–0.86 mg/mL and MIC_{HMW} = 0.6–1.0 mg/mL) and *F. solani* (MIC_{LMW}= 0.86–1.2 mg/mL and MIC_{HMW} = 0.5–1.2 mg/mL) compared to the solution form (MIC = 3 mg/mL for both MWs and species). The authors established a statistical linear relationship between MW and particle size/zeta potential, thus provided an avenue for the manipulation of physicochemical properties of NPs to maximize its ability to penetrate the cells, trigger leakage of intracellular component, eventually killing the fungi and extend safety of the grains.

Researchers [132–135] proposed the fundamental mechanism contributing to interaction of negatively charged surface components of fungi and bacteria with the positively charged NH^{3+} groups of glucosamine (chitosan), which alters cell surface, and trigger leaking of intracellular substances, resulting in the impairment of vital physiological activities thus killing the microorganism. The inability of the second amino groups on N-acetylation of chitosan oligomers to donate positive chargeresult in the inhibition of its fungistatic activity [136]. Therefore, the contribution of NH^{3+} groups to biocidal activity cannot be ignored and should carefully be considered to maximize the effects.

The outer membrane (OM), inner core of lipopolysaccharide (LPS) molecules, and lipid components of Gram(−) bacteria are composed of anionic groups like phosphate and carboxyl, which contribute to the hydrophilic nature of the cell wall, thus creatin interaction of charges (electrostatic) with divalent cations. The OM protects Gram (−) bacteria cells from macromolecules and hydrophobic compounds (antibiotics and toxic drugs), giving Gram(−) bacteria a comparative advantage over Gram(+) bacteria. Therefore, breaching the integrity of the OM by chitosan could enhance its biocidal activity toward Gram(−) bacteria [137, 138]. On the other hand peptidoglycan (PG) and teichoic acid (TA) on the cell wall of Gram(+) bacteria have polyanionic group, which facilitates interaction via covalent bond with N-acetylmuramic acid in the PG layer, or via glycolipid- which links outer leaflet of the cytoplasmic membrane [139]. As documented by Kong et al. [120], the poly(glycerol phosphate) anion groups aid the structural stability of cell wall in addition to some membrane-bound enzymes.

LMW chitosan showed higher efficiency perforate/penetrate the microbial cell compared to HMW chitosan, which interacts with DNA to change the translation and transcription profile of genes. Chitosan binds to DNA with accurate precision, denying the organism of normal DNA transcription and mRNA synthesis, resulting in cell death [140–142].

A decrease in the induction of β-galactosidase was observed when yeast cells were exposed to chitosan. A concentration of 0.35 mg/mL chitosan reduced β-galactosidase activity by 32%. An increased in concentration (1.25 mg/mL) further led to the reduction of enzyme activity. The control experiment did not follow the trend. Likewise, the treated cells showed that chitosan greatly influenced protein biosynthesis in the yeast [130]. Previous work [143] documented cell sensitivity to chitosan, which altered the deletions of genes involved in sphingolipid (e.g., *ipt1Δ*, *skn1Δ*, *lcb3Δ*) and ergosterol (e.g., *erg3Δ*, *erg5Δ*) biosynthesis. In 1981, Hadwiger et al. [144] detected chitosan within plant cytoplasm and nucleus within 15 min after application, which indicate that chitosan can efficiently penetrate the thicker cell wall (the reason for its detection) and potentially interfered with DNA transcription and translation. This study suggests that chitosan can easily penetrate microbial cells since plants have a thicker cell wall than microbes.

Moreover, looking at the time factor (15 min), it is evident that chitosan can quickly interact with fungi and bacteria cellular DNA with subsequent inhibition of DNA transcription, as well as RNA and protein synthesis [140, 145, 146], leading to cell death. Chitosan triggered transcriptional responses when introduced to *S. cerevisiae* strain X2180-1A (MATa SUC2 mal gal2 CUP1). T-Profiler analysis showed cis-regulatory motifs apart from the environmental stress response correlated positively with expression in the chitosan-treated sample. Cin5p, Crz1p, and Rlm1p were the transcription factors associated with identified binding sites. Genes participating in cell wall organization, biogenesis, and signal transduction were also triggered in the treated sample compared to the control [134]. Some factors influencing the antimicrobial activity of chitosan is discussed above; however, Kong et al. [120] and Hosseinnejad and Jafari [147] published an excellent reviews on these factors.

4. Ultradisperse humic sapropel suspension (UDHSS) nanoparticles (UDHSS-NPs)

Sapropel is benthos found in fresh water, formed under anaerobic conditions from dead organic matter of anhydrobiotic microflora and microfauna. It is principally composed of nutrients (i.e., sugars, minerals, lipids, etc.) and organic compounds known as humic substances [148–151]. Sapropels and sapropel extracts showed antibacterial and antifungal properties in previous studies hence could used as an alternate and novel biocidal agent during grain storage. The antimicrobial properties of sapropels is attributed to the presence of humic substances [152–156]. Sapropel has become a popular raw material for therapeutic applications, production of sorbents, organic fertilizers, and food supplements [157]. UDHSS-NPs are organic NPs which exhibits potent biocidal activities due to the presence of humic substances [148]. Fulvic acids (FAs), humic acids (HAs), mumie, and humin are the principal constituents of humic substances (HSs) in sapropels [158–161], and are reportedly attribute to their biocidal properties. Many studies [152–155] have illustrated the inhibitory effects of sapropel on bacteria (*S. aureus*, *E. coli*, etc.) and yeasts (*Candida*, etc.). A micrograph of UDHSS-NPs is shown in **Figure 2** however, its characteristics were not included in the present study.

In a series of tests performed by Barakova et al. [148], experiments 2 and 3 exhibited most significant fungicidal effects on *A. niger,* a species which poses a greater threat to grain/food industries due to the potent mycotoxins it produce. A report showed that hematite NPs (hematite-HA complexes) significantly

inhibited the growth and gene expression of *P. putida* KT2440. The bactericidal activities were ascribed to the oxidative stress induced by generated ROS. It was also shown that the physicochemical properties of the NPs (e.g., surface charge and size) influenced the efficacy of the hematite-HA complexes [162]. Therefore, modification of UDHSS-NPs could improve its biocidal properties.

A group of researchers [163] assessed the fungicidal activity of HAs and FAs extracted from soils on phytopathogenic fungal species (*Physalospora piricola* (P.P), *Botrytis cinerea* (B.C), *Rhizoctonia cerealis* (R.C), *Fusarium graminearum* (F.G), *Phytophthora infestans* (P.I), *Sclerotinia sclerotiorum* (S.S), *Rhizoctonia solani* (R.S), *Cercospora arachidicola Hori* (C.H), and *Bipolaris maydis* (B.M)). The results showed that HA exhibited above 30% and 50% inhibition against B.C, R.C, F.G, P.I, and P.P, respectively. The inhibition exerted by HA on all the species was higher compared to FA except for B.C. Correlation analysis further revealed that the inhibition rates of HAs decreased significantly with time (years) ($p < 0.05$) against most tested fungi except P.I., whereas FAs showed a negative correlation with cultivation years ($p < 0.05$) against most of the tested fungi except F.G. and S.S.

Recently, Ong et al. [164] documented that HAs (10 mg L^{-1} HA) altered enzyme activity in zebrafish embryo. Physicochemical properties such as size, zeta potential, and particle dissolution influenced their actions. It was further shown that coupling HAs with NPs enhanced the activity of the composite NPs. The addition of HAs reduced the hydrodynamic diameters of all examined NP suspensions except cadmium selenide (CdSe) NPs. Ezhkov and colleagues [165] developed NP-sapropel composite with particle size 45.0–180.0 nm and investigated its effects on treated albino mice. The results showed scarring of organ walls and shedding/exfoliation of the superficial epithelial cells. Further histological analysis of the oesophagus wall showed a significant thinning of the horny substance and the removal of the stratified epithelium of the mucous membranes in areas in contact with the NPs.

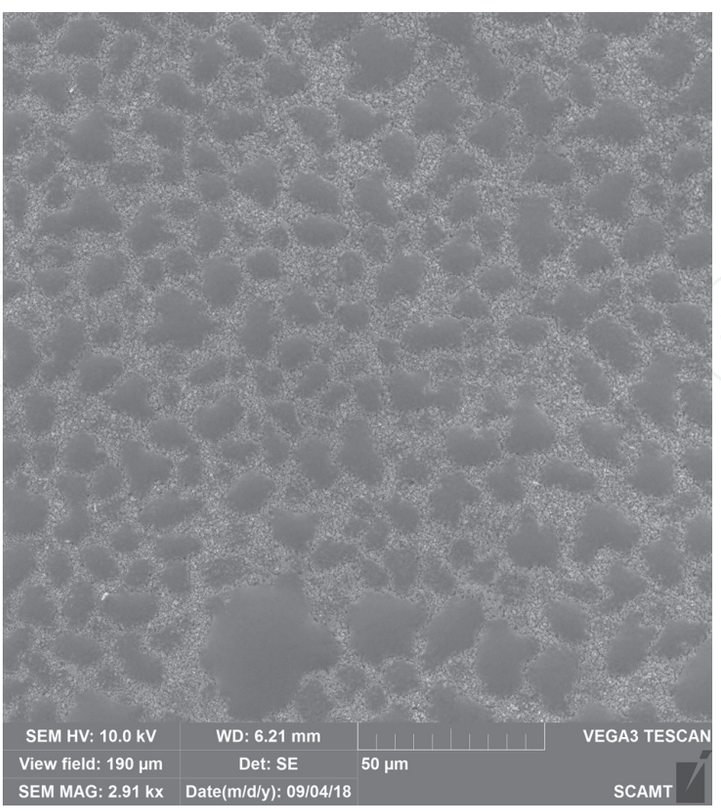

Figure 2.
UDHSS nanoparticles under a scanning electron microscope (SEM).

4.1 Mechanistic action of UDHSS-NPs antimicrobial activity

Several studies have described the biological activity of sapropel on enzymes, which confirms its antimicrobial activity. Details of these studies are discussed below in a quest to put forward a proposed mechanism by which UDHSS-NPs kill microorganisms. Environmental factors such as temperature, pH, oxygen, and moisture play a vital role in the mechanistic action of UDHSS-NPs. According to Perdue [166], HSs is complex mixture containing aliphatic, aromatic carboxyl and hydroxyl functional groups, which binds with microbial cells either on grains or in the environment (i.e., water, soil, etc.), thus alter the membrane structural intergrity and its functions. According to literature, the fungi cell walls share similarities with plant and bacterial and indeed with the extracellular matrix material of mammalian cells. The anionic surface, β1,4- and β1,3-linked polystarch forms a ribbon-like or helical (β1,3-glucan) structures which interacts with opposite charges. The cross linking of glycans of in eubacterial walls with peptides as well as phenolics and polysaccharides in plats promotes hydrogen bonding [167, 168]. Furthermore, the fungal cell wall is uniquely composed of mannoproteins, chitins, α- and β-linked glucans which serves many functions including; metabolism, ion exchange as well as providing cell rigidity and shape [169]. With the latter interacting with the HS. The interactions between HS and microbial cells depend on the lipophilicity and electric potential of the HS and cell [170], coupled with the size of the UDHSS-NPs. Microbial cells are composed of cations such as H^+, Na^+, K^+, Li^{2+}, Al^{3+}, Ca^{2+}, Cu^{2+}, or Pb^{2+} which interact with UDHSS-NPs thus penetrate the cell. As documented by Lofts et al. [171], cation-HS interactions exert control on the reactivity of cation, including its bioavailability for further reaction. Studies have shown the effects of binding metals with HS on water and soil ecosystems [172–174]. Natural and artificial HS got attracted to rice cells [175], macrophyte of *Ceratophyllum demersum*, crustaceans—*Gammarus pulex*, and vertebrates—tadpoles of *Rana arvalis* [176], which support the hypothesis that HS is charged and naturally interacts with microorganisms. When HS penetrates or is taken up by a cell, the electric potential of the cell is disrupted, denying the cell the ability to provide support in terms of rigidity, shape and metabolism, thus creating pores through which vital intracellular structures are leaked out.

In an in vivo experiment, Vigneault et al. [177] discovered that Suwannee River HA and FA enhanced the release/leakage of the fluorescent probe sulforhodamine-B (SRB) encapsulated within 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphatidyl-choline (POPC) vesicles. With regards to HA, a pH from 7.6 to 5.7 enhanced its surfactant-like effect. In conclusion, HS can alter the permeability of microbial cell, to create pores via which intracellular components are leaked out, killing the microorganism. However, the concentration, functionalisation (acylation), and pH of HS could potentially influence the biocidal activity.

According to Almatov and Akhmerov [178], 0.2–0.8 mg/mL mumie activated mitochondrial respiration and inhibited cellular succinate-oxidase and NADH-oxidase activity (mitochondrion). Similarly, mumie triggered the outflow of Ca^{2+} [160].

Previous studies [179–181] reported that mumie induced a dose-dependent elevation of superoxide dismutase, catalase, and glutathione peroxidase in rats. These enzymes are involved in the generation of ROS in an HA-induced antimicrobial or biological effects, which killed microorganisms and other grain storage pest.

A small-molecular size humic (LMSH) extracted from the feces of *Nicodrilus* and *Allolobophora rosea* enhanced the uptake of nitrate by plant roots and the accumulation of anions in the leaves. Further molecular analysis showed that LMSH influenced gene transcription in roots and long-distance effects in shoots as observed for *Mha2* and the *ZmNrt2.1* gene, respectively [182], which indicate HS can interfere with protein synthesis in microbes. FA and HA extracted from a podzol stimulated respiration in

rat liver mitochondria at concentrations between 40 and 360 mg/L. Depending on the duration of contact with mitochondria, uncoupled oxidative phosphorylation may occur subsequently affecting the growth of the microorganism [183].

A product of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine increased significantly after treatment with HA, indicating the ability of HA to inflict damage on DNA. The endonuclease activity of the viral RNA polymerase was inhibited when it came in contact with HA [184]. The concentrations (5, 10, and 15 mgL^{-1}) of HA and its organic extract significantly increased luciferase reporter gene activity in H4IIE. luc cells in a dose-dependent manner, which affected various molecular processes [185], thus killing the cell. The addition of HA (300 mg kg^{-1}) to soil stimulated the growth of bot laurel plants and rhizospheric bacteria and actinomycetes. However, high dose (3000 mg kg^{-1}), exerted an inhibitory effects [186]. The effects of HS on the hormone of *Caenorhabditis elegans* [170, 187], the sex ratio of *Xiphophorus helleri* [188], and the change in biochemical parameters of amphipod [189] were reported. These studies reiterate the potential biological effects of HS on microorganisms at the molecular level thus making them vulnerably for UDHSS-NPs.

5. Carbon-based nanoparticles/nanomaterials

Recently, carbon-based nanomaterials/particles (CNPs), which include nanotubes (i.e., double- or single-walled carbon nanotubes (DWCNT/SWCNTs)), fullerenes, and graphene oxide (GO) (**Figure 3**), have gained attention due to their potent biocidal activities. According to literature, the biocidal potency of these novel NPs is influenced by their physical/chemical properties, high adsorptive potentials, size, large surface area, and colloidal stability under wide range of pH. Increasing the NPs' surface area led to a decrease in size, with concomitant increase in adsorption and absorption (into fungi cell), which improved interaction [190–196] with subsequent inhibition of fungal growth.

The mycelia biomass and aflatoxin biosynthesis in *A. flavus* NRRL 3251 was negatively influenced at 10 μg mL^{-1} of fullerene C_{60} (fullerols $C_{60}(OH)_{24}$). The effects (growth arrest) was concentration-dependent. However, the antioxidative activity of the furrerols declined over time [197]. Hao and colleagues [198] investigated the fungicidal potentials of metal (copper oxide (CuO), ferric oxide (Fe_2O_3), and TiO_2NPs) and carbon-based NPs (multiwalled carbon nanotubes, fullerene, and reduced graphene oxide) against *Botrytis cinerea*. The results showed that all the six NPs exhibited biocidal activity with 50 mg/L of fullerene showing the strongest antifungal effects.

Reduced graphene oxide (rGO) nanosheets inhibited the mycelial growth of *A. niger*, *A. oryzae,* and *F. oxysporum* with half maximal inhibitory concentrations (IC_{50}) of 500, 500, and 250 μg/mL, respectively. The fungicidal activity as ascribed to the sharp edge of the rGO [199] which inflict injury on the cells, resulting in leaking of the cell components. Another hypothesis is that the organic functional groups on the fungi cell wall chemically interact with the ROS in rGO [200], which halts the uptake of nutrient and excretion of waste metabolites eventually killing the fungi.

Among the six carbon nanomaterials (SWCNTs, MWCNTs, GO, rGO, $C_{60,}$ and activated carbon (AC)) assessed for their fungicidal activity against pathogenic fungi (i.e., *F. graminearum* and *F. poae*), SWCNTs (500 μg/mL) exhibited the most potent activity, followed by MWCNTs, GO, and rGO respectively. However, the other two CNPs (C_{60} and AC) showed minimal activity, probably due to insufficient contact with fungal spores [201]. Conclusively, increasing the concentration of CNPs (62.5 < 125 < 250 < 500 μg/mL) increased the fungicidal potency. In a similar study, Wang et al. [202] reported that modifying the surface of MWCNTs with –OH,

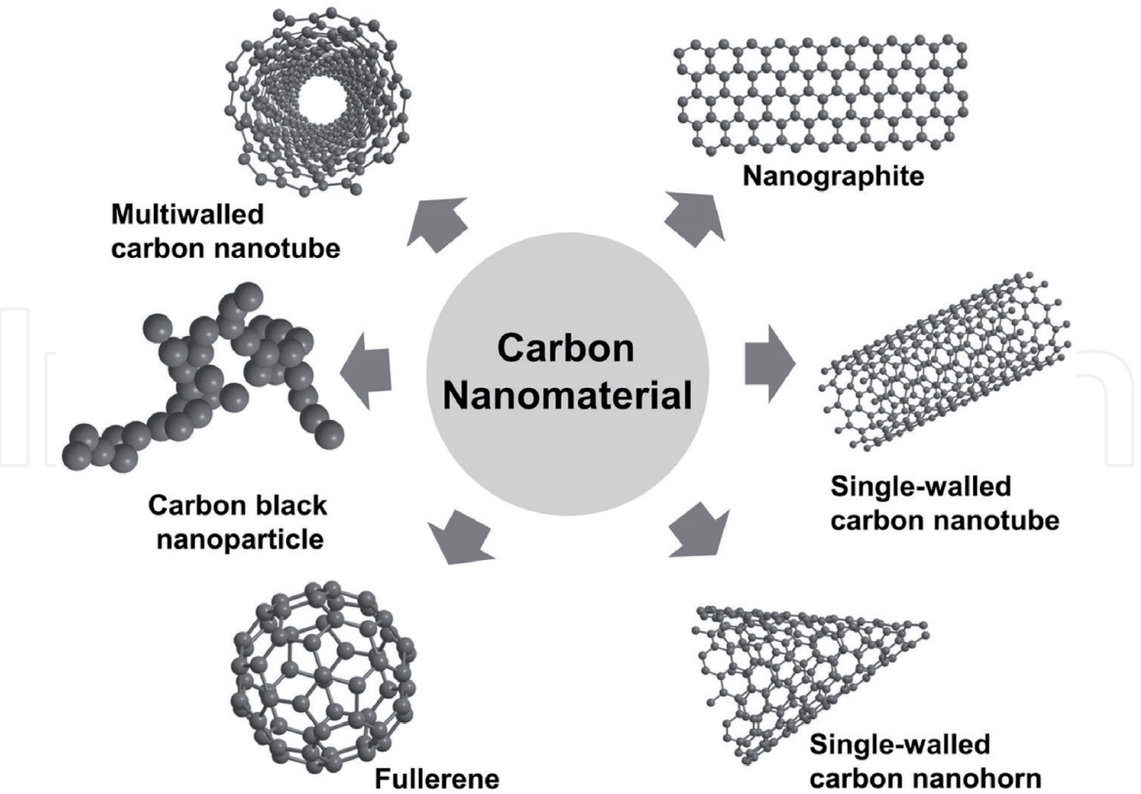

Figure 3.
Various carbon-based NPs [208].

–COOH, and $–NH_2$ improved the fungicidal activity (inhibition in spore elongation and germination) than the unmodified CNTs. It is hypothesized that modified CNTs formed a stable dispersions, which favoured interaction with spores, as a results enhanced antifungal activity. The authors observed a reduction in *F. graminearum* spore from 68.5, 54.5, 28.3, 27.4, and 29.5 μm, when 500 μg/mL MWCNTs (control), MWCNTs-COOH, MWCNTs-OH, and MWCNTs-NH_2 were applied, respectively. Moreover, previous works [203, 204] documented that biological activity of nanotubes improved upon addition of functionalized aliphatic amide (covalent) and polyethylene glycol (PEG) and/or polyoxyethylene(40)nonylphenyl ether (IGPAL) (non-covalent) chemical groups [205]. Zare-Zardini et al. [206] conducted a covalent functionalization of MWCNTs with lysine and arginine under radiation. The modified MWCNTs exhibited potent biocidal activity against all test fungi (*A. niger*, *A. fumigatus, C. albicans, P. chrysogenum, S. cerevisiae, F. culmorum, Microsporum canis, Trichophyton mentagrophytes, Trichophyton rubrum, and P. lilacinum*) compared unmodified MWCNTs. Surprisingly, the fungicidal activity of MWCNTs-arginine against all the test fungi was slightly higher than MWCNTs-lysine. The authors hypothesized that the positive charge on arginine might have enhanced the binding of NPs on the fungal membrane and altered the genetic makeup (DNA). Thus, lysine and arginine could be utilized to improve the fungicidal activity of CTNs. Recently, Katerine et al. [207] reported the fungicidal activity of cotton fabric silica-silver carbon-based hybrid NPs against *A*. sp., *Cladosporium* sp. and *Chaetomium globosum*. The fabrics with high number carbon exerted the most increased biocidal activity on *C. globosum* and *Aspergillus* sp.

5.1 Mechanistic action of CNPs fungicidal activity

The ability of CNPs to interact and integrate into fungi cells determines their fungicidal activities. Wang et al. [201] reported the importance of surface contact

of CNPS to their biocidal functionality. A transmission electron microscopy study showed CNPs interacted and integrated into spores and form an aggregation. It was hypothesized that the van der Waals force in CNTs was strong enough formed a bond with spores, as a result, triggered plasmolysis. Similarly, Zare-Zardini et al. [206] reported a strong interaction in functionalized CNTs with arginine than lysine with fungi membrane. These interactions may result in CNPs been internalized into fungi. Transmission electron microscopy (TEM) analysis showed direct evidence of nanographene been internalized in Caco-2 cells [209], which support the above finding. Moreover, cells treated with CNPs showed evidence of plasmolysis [201].

They compared images of healthy and treated (incubation for 3 h with CNPs) cell membranes of *F. graminearum* spores. They observed an intact, slick, compact, inerratic, and well-positioned cytoplasm for the untreated cell; however, after treatment the latter cell were transfigured, contracted and gathered. This shows solute lost through CNPs point of contact. Interaction of CNPs with fungi' membrane led to a decrease in membrane integrity by the stresses exerted by the electrostatic forces between the microbial outer surface and CNTs, resulting in membrane oxidation [210]. CNPs are reported to be a contributing factor to the over-generation of ROS, which could trigger fungal cells to enter oxidative stress, causing excessive impairment to cellular components and permanent DNA laddering that could potentially lead biocidal activity against the cells [211, 212]. In contrast, Saha et al. [209] found that all CNPs (C1, C2, C3, C4 C5) assessed did not contribute to ROS production in Caco-2 cells. A decrease in ATP was observed. Conclusively, CNTs applied was internalized and disrupt the functionality of mitochondria which explains the reason for the low ATP observed. However, the treatment did not influence the production of ROS.

6. Toxicological aspects of NPs

According to Higashisaka et al. [213], NPs with diameters ≤100 nm are presently been used in various applications, including food production (e.g., to improve texture). An orally ingested NP can cross the gastrointestinal barrier, absorbed into the blood, and alter normal physiological functions, thus causing adverse health is to consumer(s) [213, 214]. Ezhkov and colleagues reported acute catarrhal inflammation on esophagus, stomach, and duodenum of mice fed with sapropel-NPs at a dose of 1.8 g/kg. However, 0.3 g/kg and 1.5 g/kg dose did not manifest any toxic effects [165].

A positive correlation was established between residues of Ag-NPs in rat organs and the NP suspension applied. NPs migrated from the luminal side to the intestinal epithelial cells via endocytosis or transcytosis, which are accumulated in the mentioned organs. However, all treated rats were able to excrete the NPs from most organs except the brain and testes [214–217]. Cellular uptake of NPs is similar to mechanism of the antimicrobial activity as its also depends on size, surface charge, and dispersion or aggregation state [218, 219]. Rhodamine B (RhB) labeled carboxymethyl chitosan grafted NPs (RhBCMCNP) and chitosan hydrochloride grafted NPs (RhB-CHNP) bearing positive or negative charges used as model chitosan to elucidate the effects of particle size and surface charge on the cellular uptake of NPs revealed that the surface charges were attracted to the macrophages, and could be attributed to the electrostatic interactions between particles and phagocytic cells. Besides, different cell lines, irrespective particle size, and surface charge difference influence the uptake of NPs [219]. Kim et al. [220] detected traces of Ag-NPs in blood, liver and other organs after they orally fed rats at a dose of 30, 125, or 500 mg/kg BW/day. A significant ($p < 0.05$)

dose-related decrease in the bodyweight of high-dose male rats at the fourth, fifth, and seventh weeks was observed; however, no significant dose-dependent changes in the female rats. Further hematological assays showed a significant increase ($p < 0.01$) in cholesterol in the both high-dose male and female rats. A significant increase ($p < 0.01$) in alkine phosphotase (ALP) was also indicated for the high-dose female rats. The authors reported no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) as 30 mg/kg BW/day and 125 mg/kg BW/day, respectively. Treatment with NPs of diameters ranging from 25 to 80 nm at a dose of 5000 mg/kg body weight altered the levels of alanine transaminase, aspartate transaminase, blood urea nitrogen, and lactate dehydrogenase, along with lesions on the liver and kidneys of female mice. Myocardial damage associated with groups showing a notable changes in serum LDH and alpha-HBDH levels compared to the control experiment. Also, a biodistribution test disclosed that TiO_2 was predominantly retained in the liver, spleen, kidneys, and lung tissues, indicating TiO_2-NPs were transported via endocytosis to other tissues and organs after their uptake by the gastrointestinal tract [221]. Contradictory finding was reported by Warheit et al. [222] where no adverse effects were manifested after orally fed rats with TiO_2-NPs. However, the NOAEL on rats exposed for 90 days was >1000 mg/kg BW/day. In a similar study, Sharma et al. [223] divided male Swiss albino mice into three groups (group 1—vehicle control (water); group 2—ZnO nanoparticles (300 mg/kg body weight); group 3—ZnO nanoparticles (50 mg/kg)) and fed them with 50 and 300 mg/kg b.wt. ZnO-NPs for 14 consecutive days. ZnO-NPs induced oxidative stress, which damage the DNA and apoptosis in the mouse liver. Additionally, elevated levels of ALT and ALP serum and subsequent pathological lesions were observed in the treated mice. Lastly, at a higher dose (300 mg/kg) of ZnO-NPs, a significant ($p < 0.05$) induction of lipid peroxidation was observed in the liver, brain, and kidney (**Figure 4**) of the treated mice in comparison with the control test. Cho et al. [224] discovered ZnO-NPs had a higher absorption efficiency than TiO_2-NPs in rats. ZnO-NP concentrations in the liver and kidney were significantly higher compared to the control, whereas with TiO_2-NPs, no dramatic increase was detected in the sampled organs. In the feces, very high and low concentrations of Ti and Zn were detected, respectively. The concentration of ZnO in the spleen and brain was minimally elevated. Similarly, Ti concentrations were not drastically increased in urine; in contrast, it was Zn levels, that remarkably

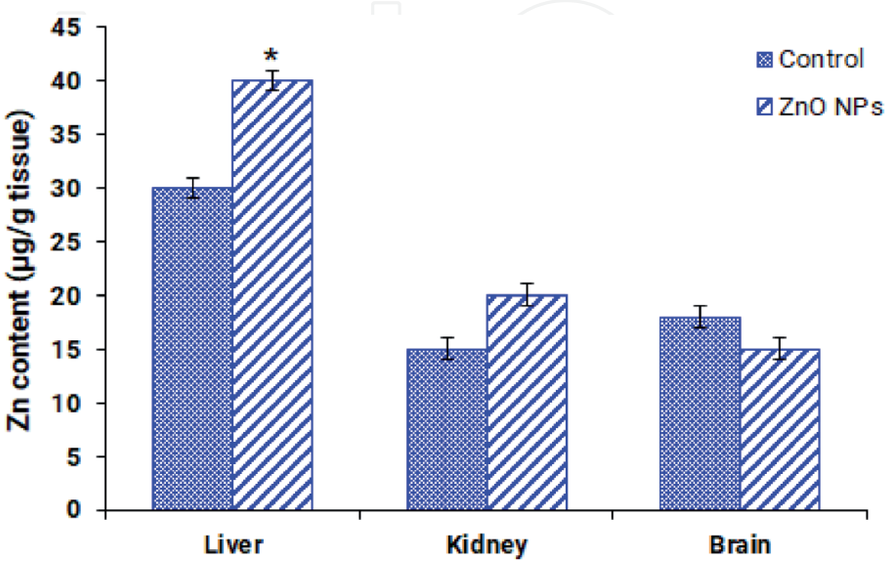

Figure 4.
*Zinc content in selected tissue of the mice (n = 5) after oral administration of ZnO nanoparticles (NPs) (300 mg/kg) for 14 consecutive days. Data represent mean±S.E.M. of three animals. *$p < 0.05$, compared to control. Modified with permission from ref 4495441125809.*

changed. Therefore, the absorption of various NPs could be attributed to the higher dissolution rate in the acidic gastric fluid; however, this might not be applicable when NPs are utilized during grain storage. When a stored grain undergoes sun drying, milling, etc., the levels of NPs may decrease to a level that could not affect the consumer health. Moreover, many NPs have received approval for application in many fields. Nevertheless, rigorous studies are warranted to expound on any risks or the safety of NPs use in grain storage. According to Zare-Zardini et al. [206], CNPs appeared less toxic to humans and animals compared to metal NPs and are therefore the better alternative and a novel method for reducing mycotoxin biosynthesis in grains.

7. Proposed methods of applying NPs during grain storage

1. The first method is direct processing of grains with solutions of the required concentration of NPs. To achieve this, biocompatible NPs in an aqueous dispersion medium with pH values close to neutral should be used.

2. Treatment with aerosols NPs could also be used if the NPs are dispersed evenly over the granary or silos. The aerosols to apply should be modified to prevent aggregation on grains. Using aerosols saves time and labor since additional drying is not required.

3. The use of packages made from NPs during storage, transportation, and sale will extend the shelf life of grains. Alternatively, NPs formulated cubes could be place in jute bags with grains; however, periodic mixing is required to distribute the NPs.

4. In our opinion, one of the most inexpensive methods is the use NPs in the production of materials for granaries, as well as treating interior and exterior surfaces of the storage facilities.

8. Conclusions

The ability of NPs to suppress the synthesis of mycotoxins in fungi and other microorganisms could be a breakthrough to curbe the issue of aflatoxin prevalence worldwide. NPs displayed excellent antifungal activity against important fungal species which contaminate grains with toxins during storage. The concentration, volume, type, and illumination (sunlight) significantly influenced the biostatic activity of NPs. Hence, these factors should carefully be considered when applying NPs in grain storage. The proposed NPs are environmentally friendly and pose no threat to consumer compared to some conventional methods of grain preservation. Several ex vivo, in vivo, and in vitro studies supports these claims. Moreover, NPs are biocompatible to the human system hence their usage in the food industry. Despite safety of NPs guaranteed by international safety organizations such as the Food Safety Authority, routine testing is required to understand the impact it has on grain nutritional, sensory, and other physicochemical parameters.

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Disclosure statement

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