CHAPTER

Copper nanomaterials for eliminating the risk of mycotoxins

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1 Introduction

Mycotoxins are low molecular weight secondary metabolites produced by mycotoxigenic fungi, which often contaminate agricultural products (Brunel et al., 2013). Mycotoxins are ubiquitous and caused by *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Cladosporium* genera. Mycotoxins are difficult to eradicate and causes serious agricultural losses thereby directly affecting growing crops intended for market—gross domestic product (GDP) of a country. For many years, this has become a global grand challenge impacted by increasing global population, loss of arable land, and gruesome reality of the consequences of climate change.

This is exacerbated by the toxicity and health problems associated with the consumption of mycotoxin contaminated foods and feeds contaminated by mycotoxin. The most prevalent and major mycotoxins include aflatoxins (AFs), fumonisins (FBs), patulin, ochratoxins (OT), zearalenone (ZEA), and trichothecenes including deoxynivalenol (DON) and T-2 toxin found in food, posing unpredictable and ongoing food safety and security problems worldwide. Some of these mycotoxins [e.g., aflatoxin B1 (AFB_1), citrinin, fumonisin B1 (FB_1), ochratoxin A (OTA), patulin, T-2 toxin, and ZEA] have been reported to cause neoplasia (cancer) in humans and animals, in addition to kidney, gastrointestinal, urogenital, vascular, and neurological diseases. This is attributed to the chemical nature of some mycotoxins being highly liposoluble, where they can easily be absorbed from the gastrointestinal and respiratory tract into the blood stream, thereby dissimilating throughout the body and other organs (e.g., liver and kidneys) (Adam et al., 2017; Çelik, 2019).

It is important to acknowledge that multiple contaminations are possible as a single fungus (such as *A. parasiticus*, *A. Flavus*, and *F. graminearum*) can produce several kinds of mycotoxins within one type of food or feed ingredient; therefore, several types of mycotoxins can be present in the same food or feed ingredient—multicontamination. Exposure of farm animals (mainly cattle, pigs, and poultry) to

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mycotoxins through ensiled by-product feed is poorly regulated and detailed. This negligence can carryover residual mycotoxins in animals' products, thereby resulting in an indirect dietary exposure to humans through the consumption of contaminated animal products (such as eggs for poultry, milk for mammals, and meat). The objectives of this chapter are to provide insight on (i) the significant role of copper (Cu) and Cu-based nanomaterials fungicides for reducing mycotoxin contamination, (ii) Cu antifungal activity against mycotoxigenic fungi, and (iii) the future recommendations for Cu nanofungicides for an intricate understanding of the comprehensive regulatory framework for their utilization in agriculture, food, and feed sectors.

2 Copper fungicides

The primary efforts for eliminating the risk of mycotoxins have involved the use of fungicides against fungal manifestation as an indirect approach in preventing mycotoxin contamination. The use of fungicides has been the first defense strategy against mycotoxigenic fungi, these fungicides are classified as either systemic or nonsystemic. Systemic fungicides are absorbed into the plants, while nonsystemic fungicides do not translocate into the crops being protected but merely acting upon the protection of the plant's surface against fungal colonization. Conventional fungicides exhibit a number of challenges such as (i) ineffectiveness overtime amid the onset of fungicide resistance, (ii) limitation on fungi, as they are not effective against bacteria, nematodes, or viral diseases, and (iii) highly toxicity on plants and the overall environment.

Moreover, commonly employed fungicides are not approved for use in organic farming. One of the major alternatives is the utilization of copper (Cu) fungicides, which included copper sulfate ($CuSO_4$) also known as bluestone due its color and copper derivatives (Bordeaux mixture: $CuSO_4$ and lime water and Burgundy mixture: $CuSO_4$ and sodium carbonate). Copper is recognized as a potent antimicrobial metal with a comprehensive control, and some Cu-based fungicides are classified into both inorganic and organic fungicides are approved for organic farming practices (Adisa et al., 2019; Brunel et al., 2013; Sidhu et al., 2017). Copper-based fungicides are categorized as copper hydroxide fungicide (COH), copper oxychloride fungicide (COC), and copper oxide fungicide (COX) and their application can be through suspension concentrate, wettable powder, and water granule. According to the market analysis by MarketWatch (2021), in 2020, the global Cu fungicide market was valued at 796.6 million USD and is expected to grow at a compound annual growth rate (CAGR) of 4.0% (during 2021–26 forecast) to reach 1051.5 million USD by the end of 2026.

The use of Cu is also beneficial because it is much cheaper, less toxic than its counterpart silver (Ag), and have been reported to be effective against a variety of pathogenic fungi (*A. carbonarius*, *A. fumigatus*, *A. niger*, *Alternaria solani*, *F. expansum*, etc.) (Khamis et al., 2017; Nath et al., 2019). Oziengbe and Osazee (2012) reported on the effectiveness of $CuSO_4$ against *Colletorichum gleosporioides*, which causes Anthracnose in mango fruit (*Mangifera indica*). The results revealed that $CuSO_4$ at 0.8 parts per million (ppm) significantly reduced the growth of *C. gleosporioides* and induced potent defense reactions in mango fruit.

Besides, Cu is one of the most essential micronutrient metals/minerals required by plants, animals, and humans (Tang et al., 2019). For plant's growth and development, the concentration of Cu ranges between 0.05 and 0.5 ppm, with most tissues (e.g., vascular, dermal, and ground tissue cells) between 3 and 10 ppm (Adisa et al., 2019). Furthermore, Cu participates in various physiological processes such as the formation of chlorophyll and photosynthesis assisting in plant respiration and metabolism of carbohydrates and proteins. Some of the enzymatic reactions use Cu as a cofactor, this includes activation of many metalloproteins and those involved in lignin synthesis (Fig. 1). The source of Cu for plants is essentially from fertilizers and several fungicides, which contain Cu as their active ingredient. Foods that contribute a majority of Cu consumed by animals and humans, include three major dietary sources; that is, seafood (e.g., oysters and other shellfish), organ meats (e.g., kidneys and liver), and dark leafy greens, whole grains, legumes (e.g., beans and lentils), nuts, potatoes, and dried fruits (e.g., prunes, cocoa, and black pepper). Cu as a nutritional dietary supplement in animal feeds is categorized as "generally recognized as safe" (GRAS) according to the US Food and Drug Administration (USFDA) and the European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) (He et al., 2019).

In humans, the adult body contains between 1.4 and 2.1 mg of Cu per kilogram of body weight (kg/bw), hence a healthy human weight of 60 kg contains approx. 0.1 g of Cu. For humans and animals, Cu is found in the liver, brain, heart, kidneys, and skeletal muscle, it assists in maintaining various physiological and metabolic processes (absorption of iron), promotes strong and healthy bones (collagen), ensures proper nervous system homeostasis, and plays a role in energy production. However, this small amount is essential to the overall well-being of humans. In agriculture, the consistent use and effectiveness of these Cu fungicides poses several challenges such as toxicity to plants, where the Cu accumulates in the roots and restricts root growth by burning the root tips and thereby causing excess lateral root growth; and Cu build up in sediments and cause long-term soil contamination (Konappa et al., 2021; Nath et al., 2019; Tegenaw et al., 2015).

Therefore, Cu impairs nutrient deficiency, chlorosis, resulting in hyperaccumulator plants and, in more severe cases, tissue necrosis and plant/crop death. The high amounts of Cu through Cu fungicides have been reported to affect crop productivity and yield, in addition to the onset of fungicide resistance from mycotoxigenic fungi (Gogos et al., 2012). Excess Cu can compete with plant uptake of iron (Fe) and sometimes molybdenum (Mo) or zinc (Zn), which are equally essential for the plant's growth and development. Moreover, Cu compounds can leach from the agricultural application into aquatic environments via groundwater and can threaten aquatic species and other organisms; thus, posing a serious environmental hazard.

To minimize the abovementioned challenges, researchers are constantly searching for alternatives to optimize Cu fungicides with limited to no toxicity effects, thereafter. Chelated Cu complexes (e.g., copper-8-quinolinolate) are known to be nonreactive with other chemical constituents in an aqueous medium. Young et al. (2016) evaluated the potential fungicidal activity of Cu^{2+} complexation with

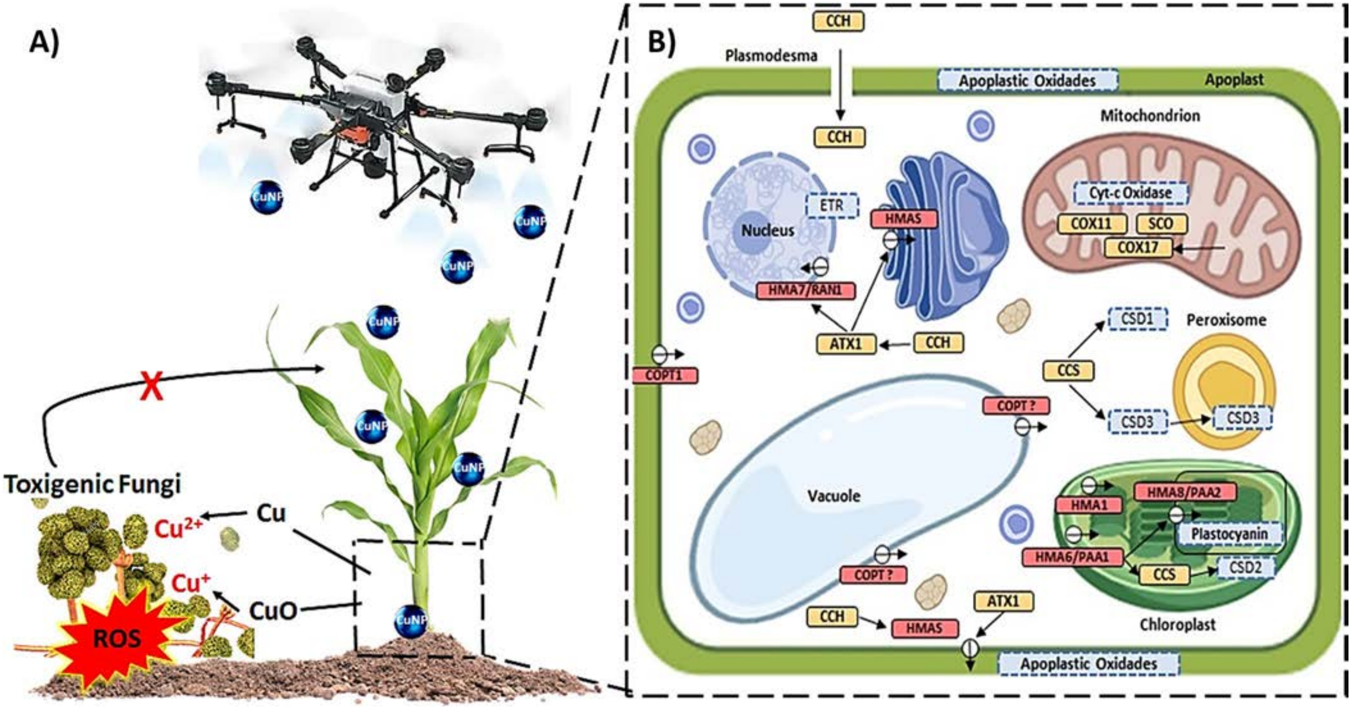


FIG. 1

The pivotal role Cu plays in agriculture (A) antifungal activity of Cu nanoformulations through the release of Cu2^{+} and Cu^{+} ions and the generation of reactive oxygen species (ROS) and (B) Cu, cofactor functionality for a variety of enzymatic reactions. Cu participates and/or linked with proteins (*blue*), transporters (*rose*), and Cu-metallo chaperones (*orange*).

salicylaldehyde benzoylhydrazone (SBH) for Cu^{2+} release against pathogenic fungi thereby minimizing their propensity of producing mycotoxins. Their experiment compared commercial DuPont Kocide 3000 [active ingredient: $Cu(OH)_2$], Kocide 3000/SBH mixtures, and Cu^{2+}-SBH complex for their fungicidal effectiveness. Results revealed that the Cu^{2+}-SBH complex improved Cu^{2+} delivery at low concentrations compared to Kocide 3000 and Kocide 3000/SBH mixtures, this can be a strategy to mitigate the environmental impact of Cu fungicides. Most recently, work by Melendez et al. (2020) from 15 farms in New Jersey, United States that used Cu fungicides (e.g., cuprous oxide and copper hydroxide) using soil analysis reported elevated soil Cu levels, which resulted from Cu accumulation with the potential to become toxic to sensitive crops and impact soil health.

3 Nanotechnology: Nano-Cu significance in mycotoxin and environment

Nanotechnology has rendered precise capability is revolutionizing every field and industry it influences from biomedical to agricultural applications, the latter being agri-nanotechnology, providing a potential to cope with food production, safety, and security against fungal and mycotoxin manifestations (Adisa et al., 2019; Agrimonti et al., 2021; Castro-Mayorga et al., 2020; Konappa et al., 2021; Mishra et al., 2017; Tanwar and Sushil, 2019). Nanomaterials, because of their captivating properties are considered new chemical entities from their bulk counterparts. In agriculture, the pronounced use of nanomaterials especially Ag, Cu, Fe, and Zn-based nanoformulations has been on nanofertilizers and nanopesticides for the protection against mycotoxigenic fungi and other pathogenic organisms with subsequent increase in crop productivity and health (Abd-Elsalam et al., 2019; Adisa et al., 2019; Agrimonti et al., 2021; Castro-Mayorga et al., 2020). The lack of science-based regulatory frameworks for nanomaterials has made it difficult to regulate the use of nanomaterials (Dimitrijevic et al., 2015; Mishra et al., 2017). Table 1 shows the commercial Cu nanoformulations utilized in agriculture.

In a recent literature review by Zhang et al. (2020), the authors describe the need to develop methods for the identification of mycotoxins and mycotoxins masked by means of separation with greater efficiency and biorecognition molecules with greater specificity and sensitivity. The development of standard detection methods using nanomaterials provides an ultrasensitive and multiplexed detection system for multiple mycotoxins in a single test is paramount. Masked mycotoxins are difficult to detect with conventional standards due to their biotransformation. To date, many Cu nanomaterials and nanocomposites have been promoted as antifungal agents; however, there is an urgent need to develop multifunctional nanocomposites able to detect small concentrations of mycotoxins simultaneously with the added functionality of adsorption and detoxification with limited to no ecotoxicity.

Work by Song et al. (2018) utilized dsDNA [ochratoxin A (OTA)-aptamer]-templated CuNPs as label-free fluorescence indicators in biosensor for sensitive

Table 1 Current commercial Cu nanoformulations utilized in agriculture.

Commercial names	Manufacturers	Current status and legislation compliance	Nanomaterial composition	Application and function
Saula Drip 10-40-10	Bio Nano Technology, Giza, Egypt	Commercialized	Minor elements, (iron, zinc, manganese, **Cu**, boron) NPs	Nanofertilizer, fertilizer
NANOCU		Commercialized	Nano **Cu**, adjuvants and chelating materials	Nanopesticides, fungicide and bactericide
ZENGA		Commercialized	**Cu**, Mitalaxil, Mancozeb	Nanopesticides, fungicide, and bactericide
NovaLand-Nano	Land Green & Technology Co., Ltd., Taiwan	Commercialized	Microelements as Mn, **Cu**, Fe, Zn, Mo, N NPs	Nanofertilizer, fertilizer

detection of OTA. Where the dsDNA serves as the template for CuNP synthesis exhibiting high fluorescence quantum yield and in the presence of OTA, aptamer forms an OTA-aptamer, and many dsDNA-templated CuNPs are degraded into mononucleotides by the RecJf exonuclease (single-stranded DNA-specific exonuclease) resulting in low fluorescence. The system had a low detection limit (LOD) of 5 parts per billion (ppb). Recent similar work by Chen et al. (2021) developed and investigated a highly specific and ultrasensitive assay that used copper monosulfide nanoparticles (CuSNPs) conjugated to an anti-OTA antibody (CuS-AbNPs) as fluorescent probes for the specific and sensitive detection of OTA in coffee, corn, and soybean samples. The fluorescent probe detected Cu^{2+} dissolution/release; briefly, OTA present in the sample binds with CuS-AbNPs which causes the release of Cu^{2+}, thereby activating the Cu^{2+} fluorescent probe. Results revealed that the assay can detect 0.1–100 ppb OTA with LOD of 0.001 ppb and a detection time of 170 min. This provides the opportunity for OTA quantification for food safety, quality assurance/control, and exemplifies the vast application of CuNPs for the detection of mycotoxins (Alghuthaymi et al., 2021).

In agriculture, a vast majority of Cu nanomaterials are used as antimicrobial agents has provided beneficial advantages over their bulk Cu counterparts (Abd-Elsalam et al., 2019; Chellaram et al., 2014) to produce more healthy foods without mycotoxin contamination (Dimitrijevic et al., 2015). This is through the antimicrobial activity of Cu nanomaterials is attributed by the release of Cu^{2+} and Cu^{+} ions and their ease functionalization via encapsulation of bioactive compounds resulting in improved food storage, increased shelf life correlating to protection against fungal contamination and mycotoxins, thus improved food safety and security (Nath et al., 2019).

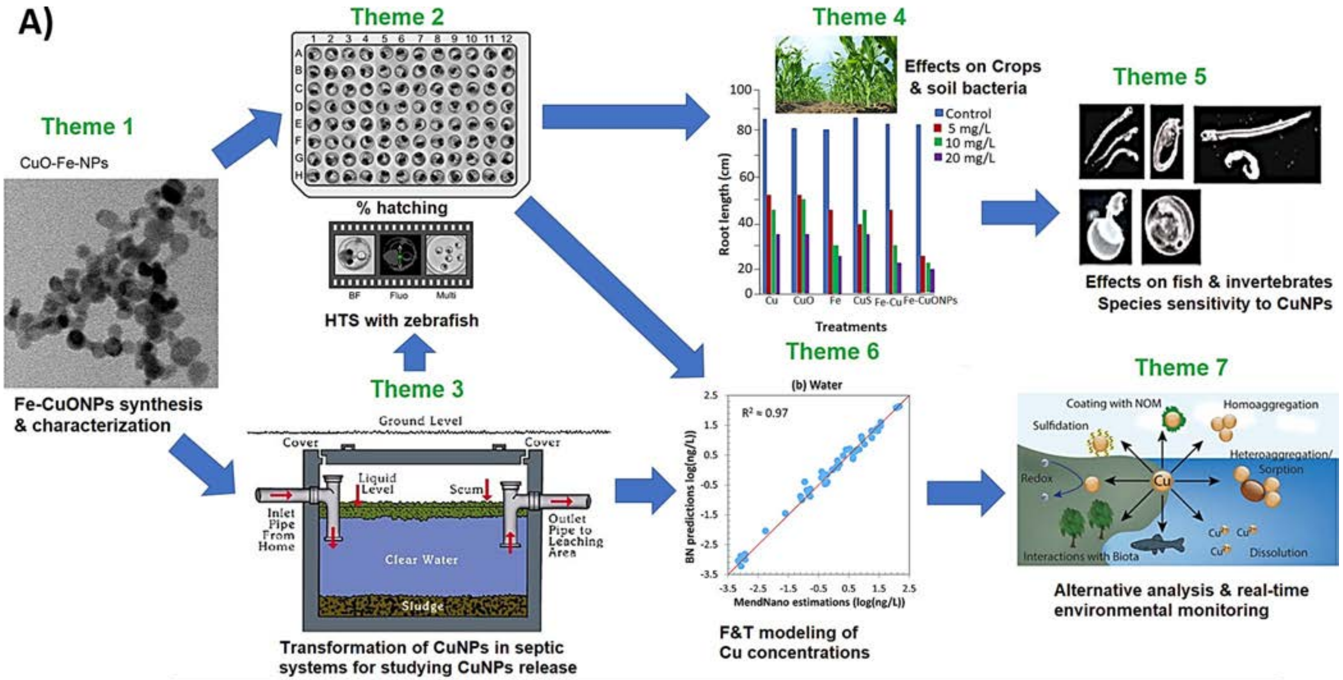
There are several Cu-based fungicidal products in the market which lack significant information (e.g., chemical composition, mineral speciation, toxicological data, and surface chemistry and reactivity) which is critical for accurately evaluating the ecotoxicological risks associated with these products (Jesmin and Chanda, 2020; Nile et al., 2020). Tegenaw et al. (2015) examined Cu-based fungicidal products (product A and B) and reference compounds (metallic Cu, Cu_2O, CuO, $CuCl_2$, and aqueous Cu^{2+}) to evaluate their mineral speciation and particle size, which correlate to their potential environmental implications (Keller et al., 2017). Their results revealed that product A and B contained 360 and 310 g of Cu kg^{-1}, respectively, which was attributed to the presence of cornetite [$Cu_3(PO_4)_2$], malachite [$Cu_2CO_3(OH)_2$], spertiniite [$Cu(OH)_2$], and tenorite (CuO).

Mineral speciation analysis revealed that product A, dominant Cu species was spertiniite and product B was dominated by tenorite (approx. 30%, < 450 nm). Product A and B had Cu nanoparticles that were 90 and 25 nm in size, respectively. Furthermore, product B had a higher toxicological impact than all the tested samples, attributed to their size compared to product A. Under environmental parameters (e.g., background electrolytes, ionic strength, and pH), both products were impacted. The authors also highlighted the importance of the charge-driven stability (electrostatics) of the nanomaterials, which is a proxy for predicting their fate and mobility within the environment. Results also pointed out that CuO was found to pose more risks than other Cu species, because of their genotoxic potential. The consensus of the work suggests that the type of chemical speciation used in the nanofungicide formulations must be also taken into consideration together with other factors such as type of synthesis, particle size, stability, and reactivity, which dictates the overall ecotoxicology of the nanofungicides (Jesmin and Chanda, 2020).

Another study by Maqsood et al. (2020) evaluated the antifungal activity of CuNPs against *A. niger*. Their results showed maximum inhibitory effect at 1.5% of CuNP concentration. In addition, Kolackova et al. (2021) evaluated the delivery of different Cu-based fungicides [CuO, $CuSO_4$, Cu-EDTA, CuO-nanopowder (< 50 nm)] using superabsorbent polymers on the effects of foliar application for nutritional characteristics (i.e., percentage of ash, crude protein, crude fat and crude fiber, acid detergent fiber, ash-free neutral detergent fiber, lignin, cellulose, and starch) and effects against mycotoxin (DON and T-2 toxin) contamination on wheat kernel. Their results revealed that the highest nutritional content was observed with treatment of Cu-nano, while all treatments were effective at controlling DON and T-2 toxin production. It is imperative to restrict mycotoxins in the food chain without affecting quality/nutritional characteristics of food, and the environment such as microbes in soil and water (Agrimonti et al., 2021; Jesmin and Chanda, 2020).

The University of California Center on the Environmental Implications of Nanotechnology conducted a systematic empirical study of the potential risks of CuNPs used in agriculture as fungicidal agents (Keller et al., 2017). The study focused on six Cu-based materials: (i) nano-Cu (nCu), (ii) micro-Cu (μCu), (iii) nano-CuO (nCuO), (iv) micro-CuO (μCuO), (v) CuPro ($nCu(OH)_2$-a), and (vi) Kocide 3000 ($nCu(OH)_2$-b). The results from the study revealed $> 95\%$ of Cu released accumulates to potentially toxic levels (> 0.5 ppm) into the environment entered the soil and aquatic sediments, and Cu^{2+} even at low concentrations exhibited higher toxicity especially in aquatic organisms, especially freshwater daphnids and marine amphipods which are more susceptible to CuNP toxicity than terrestrial plants as shown in Fig. 2. El-Abeid et al. (2020) demonstrated the use of graphene oxide nanosheet-decorated CuONPs (rGO-CuONPs) against *F. oxysporum*. Results revealed comparative antifungal activity of rGO-CuONPs was effective at 1 ppm without any phytotoxicity, while conventional fungicide Kocide 2000 was at 2500 ppm.

Shah and Mraz (2020) reported that 20 ppb of CuNPs or 100 ppb of $CuSO_4$ exposed to juvenile rainbow trout (*Oncorhynchus mykiss*) caused organ injuries (gill, gut, liver, kidney, brain, and muscle) according to the pathological findings. A review by Malhotra et al. (2020) summarized the toxicity of metallic Cu, CuNPs, and CuONPs on various fish species, data related to their toxicity are still limited. The results are contradictory where some studies reported CuNPs to be less toxic than $CuSO_4$, while others reported otherwise. Interestingly, Fadl et al. (2020) reported on the binding affinity of CuONPs and hydrated sodium aluminum silicate nanoparticles (HSCASNPs) as adsorbents at 500 ppm for OTA in Nile tilapia fish fed on OTA contaminated diet. Collectively, these results provide insight on the environmental risk predictions to assess impacts, and approaches to mitigate the toxicity of Cu nanoformulations while promoting beneficial uses of Cu-nanofungicides.



B) Cu^{2+} > nano Cu(0) > nano $Cu(OH)_2$ > nano CuO > micron-scale Cu compounds

FIG. 2

Copper nanoparticles (CuNPs) lifecycle (A) Theme 1: acquisition and characterization of the Cu particles, followed by distribution of the characterized materials to other themes; Theme 2: high throughput screening, which served to design and prioritize studies in Themes 4 (terrestrial toxicity) and Theme 5 (aquatic toxicity). In parallel, Theme 3 conducted life cycle material flow analyses to determine likely release estimates, exposure concentrations, and doses for use in Themes 4 and 5. Exposure and toxicological data were transferred to Theme 6 to model risk based on expected concentrations/doses and hazards. Theme 7 conducted alternative analyses workshops for copper in paints. The project outcomes were (1) release estimates for various CuNP applications; (2) assessment of likely exposure pathways and concentrations; and (3) ranking of toxicity of different species of CuNPs, micron-scaled Cu particles, and Cu salts and (B) comparative toxicity of CuNPs, μ-scaled Cu particles and Cu salts.

Reproduced from Keller, A.A., Adeleye, A.S., Conway, J.R., Garner, K.L., Zhao, L., Cherr, G.N., Hong, J., Gardea-Torresdey, J.L., Godwin, H.A., Hanna, S., Ji, Z., Kaweeteerawat, C., Lin, S., Lenihan, H.S., Miller, R.J., Nel, A.E., Peralta-Videa, J.R., Walker, S.L., Taylor, A.A., et al., 2017. Comparative environmental fate and toxicity of copper nanomaterials. NanoImpact 7, 28–40. https://doi.org/10.1016/j.impact.2017.05.003 with permission from Elsevier.

Table 2 Antifungal activity of $NCuS_{1-3}$ and standard fungicide (Captan).

	A. alternata		*D. oryzae*		*C. lunata*	
Treatments	**ED_{50}**	**ED_{90}**	**ED_{50}**	**ED_{90}**	**ED_{50}**	**ED_{90}**
$NCuS_1$	6.7	9.6	8.0	10.0	8.0	10.5
$NCuS_2$	8.3	11.0	7.0	9.5	11.5	14.0
$NCuS_3$	5.5	8.3	5.0	7.0	6.0	8.0
Captan[a]	240	430	250	390	270	400

[a] *Standard fungicide (N-trichloromethyl-thio-4-cyclohexene-1,2-dicarboximide), ED_{50}, effective dose at 50% fungal inhibition, ED_{90}, effective dose at 90% fungal inhibition.*

Sidhu et al. (2017) developed copper sulfide nano-aquaformulation ($NCuS_{1–3}$) with different capping agents for their antifungal activity against *Alternaria alternata*, *Drechslera oryzae*, and *Curvularia lunata* and their impact on seed quality of rice grains (*Oryzae sativa*). The NCuS, naked CuS (no stabilizing agent); $NCuS_1$, stabilized with polyvinyl pyrrolidone (PVP); $NCuS_2$, stabilized with 4-aminobutyric acid (GABA); and $NCuS_3$, stabilized with trisodium citrate and their average size range of 14, 12, 10, and 8 nm, respectively. Antifungal activity results are summarized in Table 2 (adapted from Sidhu et al., 2017) and $NCuS_3$ at 7 ppm for 2 h significantly reduced seed rot and seedling blight, favorable effect to accelerate seed germination and seedling growth.

On the other hand, green nanotechnology provides pronounced strategies to circumvent the challenges experienced with conventional Cu fungicides (Tanwar and Sushil, 2019). This can be done through the green synthesis of copper sulfate, copper, and copper oxide nanoparticles ($CuSO_4$-NPs, CuNPs, and CuONPs, respectively) using bioactive constituents from plants. Ideally, this would produce Cu nanofungicides with high efficacy at low concentrations, stability, low cost, biocompatibility, and concomitantly low ecotoxicity to crops, animals, humans, and the overall ecosystem (Jesmin and Chanda, 2020; Mishra et al., 2017; Tegenaw et al., 2015). Cu nanofungicides would provide a multifarious approach with armaments potential as nanofertilizers for enhanced plant/crop growth stimulation while proffering plant/crop protection from mycotoxins (Konappa et al., 2021).

4 Cu antifungal activity

The activity of Cu nanomaterials as antimicrobial agents is attributed through the electrostatic forces exerted by Cu^{2+} ions on the outer plasma membrane composed of lipopolysaccharide, eliciting significant permeability thus affecting several essential membrane-dependent protein transport systems (e.g., ATP-powered pumps, channel proteins, and transporters). Cu-induced disruption of membrane integrity inevitably results in cellular death, thereby reducing fungal growth eliminating the risk of mycotoxins (Borkow and Gabbay, 2009). The Cu^{2+} ions released from Cu nanofungicides impose structural conformational changes in protein folding resulting in inhibition or impaired biological activities, thus leads to substantial protein alterations and cleavage (e.g., Cu^{2+} can result in the inactivation of the vaccinia H1-related tyrosine phosphatase and stoichiometric concentrations of Cu ions can inhibit HIV-1 protease activity) (Kim et al., 2000).

Furthermore, Cu ions can intercalate with DNA, causing helical structure deformation and denaturation and this is a result of guanine-specific covalent binding affinity of Cu^{2+} ions facilitating a Cu^{2+}-induced oxidative DNA damage. DNA damage is further intensified by repeated cyclic redox reactions through the generation of reactive oxygen species (ROS), where Cu^{2+} and Cu^{+} catalyze H_2O_2 to hydroxyl radicals (•OH) through the Fenton oxidation reaction (Dimitrijevic et al., 2015). The redox Cu^{2+} and Cu^{+} are responsible for preventing the germination of fungal spores thereby eliminating the risks of mycotoxin manifestation (Gogos et al., 2012). It is worth noting that once Cu nanofungicides ($>$ 95% of Cu) applied in the field to mitigate mycotoxigenic fungal contamination, it takes approximately hours to weeks for their oxidization, dissolving, and forming chemical speciation of CuS and other insoluble Cu complexes depending on the soil, water chemistry (e.g., alkalinity, salinity, organic matter content, presence of sulfide, and other complexing ions) is released into the environment enters soil and aquatic sediments (Keller et al., 2017).

5 Green Cu nanofungicides

The synthetic repertoire of Cu nanoparticles is very challenging due to the rapid oxidation of CuNPs to CuONPs. Several green synthetic routes have been reported, this includes chitosan-Cu (Chit-Cu) nanogels and nanohydrogels complexation for inhibiting *F. graminearum* through the synergistic effect of chitosan and Cu (Alghuthaymi et al., 2021; Atiq et al., 2020). This provides a platform for the generation of biodegradable nanofungicides. Brunel et al. (2013) developed Chit-Cu^{2+} nanohydrogels as a biocompatible, bioactive, and pH-sensitive system for Cu^{2+} release against *F. graminearum*. They reported that at optimum conditions, the optimal adoption of Cu^{2+} (300 mg) was complexed to 1 g of chitosan via neutralized amino groups. The authors revealed that the slow release of Cu^{2+} was facilitated through two mechanisms as shown in Fig. 3.

The announced benefit of the Chit-Cu^{2+} nanohydrogels which include the ease in the complexation of chitosan with Cu^{2+} ions provides a thermodynamically favorable reservoir for slow Cu^{2+} release; chitosan has been reported to demonstrate antimicrobial activity, and is also known to be a plant growth promoter. Taken together with antimicrobial and biological activity of Cu, Chit-Cu^{2+} nanohydrogels provide a culminating synergistic complementary antifungal repertoire against mycotoxigenic fungi, subsequently eliminating the risk of mycotoxins.

Maize (*Zea mays* L.) is one of the major staple crop worldwide surpassing wheat and rice; however, it is susceptible to different fungal (*A. flavus*, *F. verticillioides*,

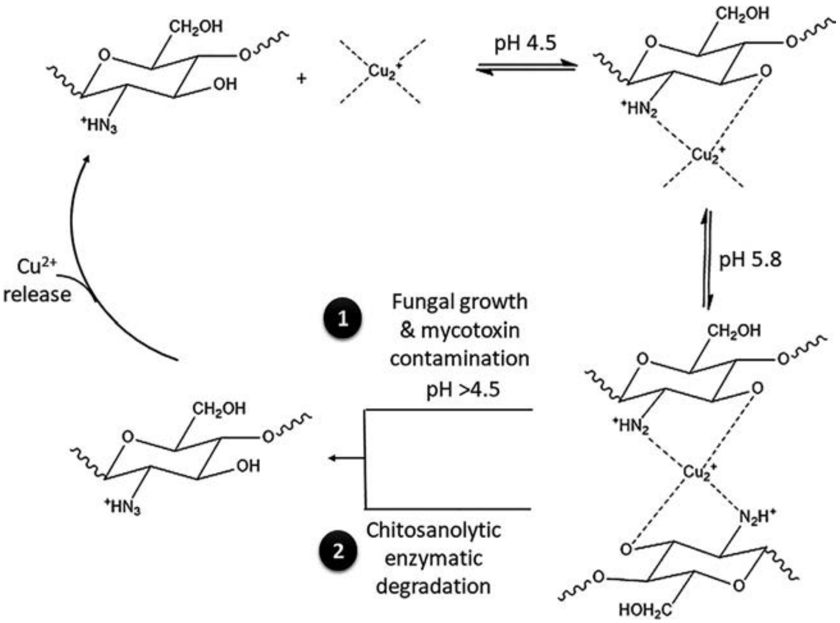


FIG. 3

Mechanism of Cu^{2+} complexation with chitosan and the release of Cu^{2+} through (1) acidification caused by fungal growth and mycotoxin contamination which aided the protonation of the amino groups, concomitantly releasing the bound Cu^{2+} from chitosan and (2) chitosanolytic enzymatic degradation of chitosan nanogels by fungal growth which causes a release of bound Cu^{2+}.

and *F. graminearum*) contamination both in field and during storage; therefore, is prone to multimycotoxins such as AFB_1, FBs, and DON contamination (Giorni et al., 2019). Choudhary et al. (2017) developed biodegradable chitosan-Cu nanoparticles (Chit-CuNPs) to enhanced innate immunity and plant growth stimulate activities of maize against various fungal diseases like curvularia leaf spot (CLS) disease caused by *Curvularia lunata*, which can also be applicable for eliminating mycotoxin contamination.

The developed Chit-Cu nanocomplex generated a well-coordinated synergy between chitosan and Cu, allowing for dynamic bioactivities of chitosan and Cu to be realized using cofactor functionality of various enzymes engaged in electron transport and redox reactions. Their findings revealed that the Chit-CuNPs were formed through CN bonding, with pH- and time-dependent Cu release facilitated by the protonation and deprotonation of the amino group of chitosan. At pH 4.5, Cu release was rapidly release from the nanocomplex, whereas at pH > 4.5 a gradual and persistent Cu release was observed. Their results revealed that the Chit-CuNPs were through C–N bonding with the pH- and time-dependent release of Cu facilitated by protonation and deprotonation of the amino group of chitosan, at pH < 4.5

expedited Cu release and pH > 4.5 resulted in slow and sustained release of Cu, corroborating the results repeated by Brunel et al. (2013). In addition, Chit-CuNPs enhanced plant defense responses through increased superoxide dismutase (SOD), peroxidase (POD), polyphenol oxidase, (PPO), and phenylalanine ammonia-lyase (PAL) activities (Fig. 4).

Pariona et al. (2019) synthesized CuNP fungicide using ascorbic acid and evaluated their antifungal activity against *F. solani*, *Neofosicoccum* sp., and *F. oxysporum.* Their results revealed that CuNPs induced mycelium morphological change through cell membrane damage and intracellular ROS generation. The utilization of green nanotechnology using phytochemicals from plants or crops susceptible to fungal and mycotoxin contamination to produce nanoformulations as nanomicrobicidal agents for synergistic protection will be an effective benign strategy. This notion was demonstrated by Ponmurugan et al. (2016), where they synthesized CuNP synthesized extracellularly using *Streptomyces griseus* to evaluate their antifungal activity against *Poria hypolateritia*, which causes red root-rot disease in tea plants. Their CuNPs were compared with widely used commercial systemic fungicide carbendazim and bulk Cu to assess their effectiveness as fungicide and soil quality after treatment. The results showed that the produced CuNPs were < 50 nm, fungicidal activity was recorded at 57.2% for carbendazim, followed by 52.7% at 2.5 ppm for CuNPs, and 45.3% bulk Cu.

Abd-Elsalam et al. (2020) developed a nanocomposite hydrogel composed of copper and chitosan (Cu-Chit/NC hydrogel) prepared by metal vapor synthesis (MVS). This study demonstrated the antifungal activity of the nanocomposite hydrogel against aflatoxigenic strains of *A. Flavus*, at 240 ppm attributed by the inactivation of *A. flavus* mycelia growth, subsequently reducing the production of AFs. The results demonstrate that Cu-Chit/NCS hydrogel is an innovative nano-biopesticide that can be used not only as an effective fungicide against plant pathogens but also as an effective agent for the management of toxigenic fungi in food and feed.

Carbendazim showed superior activity, however, CuNPs in addition to the fungicidal activity provided maximum leaf yield and improved soil macronutrients (total organic carbon, total nitrogen, phosphorus, and potassium) compared to the other treatments. In another study by Devipriya and Roopan (2017) produced 30 nm CuONPs synthesized using *Cissus quadrangularis* and evaluated their antifungal activity against *A. niger* and *A. flavus*, well-known mycotoxigenic fungi; notorious for producing aflatoxins and ochratoxins. The CuONPs exhibited better antifungal activity (> 80% inhibition at 500 ppm) against *A. niger* and *A. flavus*, while carbendazim showed 40% at 500 ppm. This knowledge provides a clear indication of the importance of developing benign Cu nanofungicides for agricultural practices against mycotoxigenic fungi and other pathogenic organisms that pose significant challenges in food safety and security.

Asghar et al. (2020) conducted a study comparing the antimicrobial activity of three different metallic nanoparticles (Ag, Cu, and Fe) synthesized via green chemistry using leaf extracts of *Syzygium cumini.* Results revealed that AgNPs showed

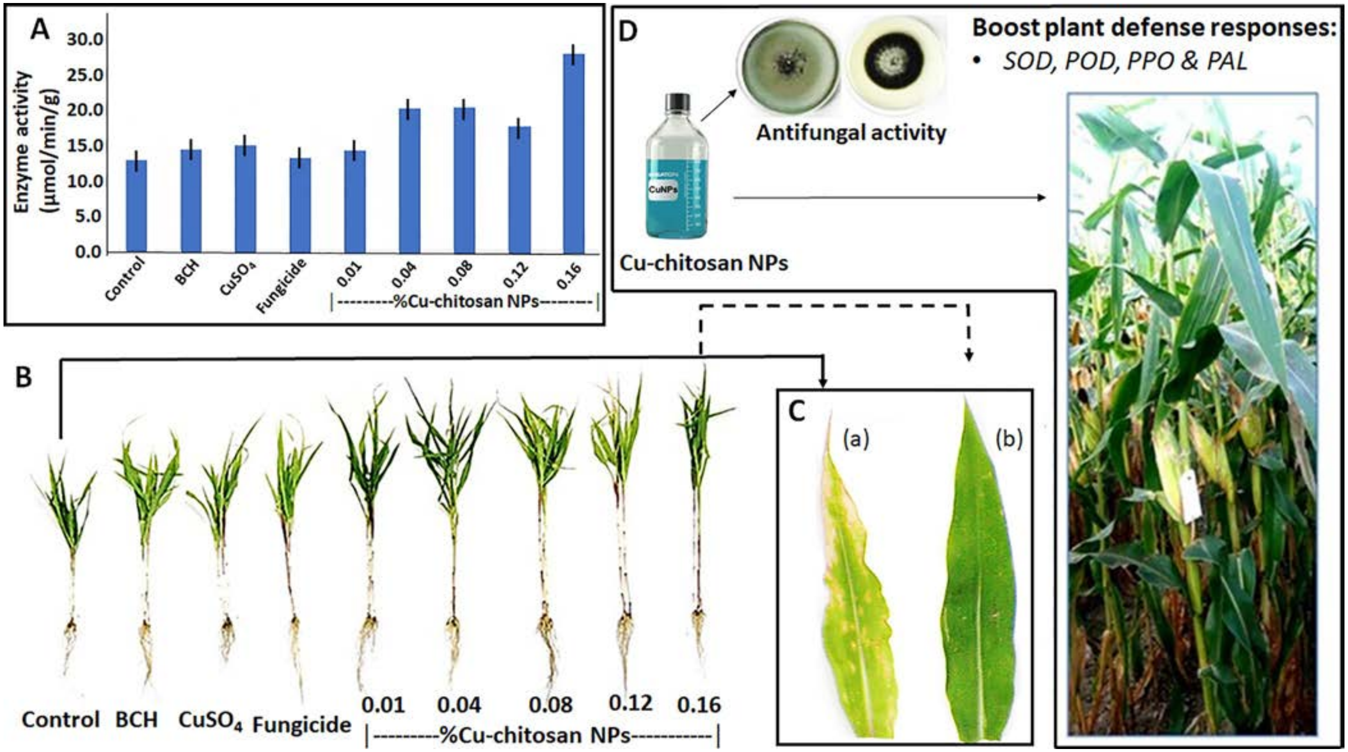


FIG. 4

Chitosan-Cu nanoparticles (Chit-CuNPs) antifungal and plant growth activities (A) effect of Chit-CuNPs on enzymes (SOD, POD, PAL, PPO) activity, (B) plant growth effects of Chit-CuNP treatment, (C) CLS disease symptoms on maize plant leaf (a) necrotic lesions in control, (b) no lesions on Chit-CuNPs (0.16%) treated leaf, and (D) in-field translational application model of Chit-CuNPs for enhanced defense responses and plant growth in maize.

Reproduced from Choudhary, R.C., Kumaraswamy, R.V., Kumari, S., Sharma, S.S., Pal, A., Raliya, R., Biswas, P., Saharan, V., 2017. Cu-chitosan nanoparticle boost defense responses and plant growth in maize (Zea mays L.). Sci. Rep. 7(1), 1–11. https://doi.org/10.1038/s41598-017-08571-0 with permission from Springer Nature, Creative Commons.

excellent antimicrobial activities against methicillin and vancomycin resistance bacterial strains *Staphylococcus aureus* and aflatoxigenic fungi *A. flavus* and *A. parasiticus*. Additionally, the production of AFs was significantly inhibited by the treatments, with AFB_1 adsorption capability on NPs observed in the following order FeNPs > CuNPs > AgNPs. Thus, metallic NPs obtained by green nanotechnology can be used with AF adsorbent and provide a promising avenue for the detoxification of AFB_1 in human food and animal feed.

Researchers have developed a method of mycogenic synthesis of zinc oxide (ZnO) and copper oxide (CuO) nanoparticles using *Penicillium chrysogenum* (Mohamed et al., 2020). The synthetic route for the production was facilitated by secreted extracellular proteins related to nitrate reductase during fungal growth. The produced ZnONPs and CuONPs exhibited increased antimicrobial activity against Gram-positive and Gram-negative bacteria, antibiofilm effects, and phytopathogenic fungal strains (*F. solani*, *F. oxysporum*, *Sclerotium sclerotia*, and *A. terreus*). Results showed that CuONPs antifungal activity was stronger than ZnONPs at 10 mg/mL. The CuONPs and ZnONPs were also effective *A. solani*, *A. niger*, *F. oxysporum*, *Pythium ultimum*, and *A. alternata*. The proposed mechanism of activity of nanomaterials to inhibit fungal growth was multifunctional through affecting cell functions, causing morphological changes in fungal hyphae, preventing the expansion of conidia and conidiophores, and consequently, leading to cell death.

Pérez-de León et al. (2020) investigated the comparative antifungal and antifumonigenic activities between CuNPs (2.5 ± 0.3 nm) and AgNPs (17 ± 1.5 nm) obtained by the chemical reduction method. Results revealed that both these nanoparticles exhibited antifungal activity against *F. verticillioides*, at 125 and 75 ppm for CuNPs and AgNPs, respectively. Antifumonigenic activity of CuNPs, completely inhibited FB_1 production at ≥ 100 ppm, while AgNPs only suppressed FB_1 biosynthesis at ≥ 20 ppm. The authors suggest that the activity of treatment with CuNPs and AgNPs occurred due to changes caused in the structure of the hyphae, such as the interference in mycelial growth, loss of contour and uniformity of the hyphae, and rupture of the hyphae, resulting in a significant reduction in the biosynthesis of FB_1. It is worth noting, since AgNPs were performed better than CuNPs, CuNPs for agricultural applications are more superior than AgNPs because of its added advantage of being an essential micronutrient for plant growth promoter.

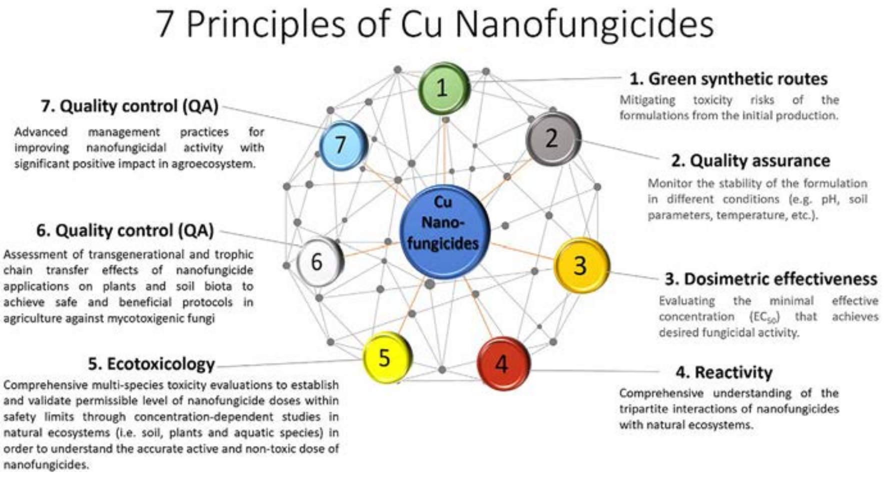
Researchers have developed an innovative approach using sucrose as a carbon source to obtain copper oxide nanocomposites (CuO/C) with a particle size of 50 nm (Roopan et al., 2019). The antifungal activities of the CuO/C nanocomposite were evaluated against *A. niger* and *A. flavus*. Antifungal activity of CuO/C nanocomposite at 1000 ppm, inhibited *A. flavus* and *A. niger* by 70% and 90%, respectively. Another study by Safaei et al. (2019) developed an alginate-CuO bionanocomposite and evaluated the antifungal activity against *A. niger*. Although the efficacy against the production of mycotoxins has not been evaluated, the alginate-CuO nanocomposite produced by green synthesis has demonstrated satisfactory antifungal efficacy, in addition to biocompatibility for biomedical, pharmaceutical, nutritional, agricultural, and environmental applications.

6 Future recommendations for Cu nanofungicides

The development and production of Cu nanofungicides require a comprehensive understanding of the multitude of the tripartite interactions of Cu nanoparticles with plant, soil, and soil microbes in order to assess and govern reliable ecotoxicology risk assessments and risk management factors of Cu nanofungicides destined for agricultural applications for effective protection against mycotoxigenic fungal contamination. It is only through this notion that sufficient scientific evidences would provide the basis of legislative frameworks, regulatory entities [e.g., USFDA, Food and Agriculture Organization (FAO), US Environmental Protection Agency (USEPA), Organization for Economic Cooperation and Development (OECD), International Standard Organization (ISO), and Registration, Evaluation, Authorization and Restriction of Chemicals (REACH)] for specific guidelines and provisions bestowing Cu nanofungicides utilization for agriculture, food, and feed sectors (Fig. 5). The intricate understanding of the comparative environmental fate and ecotoxicology of Cu nanomaterials interactions for eliminating the overall risk of mycotoxicology.

7 Conclusion

The use of Cu nanoformulation produced through green nanotechnology provides a remarkable strategy to reduce the amount of Cu introduced into agroecosystems, thus limiting ecotoxicological risks but still providing significant Cu for antifungal activity and micronutrient as a cofactor required by plants to facilitate a cascade of essential pathways. Albeit benefits have been since with the utilization of Cu



1. Green synthetic routes
Mitigating toxicity risks of the formulations from the initial production.

2. Quality assurance
Monitor the stability of the formulation in different conditions (e.g. pH, soil parameters, temperature, etc.).

3. Dosimetric effectiveness
Evaluating the minimal effective concentration (EC_{50}) that achieves desired fungicidal activity.

4. Reactivity
Comprehensive understanding of the tripartite interactions of nanofungicides with natural ecosystems.

5. Ecotoxicology
Comprehensive multi-species toxicity evaluations to establish and validate permissible level of nanofungicide doses within safety limits through concentration-dependent studies in natural ecosystems (i.e. soil, plants and aquatic species) in order to understand the accurate active and non-toxic dose of nanofungicides.

6. Quality control (QA)
Assessment of transgenerational and trophic chain transfer effects of nanofungicide applications on plants and soil biota to achieve safe and beneficial protocols in agriculture against mycotoxigenic fungi

7. Quality control (QA)
Advanced management practices for improving nanofungicidal activity with significant positive impact in agroecosystem.

FIG. 5

The seven principles of the production, assessment, and application of Cu nanofungicides for eliminating mycotoxin contamination and other agricultural pathogens.

nanofungicides over conventional fungicides. It is imperative to invest in the understanding of the ecotoxicology of the Cu nanoformulation lifecycle. The architectural design of Cu nanofungicides can influence the physiochemical and fungicidal/fungistatic properties attributed by bioavailability and cupric ion release as a function of antifungal activity and overall toxicity profile. To avert toxicity, it is important to consider green nanotechnologies and dosimetric calculations. This amalgamation of a green sustainable nanotechnology will result in enhanced fungicidal activity thereby eliminating the risk of mycotoxins with improved crop/food productivity and management without imposing ecotoxicological, nano-phytotoxicity effects in agroecosystems (crops, soil), animal, and human.

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References

Abd-Elsalam, K.A., El-Naggar, M.A., Ghannouchi, A., Bouqellah, N.A., 2019. Nanomaterials and ozonation: safe strategies for mycotoxin management. In: Nanomycotoxicology: Treating Mycotoxins in the Nano Way. Elsevier Inc., https://doi.org/10.1016/B978-0-12-817998-7.00013-6.

Abd-Elsalam, K.A., Alghuthaymi, M.A., Shami, A., Rubina, M.S., Abramchuk, S.S., Shtykova, E.V., Vasil'kov, A.Y., 2020. Copper-chitosan nanocomposite hydrogels against aflatoxigenic *Aspergillus flavus* from dairy cattle feed. J. Fungi 6 (3), 1–20. https://doi.org/10.3390/jof6030112.

Adam, M.A.A., Tabana, M.Y., Musa Binti, K., Sandai Anak, D., 2017. Effects of different mycotoxins on humans, cell genome and their involvement in cancer (review). Oncol. Rep. 37 (3), 1321–1336. https://doi.org/10.3892/or.2017.5424.

Adisa, I.O., Pullagurala, V.L.R., Peralta-Videa, J.R., Dimkpa, C.O., Elmer, W.H., Gardea-Torresdey, J.L., White, J.C., 2019. Recent advances in nano-enabled fertilizers and pesticides: a critical review of mechanisms of action. Environ. Sci. Nano 6 (7), 2002–2030. https://doi.org/10.1039/c9en00265k.

Agrimonti, C., Lauro, M., Visioli, G., 2021. Smart agriculture for food quality: facing climate change in the 21st century. Crit. Rev. Food Sci. Nutr. 61 (6), 971–981. https://doi.org/10.1080/10408398.2020.1749555.

Alghuthaymi, M.A., Rajkuberan, C., Rajiv, P., Kalia, A., Bhardwaj, K., Bhardwaj, P., Abd-Elsalam, K.A., Valis, M., Kuca, K., 2021. Nanohybrid antifungals for control of plant diseases: current status and future perspectives. J. Fungi 7 (1), 1–20. https://doi.org/10.3390/jof7010048.

Asghar, M.A., Zahir, E., Asghar, M.A., Iqbal, J., Rehman, A.A., 2020. Facile, one-pot biosynthesis and characterization of iron, copper and silver nanoparticles using *Syzygium cumini* leaf extract: As an effective antimicrobial and aflatoxin B1 adsorption agents. PLoS One 15 (7), e0234964. https://doi.org/10.1371/journal.pone.0234964.

Atiq, M., Naeem, I., Sahi, S.T., Rajput, N.A., Haider, E., Usman, M., Shahbaz, H., Fatima, K., Arif, E., Qayyum, A., 2020. Nanoparticles: a safe way towards fungal diseases. Arch. Phytopathol. Plant Protect., 1–12. https://doi.org/10.1080/03235408.2020.1792599.

Borkow, G., Gabbay, J., 2009. Copper, an ancient remedy returning to fight microbial, fungal and viral infections. Curr. Chem. Biol. 3 (3), 272–278. https://doi.org/10.2174/187231309789054887.

Brunel, F., El Gueddari, N.E., Moerschbacher, B.M., 2013. Complexation of copper(II) with chitosan nanogels: toward control of microbial growth. Carbohydr. Polym. 92 (2), 1348–1356. https://doi.org/10.1016/j.carbpol.2012.10.025.

Castro-Mayorga, J.L., Cabrera-Villamizar, L., Balcucho-Escalante, J., Fabra, M.J., López-Rubio, A., 2020. Applications of nanotechnology in agry-food productions. Nanotoxicity, 319–340. https://doi.org/10.1016/b978-0-12-819943-5.00015-4.

Çelik, K., 2019. The efficacy of mycotoxin-detoxifying and biotransforming agents in animal nutrition. In: Nanomycotoxicology: Treating Mycotoxins in the Nano Way, pp. 271–284, https://doi.org/10.1016/B978-0-12-817998-7.00012-4.

Chellaram, C., Murugaboopathi, G., John, A.A., Sivakumar, R., Ganesan, S., Krithika, S., Priya, G., 2014. Significance of nanotechnology in food industry. APCBEE Procedia 8 (Caas 2013), 109–113. https://doi.org/10.1016/j.apcbee.2014.03.010.

Chen, R., Sun, Y., Huo, B., Zhao, X., Huang, H., Li, S., Bai, J., Liang, J., Gao, Z., 2021. A copper monosulfide-nanoparticle-based fluorescent probe for the sensitive and specific detection of ochratoxin A. Talanta 222, 121678. https://doi.org/10.1016/j.talanta.2020.121678.

Choudhary, R.C., Kumaraswamy, R.V., Kumari, S., Sharma, S.S., Pal, A., Raliya, R., Biswas, P., Saharan, V., 2017. Cu-chitosan nanoparticle boost defense responses and plant growth in maize (*Zea mays* L.). Sci. Rep. 7 (1), 1–11. https://doi.org/10.1038/s41598-017-08571-0.

Devipriya, D., Roopan, S.M., 2017. *Cissus quadrangularis* mediated ecofriendly synthesis of copper oxide nanoparticles and its antifungal studies against *Aspergillus niger*, *Aspergillus flavus*. Mater. Sci. Eng. C 80, 38–44. https://doi.org/10.1016/j.msec.2017.05.130.

Dimitrijevic, M., Karabasil, N., Boskovic, M., Teodorovic, V., Vasilev, D., Djordjevic, V., Kilibarda, N., Cobanovic, N., 2015. Safety aspects of nanotechnology applications in food packaging. Proc. Food Sci. 5, 57–60. https://doi.org/10.1016/j.profoo.2015.09.015.

El-Abeid, S.E., Ahmed, Y., Daròs, J.A., Mohamed, M.A., 2020. Reduced graphene oxide nanosheet-decorated copper oxide nanoparticles: a potent antifungal nanocomposite against fusarium root rot and wilt diseases of tomato and pepper plants. Nanomaterials 10 (5). https://doi.org/10.3390/nano10051001.

Fadl, S.E., El-Shenawy, A.M., Gad, D.M., El Daysty, E.M., El-Sheshtawy, H.S., Abdo, W.S., 2020. Trial for reduction of Ochratoxin A residues in fish feed by using nano particles of hydrated sodium aluminum silicates (NPsHSCAS) and copper oxide. Toxicon 184 (May), 1–9. https://doi.org/10.1016/j.toxicon.2020.05.014.

Giorni, P., Bertuzzi, T., Battilani, P., 2019. Impact of fungi co-occurrence on mycotoxin contamination in maize during the growing season. Front. Microbiol. 10, 1265. https://www.frontiersin.org/article/10.3389/fmicb.2019.01265.

Gogos, A., Knauer, K., Bucheli, T.D., 2012. Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. J. Agric. Food Chem. 60 (39), 9781–9792. https://doi.org/10.1021/jf302154y.

He, X., Deng, H., Hwang, H.M., 2019. The current application of nanotechnology in food and agriculture. J. Food Drug Anal. 27 (1), 1–21. https://doi.org/10.1016/j.jfda.2018.12.002.

Jesmin, R., Chanda, A., 2020. Restricting mycotoxins without killing the producers: a new paradigm in nano-fungal interactions. Appl. Microbiol. Biotechnol. 104 (7), 2803–2813. https://doi.org/10.1007/s00253-020-10373-w.

Keller, A.A., Adeleye, A.S., Conway, J.R., Garner, K.L., Zhao, L., Cherr, G.N., Hong, J., Gardea-Torresdey, J.L., Godwin, H.A., Hanna, S., Ji, Z., Kaweeteerawat, C., Lin, S., Lenihan, H.S., Miller, R.J., Nel, A.E., Peralta-Videa, J.R., Walker, S.L., Taylor, A.A., et al., 2017. Comparative environmental fate and toxicity of copper nanomaterials. NanoImpact 7, 28–40. https://doi.org/10.1016/j.impact.2017.05.003.

Khamis, Y., Hashim, A.F., Margarita, R., Alghuthaymi, M.A., Abd-Elsalam, K.A., 2017. Fungicidal efficacy of chemically-produced copper nanoparticles against *Penicillium digitatum* and *Fusarium solani* on citrus fruit. Philipp. Agric. Sci. 100 (1), 69–78.

Kim, J.-H., Cho, H., Ryu, S.-E., Choi, M.-U., 2000. Effects of metal ions on the activity of protein tyrosine phosphatase VHR: highly potent and reversible oxidative inactivation by Cu^{2+} ion. Arch. Biochem. Biophys. 382 (1), 72–80. https://doi.org/10.1006/abbi.2000.1996.

Kolackova, I., Gruberova, H.A., Kratochvil, O., Baholet, D., Skladanka, J., Jancar, J., Skarpa, P., 2021. Effect of foliar copper-containing superabsorbent polymers on nutritional characteristics and mycotoxin contamination of wheat kernel. Acta Univ. Agric. Silvic. Mendel. Brun. 69 (1), 71–78. https://doi.org/10.11118/ACTAUN.2021.007.

Konappa, N., Krishnamurthy, S., Arakere, U.C., Chowdappa, S., Akbarbasha, R., Ramachandrappa, N.S., 2021. Nanofertilizers and nanopesticides: recent trends, future prospects in agriculture. In: Jogaiah, S., Singh, H.B., Fraceto, L.F., de Lima, R. (Eds.), Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp. 281–330, https://doi.org/10.1016/B978-0-12-820092-6.00012-4 (Chapter 12).

Malhotra, N., Ger, T.R., Uapipatanakul, B., Huang, J.C., Chen, K.H.C., Der Hsiao, C., 2020. Review of copper and copper nanoparticle toxicity in fish. Nanomaterials 10 (6), 1–28. https://doi.org/10.3390/nano10061126.

Maqsood, S., Qadir, S., Hussain, A., Asghar, A., Saleem, R., Zaheer, S., Nayyar, N., 2020. Antifungal properties of copper nanoparticles against *Aspergillus niger*. Sch. Int. J. Biochem. 03 (04), 87–91. https://doi.org/10.36348/sijb.2020.v03i04.002.

MarketWatch, 2021. Copper Fungicides Market 2021: Analytical Overview, Key Players, Growth Factors, Demand, Market Size, Trends and Forecast to 2026 with Top Countries Data. The Expresswire. https://www.marketwatch.com/press-release/copper-fungicides-market-2021-analytical-overview-key-players-growth-factors-demand-market-size-trends-and-forecast-to-2026-with-top-countries-data-2021-03-21.

Melendez, M.V., Heckman, J.R., Murphy, S., D'amico, F., 2020. New Jersey farm soil copper levels resulting from copper fungicide applications. HortTechnology 30 (2), 268–272. https://doi.org/10.21273/HORTTECH04494-19.

Mishra, S., Keswani, C., Abhilash, P.C., Fraceto, L.F., Singh, H.B., 2017. Integrated approach of agri-nanotechnology: challenges and future trends. Front. Plant Sci. 8 (April), 1–12. https://doi.org/10.3389/fpls.2017.00471.

Mohamed, A.A., Abu-Elghait, M., Ahmed, N.E., Salem, S.S., 2020. Eco-friendly mycogenic synthesis of ZnO and CuO nanoparticles for in vitro antibacterial, antibiofilm, and antifungal applications. Biol. Trace Elem. Res. https://doi.org/10.1007/s12011-020-02369-4.

Nath, A., Molnár, M.A., Albert, K., Das, A., Bánvölgyi, S., Márki, E., Vatai, G., 2019. Agrochemicals from nanomaterials—synthesis, mechanisms of biochemical activities and applications. Compr. Anal. Chem. 84, 263–312. https://doi.org/10.1016/bs.coac.2019.04.004.

Nile, S.H., Baskar, V., Selvaraj, D., Nile, A., Xiao, J., Kai, G., 2020. Nanotechnologies in food science: applications, recent trends, and future perspectives. Nano-Micro Lett. 12 (1). https://doi.org/10.1007/s40820-020-0383-9. Springer, Singapore.

Oziengbe, E.O., Osazee, J.O., 2012. Antifungal activity of copper sulphate against *Colletotrichum gloeosporioides*. J. Asian Sci. Res. 2 (12), 835–839. http://aessweb.com/past-issue.php?id=5003%0Ahttps://www.cabdirect.org/cabdirect/abstract/20133089771.

Pariona, N., Mtz-Enriquez, A.I., Sánchez-Rangel, D., Carrión, G., Paraguay-Delgado, F., Rosas-Saito, G., 2019. Green-synthesized copper nanoparticles as a potential antifungal against plant pathogens. RSC Adv. 9 (33), 18835–18843. https://doi.org/10.1039/c9ra03110c.

Pérez-de León, A., Plasencia, J., Vázquez-Durán, A., Méndez-Albores, A., 2020. Comparison of the in vitro antifungal and anti-fumonigenic activities of copper and silver nanoparticles against *Fusarium verticillioides*. J. Clust. Sci. 31 (1), 213–220. https://doi.org/10.1007/s10876-019-01638-0.

Ponmurugan, P., Manjukarunambika, K., Elango, V., Gnanamangai, B.M., 2016. Antifungal activity of biosynthesised copper nanoparticles evaluated against red root-rot disease in tea plants. J. Exp. Nanosci. 11 (13), 1019–1031. https://doi.org/10.1080/17458080.2016.1184766.

Roopan, S.M., Devi Priya, D., Shanavas, S., Acevedo, R., Al-Dhabi, N.A., Arasu, M.V., 2019. CuO/C nanocomposite: synthesis and optimization using sucrose as carbon source and its antifungal activity. Mater. Sci. Eng. C 101, 404–414. https://doi.org/10.1016/j.msec.2019.03.105.

Safaei, M., Taran, M., Imani, M.M., 2019. Preparation, structural characterization, thermal properties and antifungal activity of alginate-CuO bionanocomposite. Mater. Sci. Eng. C 101, 323–329. https://doi.org/10.1016/j.msec.2019.03.108.

Shah, B.R., Mraz, J., 2020. Advances in nanotechnology for sustainable aquaculture and fisheries. Rev. Aquac. 12 (2), 925–942. https://doi.org/10.1111/raq.12356.

Sidhu, A., Barmota, H., Bala, A., 2017. Antifungal evaluation studies of copper sulfide nano-aquaformulations and its impact on seed quality of rice (*Oryzae sativa*). Appl. Nanosci. 7 (8), 681–689. https://doi.org/10.1007/s13204-017-0606-7.

Song, C., Hong, W., Zhang, X., Lu, Y., 2018. Label-free and sensitive detection of Ochratoxin A based on dsDNA-templated copper nanoparticles and exonuclease-catalyzed target recycling amplification. Analyst 143 (8), 1829–1834. https://doi.org/10.1039/c8an00158h.

Tang, H., Xu, M., Luo, J., Zhao, L., Ye, G., Shi, F., Lv, C., Chen, H., Wang, Y., Li, Y., 2019. Liver toxicity assessments in rats following sub-chronic oral exposure to copper nanoparticles. Environ. Sci. Eur. 31 (1). https://doi.org/10.1186/s12302-019-0214-0.

Tanwar, A., Sushil, 2019. Role and effects of nanotechnology used in pesticides and agriculture field. AIP Conf. Proc. 2142 (August). https://doi.org/10.1063/1.5122581.

Tegenaw, A., Tolaymat, T., Al-Abed, S., El Badawy, A., Luxton, T., Sorial, G., Genaidy, A., 2015. Characterization and potential environmental implications of select Cu-based fungicides and bactericides employed in U.S. markets. Environ. Sci. Technol. 49 (3), 1294–1302. https://doi.org/10.1021/es504326n.

Young, D., Avila-Adame, C., Webster, J., Olson, B., Ouimette, D., 2016. Enhancing the Efficacy of Copper Fungicides Through Synergism with Salicylaldehyde Benzoylhydrazones. VIII. Modern Fungicides and Antifungal Compounds, vol. VII Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978-3-941261-15-0, pp. 273–278.

Zhang, X., Li, G., Wu, D., Liu, J., Wu, Y., 2020. Recent advances on emerging nanomaterials for controlling the mycotoxin contamination: from detection to elimination. Food Front. 1 (4), 360–381. https://doi.org/10.1002/fft2.42.