CHAPTER

Silver nanoparticles applications and ecotoxicology for controlling mycotoxins

23

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23.1 Introduction

The agricultural and food systems sector suffers from a vast array of contaminations resulting in losses in agricultural productivity, which accounts to approx. 1 billion metric tons of foods (i.e., crops, cereals, and food derivatives/products) destined for human and animal consumption amid global food demands and evolutional climate change patterns (Yin et al., 2018). Of these contaminations, mycotoxins account for 25% and the most frequent contaminant according to the Food and Agriculture Organization (FAO). Food insecurity is still associated with high mortality rates, which are exacerbated by mycotoxins, especially in developing countries. Increased global food demands are inevitable according to projected population growth of 9.7 billion by 2050 and 10.9 billion by 2100 (United Nations, 2019). Fungi ubiquitously occur in different environmental conditions and produce toxigenic mycotoxins that may have detrimental toxic effects to both human and animal health. Fungi can invade crops with subsequent production of mycotoxins at different stages of the food chain, during preharvesting, harvesting, processing, transport, and storage (Abd-Elsalam et al., 2017; Thipe et al., 2018). The biological activities of mycotoxins are specifically to induce toxicity, phytotoxicity, and antibiosis in food and feed, yielding a cascade of ecotoxicological ramification to human and animal health (El-Waseif et al., 2019).

The agricultural sector is manifested by problems caused by aflatoxins (AFs), ochratoxins (OT), fumonisins (FUM), trichothecenes, and zearalenone (ZEN), with aflatoxin B_1 (AFB_1) known to be the most dangerous mycotoxin to human health because of its high hepatocarcinogenicity (Thipe et al., 2018). Exposure through acute or chronic to

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mycotoxins via consumption of contaminated foods/feeds can result in mycotoxicosis, provoking problems such as cancers and other generally irreversible effects. Over time, chronic exposure to mycotoxin can result in the deterioration of target organs such as the liver or lungs in animals and human beings (Cinar and Onbasi, 2019). Despite scientific advances, mycotoxins are endocrine and economic disruptors as they impose a tremendous burden on the healthcare system and economic losses from fungal infections with subsequent mycotoxins contamination. The staggering incidences of mycotoxins prompt consistent mandatory measures to monitor and control mycotoxins in the food segment. Prevention of mycotoxin manifestation can be achieved by mitigating the fungal growth using chemical compounds such as fungicides to protect crops. Nevertheless, these substances are not entirely efficient and present secondary toxicity due to 90% of fungicides is susceptible to runoff, thus leaching to the water supply that can increase costs and greater damage to the environment (Zubrod et al., 2019). Moreover, pesticides also affect human/animal health and plants, and this is due to their high toxicity and non-biodegradable properties (Abd-Elsalam et al., 2017; Abd-Elsalam and Alghuthaymi, 2015).

As we foster innovative solutions against mycotoxin, new technologies such as nanotechnology could be the solution to eliminating mycotoxins with no to minimal ecotoxicology. To circumvent the abovementioned challenges, nanotechnology and material sciences have received considerable attention due to the properties and feasibility of engineered nanomaterials in various applications (e.g., antiseptics and disinfectants, cosmetics, electronics, textiles, diagnostic imaging, and targeted drug delivery). This provides innovative, creative, and safe solutions for the detection, control, and prevention of mycotoxins. The high surface-to-volume ratio exhibited by nanomaterials exposes surface atoms, thus allowing multifunctionalization with active compounds at low concentration for increased efficacy (Yin et al., 2018). The minimal utilization of nanoparticles to obtain good efficiency in plant protection permits low toxic effects on the environment and human/animal health. Among the nanomaterials developed, metallic nanoparticles are known as the most promising nanomaterials because of their antimicrobial characteristics and ability to target plant pathogens, sparing good microorganisms and insects within the soil and aquatic microbiota (Abd-Elsalam et al., 2017).

Nanoparticles can originate from anthropogenic or natural sources or intentionally produce for different purposes. The popularity of nanoparticles, especially silver nanoparticles (AgNPs), which are one of the most prevalent nanoparticles, stems from their antimicrobial properties (controlling diverse and multidrug-resistant microorganisms), which makes them ideal substitutes for fungicides in agriculture as a new class of agrochemicals, antimicrobials, disinfectants, and food protectant (i.e., food packaging) acting over 650 different microorganisms, including mycotoxigenic fungi (Siddiqi and Husen, 2016; Tortella et al., 2020). Antimicrobial additives in various products consisting of Ag complexes, mainly AgNPs due to their high reactivity.

AgNPs have been suggested to be very effective antifungal agents against mycotoxigenic fungi, thereby ensuring agricultural sustainability and food safety toward achieving the food security in the 2030 Agenda for a sustainable development program for ending poverty and hunger to responding to climate change and sustaining natural resources, food and agriculture (FAO, 2020). Nanoparticles have offered enormous benefits in a myriad of applications due to their physicochemical properties; however, we must not be naïve in fully understanding their ecotoxicology profile before using them in agriculture and other applications (Yan and Chen, 2019). The surge in the application of AgNPs as antifungal agents in the agricultural sector has been under great concerns due to their safety and ecotoxicology and is intensified by the limited knowledge of AgNPs lifecycle analysis studies. It is paramount to fully understand the interaction between AgNPs and eukaryotic cells (e.g., animals, plants, fungus, and human) to best engineer materials effective at low doses with minimal to no toxicity on environmental and human health for ecological and ecotoxicological insight.

Ideally, AgNPs at much lower doses than those typically used in toxicological and ecotoxicological studies for application as mycotoxins inhibitors is important for food/feed and human/animal safety. This is advantageous because the low dose substantially reduces the concern of human toxicity and provides inhibition of the mycotoxins and virulence factors (Jesmin and Chanda, 2020). It is important to acknowledge that the efficacy and safety of nanoparticles including AgNPs are dependent on their size and capping/stabilizing ligand. The AgNPs-bio interaction dictates the efficacy and safety of AgNPs in agriculture as mycotoxins inhibitors. While the AgNPs interaction with fungal cell via endocytosis (clathrin-dependent and receptor-mediated) or cell surface lipid raft-associated domain (caveolae) often results in cellular and membrane damage facilitated by the dissolution of the AgNPs into Ag^+ ions producing reactive oxygen species (ROS) causing membrane rupture and leakage (Vergallo et al., 2020). This is attributed to AgNPs exhibiting several modes of action that guarantees their high efficiency as biocides. These nanoparticles can inhibit fungal growth and also interfere with mycotoxins biosynthetic pathways (Lara et al., 2015; Pietrzak et al., 2015; Thipe et al., 2020). When acting as a mycotoxin inhibitor in the toxin-producing environment (endosome), AgNPs do not necessarily inhibit the growth of the fungus. The fungicidal activity or mycotoxin inhibitory effect of AgNPs against fungi is dependent on the several factors (e.g., the concentration of AgNPs, release of Ag^+ ions, surface chemistry, size, coating ligand, shape, and polydispersity) (Jesmin and Chanda, 2020; Jogee et al., 2017). In this context, there are concerns with them leaching/migrating into the environment and entering the food ecosystem through water/soil bodies (Montes de Oca-Vásquez et al., 2020). Yan and Chen (2019) further elaborated on the importance of phytotoxicity of AgNPs, as plants are the primary constituents in the ecosystem that serves as important sources of food for both animals and humans.

Moreover, the effectiveness of AgNPs is dependent on their physicochemical properties such as their stability. This was corroborated by Mitra et al. (2019), where they demonstrated that PVP-coated AgNPs having high stability than citrated-coated AgNPs, which are unstable, exhibited inhibition of aflatoxin biosynthesis over a broader concentration range than citrated-coated AgNPs even at higher concentrations. They showed that nanoparticles with 15 nm are more easily captured by the endosome than the bigger ones (20 and 30 nm) and that the concentrations of inhibitors are directly linked to the size of the nanoparticles—the smaller the size of the AgNPs, the lower the minimum inhibitory concentration (MIC). The material coating also influences AgNPs action, and those encapsulated with PVP (36 nm) showed a higher concentration range for inhibition (from 40 to 75 ppm), while AgNPs coated with citrate presented punctual concentrations for inhibitory action: 25 ppm for 15 nm nanoparticles, 40–60 ppm for 20 nm nanoparticles, and 50 ppm for 30 nm nanoparticles (Mitra et al., 2019).

23.2 Silver nanoparticles in agriculture as antifungicides

23.2.1 Antifungal activity of commercial AgNPs

The global application of AgNPs has increased during the past few years; this is fueled by the desirable properties that AgNPs exhibit. Approximately 2000 products with nanomaterials are produced daily, with about 1/5 (435) of nanoproducts are Ag-based (Pulit-Prociak and Banach, 2016). Applications of AgNP or AgNPs formulations have seen a boom within the agricultural sector as alternative antimicrobial agents and packaging material constituents for protection against fungal growth, mycotoxins, and increasing shelf-life of various products (Liu et al., 2020). This warrants the application of AgNPs in agriculture as an alternative to toxic chemical fungicides that result in the development of resistance. New generation packing materials made of nanocomposites protect food by their antimicrobial properties. Polymers containing metal nanoparticles, including AgNPs, are used to produce these packaging materials that are also capable of reducing mycotoxin contamination by interacting with the outer cell membrane of the fungi and cause changes in their permeability and functionality (Thipe et al., 2018). Tarus et al. (2019) developed electrospun cellulose acetate and poly(vinyl chloride) nanofiber mats with AgNPs aiming to produce antifungal packaging material. Their results demonstrated that the presence of AgNPs decreased the development of mold on fruit surfaces.

Work by El-Waseif et al. (2019) evaluated the commercial prospects of AgNPs synthesized using *Streptomyces clavuligerus* against mycotoxigenic *F. oxysporum*, which produces fumonisins, zearalenone, trichothecenes, and nivalenol. Their investigation was on 4-week old tomato seedlings with pot experiment at an experimental farm station (using different methods of treatment such as foliar shoot, root immersion (RI), and foliar shoot + root immersion) for evaluating the effects of AgNPs on the percentage of disease incidence and protection of tomato plants infected with *F. oxysporum*. Results demonstrated that AgNPs have significant antifungal activity against *F. oxysporum*, in addition to the reduced percent disease index (PDI) at 8.00% and increased protection at 90.40% against infection compared to control infected tomato plants with PDI at 83.33% and no protection. Treatment with AgNPs at 60 ppm revealed optimum protection with increased phenolic level, proline amount as inhibitors of preinfection, thereby providing tomatoes with a specific level of essential resistance against *F. oxysporum*.

Villamizar-Gallardo et al. (2016) evaluated the fungicidal activity of commercial Biopure-AgNPs from NanoComposix against *A. flavus* and *F. solani* on cocoa (*Theobroma cacao*) crops. Their results revealed that Biopure-AgNPs did not significantly affect the growth of *A. flavus* and *F. solani* even at 100 ppm in liquid and solid synthetic culture media. However, in plant tissues (especially in the cortex), AgNPs inhibited the fungal growth of *A. flavus* at 80 ppm and it was noted that once fungi penetrated through the cocoa pods, their growth is unavoidable, and the effect of AgNP was reduced, while in *F. solani*, AgNPs only induced some texture and pigmentation changes. This demonstrated that AgNPs used in this study are more effective in plant tissues than in culture media, providing an opportunity for their utilization in-field application to control the growth of *A. flavus* and the production of aflatoxins.

Kotzybik et al. (2016) also investigated the use of commercial AgNPs (Citrate BioPure™ Silver) with different sizes (5, 50, and 200 nm) at varying concentrations (1, 5, 25, 50 and 100 ppm) from NanoComposix, in addition to MesoSilver® with size ≤ 0.65 nm at different concentrations (1, 2, 4, 6, and 8 ppm) from Purest Colloids. These AgNPs were tested for their influence on *P. verrucosum* growth and mycotoxin biosynthesis. Before studies were conducted on *P. verrucosum*, the authors evaluated the stability of AgNPs in the Malt Extract Agar (MEA) medium (with Tween/NaCl/Agar) used in the experiment. They noticed that Citrate BioPure™ Silver were not stable in MEA medium while MesoSilver® exhibited high stability over 7 days. Moreover, their antifungal activity studies revealed that smaller sized AgNPs (0.65 and 5 nm) application preinoculation of spore germination strongly inhibited germination and fungal growth of *P. verrucosum* with a subsequent decrease in mycotoxins biosynthesis at 2 and 5 ppm, respectively. The larger sized AgNPs were not effective, and this can be attributed to that smaller AgNPs have a high rate of Ag^+ dissolution, which is also dependent some factors (size, shape, concentration, capping agent, and the colloidal state of AgNPs) due to their high surface area to volume ratio, increased bioavailability, enhanced distribution, and toxicity of Ag compared with larger AgNPs (Ferdous and Nemmar, 2020). These AgNPs accumulate the fungal cell mycelial filaments, the Ag^+-ions/particles interfere with electron-transport-systems with subsequent production of ROS and damage to proteins associated with ATP production, lipids, and nucleic acids, ultimately modulating the intracellular signaling pathways resulting in apoptosis (Ferdous and Nemmar, 2020).

Kwak et al. (2012) evaluated the use of commercial product Pyto-patch®, which contains ≤5 nm AgNPs antifungal activity against *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Sclerotinia sclerotiorum* for controlling anthracnose. Their results demonstrated that pepper fruits sprayed with 10 ppm Pyto-patch® treatment reported 75.8% survival, whereas in the untreated pepper fruits, no survival was observed. The results obtained by Kotzybik et al. (2016) corroborate those reported by Jo et al. (2009) where they used different forms of Ag [$AgNO_3$, AgCl, AgNPs (20–30 nm), where AgNPs are denoted as Ag(p), and electrochemical Ag are denoted as Ag(e)] to evaluate their effectiveness against plant-pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea*, and their disease severity in plants for phytotoxicity. Their results revealed that plants sprayed with $AgNO_3$, Ag(e), and Ag(p) before inoculation with spores inhibited colony formation. The half-maximal effective concentration (EC_{50}) values for *M. grisea* and *B. sorokiniana* ranged from 0.8 to 1.0 ppm $AgNO_3$, 3.9 to 4.7 ppm Ag(p), and 5.6 to 6.8 ppm Ag(e) at 1 to 6 h after treatment.

The utilization of AgNPs incorporated into packaging matrices to serve as antimicrobial agents to provide enhanced material strength, and inactivation of fungi and mycotoxins is desirable. Kim et al. (2012) compared three different types of AgNPs provided by BioPlus Co. Ltd. (Korea) applied for antifungal treatment in plant pathogens. The nanoparticles were WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R, and they were tested on 18 plant-pathogenic fungi of agricultural interest. The fungi were grown in vitro on three types of agar PDA (potato dextrose), MEA (malt extract), and CMA (corn flour). AgNPs size varied from 7 to 25 nm and concentrations from 10 to 100 ppm, the most significant inhibition was in PDA with AgNPs at 100 ppm. The results of this work indicated that AgNPs have antifungal properties against these plant pathogens at various levels depending on the type of AgNPs, fungi, culture medium, and concentration.

23.2.2 AgNPs commercial applications in controlling mycotoxins

The European Union has endorsed the use of commercial products fabrication and/or formulated with AgNPs for the mitigation of mycotoxins (Kotzybik et al., 2016). Nanotechnology has been beneficial at protecting crops from contaminates and extending food shelf life with immense security through in-field stage (i.e., enhanced agricultural crop cultivation and protection practices) and cross-stage (i.e., food packaging material and nanoenabled sensors for fungal and mycotoxin detection) applications to protect during all subsequent stages (i.e., harvest, storage, processing, and transportation) to the endpoint—consumer (Yin et al., 2018). The current use of AgNPs in a variety of products for increased product shelf-life and protecting from fungal and mycotoxins contamination has spiked due to catalytic and antimicrobial properties exhibited by AgNP through multimodal activities (Ferdous and Nemmar, 2020; Jo et al., 2009). This provides added functionality and enhanced efficiency for advanced agricultural sustainability.

The application of AgNPs supplements such as spray-impregnation for foods, which are normally washed, shelled, or peeled (e.g., melons, apples, or citrus fruits) in the field or applied via dehumidifier during the storage and transport, would be ideal to provide a protective coating against fungal manifestation and mycotoxins contamination (Kotzybik et al., 2016). AgNPs are already in products of daily use; this includes Ag stabilized hydrogen peroxide for soil fumigation and sterilization, as fungicides in agriculture and agricultural spray adjuvants, and in food context, especially in packaging material and container, as shown in Table 23.1 (Bumbudsanpharoke and Ko, 2015).

The use of AgNPs and/or incorporation polymeric matrices in packaging material provides stronger packaging barriers against fungal growth and mycotoxins contamination. According to a new Polaris Market Research report released on August 17, 2020, for "Nano-Enabled Packaging Market Share, Size, Trends, and Industry Analysis Report, By

Table 23.1 Commercial AgNP nanofungicides.

Company	AgNP formulation product	Application	Accreditation/ certification
Chemtex Specialty Ltd., Vedic Orgo LLP, Anand Agro Care, Future India Chemicals, Acuro Organics Limited, India	Alstasan Silvox: Ag stabilized H_2O_2 Nano Shield fungicide Siddhi Nano: $Ag + H_2O_2$ Acurosil Nano+	Agriculture: – Open field cultivation – Protective cultivation – Animal husbandry – Aquaculture – Floriculture – Soil disinfectant Food & Beverages[a]	Certified by FDA, approved by National Toxicology Center up to 25 ppm in portable water. Approved in several countries as regarded as safe according to the EEC, WHO, and US EPA
Sistema Plastics, New Zealand	Fresher Longer™: AgNPs (25 nm) in polypropylene (PP) matrix	Food packaging: – Adhesives – Antifungal ingredients Food contact materials: – Storage containers	Phthalate and BPA free, products are not recommended for microwave
Huangshan Yongrui Biotechnology Co., Ltd., China	5–10 nm AgNP solution (20 ppm)	Household appliance, clothes, tableware & cookware, hospital appliance	Certified ISO9001, Ce, FDA, MSDS, Coa, Product Safety Test R
Nano Koloid, Poland	Nanosilver	Agriculture: – Animal husbandry – Food processing, storage and transport – Plant care and protection – Antifungal activity against *Aspergillus* spp., *Candida* spp., and *Saccharomyces*	Not mentioned
Libra Biotech, India	Nanosil Antifungal: AgNPs and peroxy acid	Agriculture via Foliar Application through a Knap Sack Sprayer and soil drenching, product is recommended for seed, soil, and plant disinfection of fungal and other related contaminations	Dermal $LD_{50} > 2000$ mg/kg Oral $LD_{50} = 2896$ mg/kg Ag rated as nontoxic according to EPA/FDA. Drinking water safe level: 180 ppb/day USA, Canada, Russia, Japan 0.05 ppm European Union 0.08 ppm Switzerland 0.10 ppm

[a] *Packaging materials; LD50, mean lethal dose of AgNPs/AgNPs formulation that is lethal for 50% of tested animal model used within the dose group.*

Type (Active, Intelligent, Others); By End-User (Food and Beverages, Pharmaceutical, Personal Care, Others); By Regions: Segment Forecast, 2018–2026." The global nanoenabled packaging market for food and beverages is estimated to grow at a compound annual growth rate (CAGR) of 12.7% to reach about $89.0 billion by 2026 (Polaris Market Research, 2020). Polyolefins are among the most widely used polymeric material for food packaging material. A polypropylene (PP) film, which is a type of polyolefin, is widely used due to its chemical inertness, brilliance, low specific weight, and transparency. In addition to PP, various grades of polyethylene and polymer blends are used as support matrices for AgNPs to create nanocomposites as *active* packaging materials for controlling food spoilage by fungi and mycotoxins.

23.2.3 Mycotoxin detection using AgNPs

AgNPs may also can be used to detect mycotoxins, and as previously mentioned, there is some difficulty and limitations in detecting some mycotoxins once they can be masked and possess some nonextractable compounds. Utilization of AgNPs for increasing the surface-enhanced Raman scattering can improve the signal amplification (Thipe et al., 2018). A majority of mycotoxins are present in trace levels (parts per billion or nanograms) and/or coexist (e.g., more than one type of mycotoxin can exist in a single food matrix), which makes their detection challenging. Biosensors can be applied in several ways in the food industry, from storage to packaging, guaranteeing safety of consumer products with the advantage of simplicity through point-of-care testing (POCT) and low cost. For mycotoxins identification, different methods may be used such as optical (surface plasmon resonance and fluorescence), piezoelectric (quartz crystal microbalance), and electrochemical (impedimetric, potentiometric, and amperometric) spectroscopies. Normally, nanomaterials are applied in these biosensors for signal amplification, improving their sensitivity. Moreover, the strategy of uniting nanoparticles and fluorescence techniques is especially interesting for mycotoxin detection (Adeyeye, 2020; Chauhan et al., 2016; Ng et al., 2016; Santana Oliveira et al., 2019). Khan et al. (2018) developed a functionalized silver nanoclusters assembled on a molybdenum disulfide thin layer for T-2 toxin detection. Anfossi et al. (2018) used gold and Ag nanoparticles to quench Quantum Dots emission by Foster resonance energy transfer and inner filter effect. This caused the decrease of the background noise caused by the Quantum Dots fluorescence used on an analytical platform such as lateral flow immunoassay with fluorescence. Biosensor development that uses AgNPs for mycotoxin detection is still at its infancy but postulated to grow as the knowledge about their ecotoxicological profile increases.

23.2.4 Green synthetic AgNP against mycotoxigenic fungi

The green production of AgNPs may also present advantages to the environment because of the absence of toxic waste byproducts. This is possible because AgNPs may be synthesized by green methods using microorganisms or plant extracts through green nanotechnology (Siddiqi and Husen, 2016). Antifungal action of Ag has been previously demonstrated against several strains such as *F. solani*, *Alternaria alternata*, *A. flavus*, and *A. ochraceus* (Abd-Elsalam et al., 2017). Jogee et al. (2017) synthesized AgNPs using 10 different plants to inhibit fungi that contaminate peanut, which includes *Aspergillus* spp., *Penicillium* spp., and *Macrophomina phaseolina,* the synthesis of AgNPs generated polydisperse particles from spherical to irregular in the range of 31–40 nm. The authors found that AgNPs mediated using *Cymbopogon citratus* leaf showed greater antifungal potential and their MIC was 2 ppm. In this case, the fungal potential of AgNPs was associated with the type of plant used in the synthesis.

Xue et al. (2016) performed optimization studies of AgNPs biosynthesis using a strain of *Arthroderma fulvum* isolated from soil. The produced bio-AgNPs were tested against *Candida* spp., *Aspergillus* spp., and *Fusarium* spp., in all species; results revealed considerable antifungal activity. The use of *A. fulvum* provides a green-sustainable, robust, and large-scalability of AgNPs production with antifungal capabilities. A study by Ashraf et al. (2020) used *Melia azedarach* leaf extract to produce AgNPs (MLE-AgNPs) against *F. oxysporum*, which produces trichothecenes mycotoxins (T-2 toxin, HT-2 toxin, diacetoxyscirpenol, and 3′-OH T-2 (TC-1)). Their results revealed that the AgNPs repressed fungal mycelia growth by 98% through the cell wall and cellular membrane damage attributed by elevated levels of ROS production and enhanced tomato seedlings without affecting plants grown, as shown in Fig. 23.1.

Abdel-Hafez et al. (2016) investigated the use of an endophytic nonpathogenic strain of *Alternaria solani* F10 (KT72914) as a reducing agent for the synthesis of AgNPs due to the vast repertoire of constituents and in turn evaluated the antifungal activity of the produced AgNPs against toxigenic *A. solani*. Their results demonstrated that AgNPs at a lower concentration (10 ppm) exhibited a higher antifungal activity when compared to standard treatment with Ridomil Gold® plus (2002.28 ppm). This justifies the use of AgNPs as antifungal agents at low concentration can become a safe, effective, and economical strategy in agriculture for controlling fungi and mycotoxin contamination. Furthermore, this would limit the onset of resistance due to the multimodality of AgNP's mechanism of action via disrupting cell walls and destroying the membrane lipid bilayer, as shown in Fig. 23.2. Moreover, AgNPs bind to nucleic acid and proteins containing sulfur to cause direct damage and inhibition of DNA replication through the release of Ag^+ ions that generates ROS and oxidative stress (Guilger-Casagrande and de Lima, 2019).

Elamawi et al. (2018) investigated *Trichoderma longibrachiatum* synthesized AgNPs and their effects against phytopathological fungi (*F. verticillioides*, *F. moniliforme*, *Helminthosporium oryzae*, *P. brevicompactum*, and *Pyricularia grisea*). Their results revealed that the produced AgNPs as an antifungal agent significantly reduced the growth of all the tested fungi by 90%. Also, Khalil et al. (2019) synthesized AgNPs through *Fusarium chlamydosporum* NG30 and *Penicillium chrysogenum* NG85 fungi to inhibit *A. flavus* and *A. ochraceus* with subsequent inhibition of aflatoxin and ochratoxin biosynthesis, respectively. The synthesis generated spherical particles with size varying between 6 and 26 nm for *F. chlamydosporum*

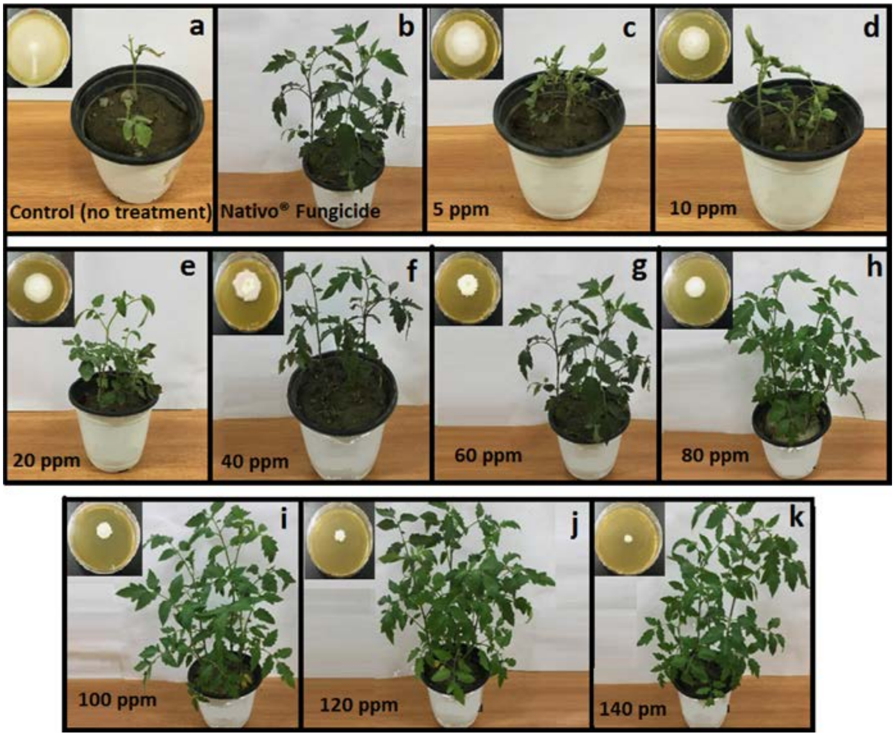

FIG. 23.1

Culture plates after 7 days of postincubation at 28°C and effect of treatment on the development of tomato plants after 45 days under greenhouse conditions infected with *F. oxysporum*: (A) control, (B) Nativo® fungicide treatment, and (C–K) antifungal activity of MLE-AgNPs at different concentrations (5, 10, 20, 60, 80, 100, 120, and 140 ppm).

Modified and reprinted by permission from Ashraf, H., Anjum, T., Riaz, S., Naseem, S., 2020. Microwave-assisted green synthesis and characterization of silver nanoparticles using Melia azedarach for the management of fusarium wilt in tomato. Front. Microbiol. 11, 1–22 distributed under the terms of the Creative Commons Attribution License (CC BY).

and 9 and 17.5 nm for *P. chrysogenum*. The MIC of *F. chlamydosporum* produced AgNPs was 45 ppm for *A. flavus* and MIC of *P. chrysogenum* produced AgNP was 48 ppm. On the other hand, for the total inhibition of aflatoxin production, the concentrations were 5.6 and 6.1 ppm for *F. chlamydosporum* AgNPs and *P. chrysogenum* AgNPs, respectively; even at these concentrations, no cytotoxicity against normal human melanocyte cells (HFB4) was observed. This study demonstrated the possibility to determine the concentration of AgNPs as fungal or mycotoxin inhibitors. Le et al. (2020) utilized phytoconstituents in leaf extracts of *Achyranthes aspera* and *Scoparia dulcis* to produce AA-AgNPs and SD-AgNPs, respectively. These AgNPs were tested for their fungicidal activity against *A. niger*, *A. flavus*, and *F. oxysporum*; they exhibited high antifungal activity against all tested fungi, with *F. oxysporum*

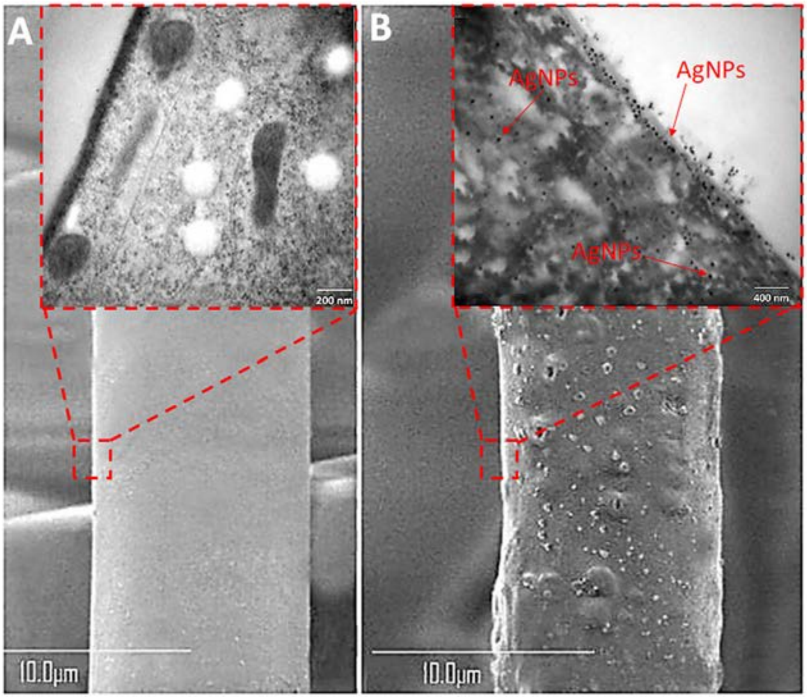

FIG. 23.2

Field emission scanning electron micrographs and corresponding high-resolution electron microscopy image insert of pathogenic *Alternaria solani* F11 (KT721909) hyphae: (A) Fungal hyphae before treatment with AgNPs which showing regular smooth surface and well-distinguished cell components and (B) fungal hyphae after treatment with AgNPs showing pores and cavities formed on the surface and AgNPs accumulation in the cytoplasm and membrane.

Modified and reprinted by permission from Abdel-Hafez, S.I.I., Nafady, N.A., Abdel-Rahim, I.R., Shaltout, A.M., Daròs, J.A., Mohamed, M.A., 2016. Assessment of protein silver nanoparticles toxicity against pathogenic Alternaria solani. 3 Biotech 6 (2), 199 distributed under the terms of the Creative Commons Attribution License (CC BY).

being the most susceptible. In a similar study, Ibrahim et al. (2020) demonstrated biosynthesized AgNPs using endophytic bacterium *Pseudomonas poae* strain CO isolated from *Allium sativum* against *F. graminearum*. The AgNPs inhibited mycelium growth, spore germination, and mycotoxin production.

Toxicity profile of AgNPs on human health was investigated by Panáček et al. (2009); this study compared the toxicity of ionic Ag and AgNPs used as a fungicide in the inhibition of *Candida* spp. Their results showed that the AgNPs were cytotoxic for human fibroblasts in concentrations higher than 30 ppm, while ionic Ag showed a lethal concentration (LC_{100}) = 1 ppm. This corroborates the results previously reported in the literature that at low concentrations, AgNPs do not present health risks. Yassin et al. (2017) evaluated the use of biosynthetic AgNPs using *P. citrinum* against aflatoxigenic *A. flavus* var. *columnaris*. Fungal growth decreased as a function of AgNPs concentration; the median effective dose (ED_{50}) and the effective dose required for 95% of the exposed population (ED_{95}) was 224.5 and 4001.8 ppm, respectively. The use of biogenic AgNPs production using fungi can be a feasible route for controlling fungal growth and mycotoxin production (Guilger-Casagrande and de Lima, 2019).

23.3 Toxicology profile of AgNPs

23.3.1 Ecotoxicology and phytotoxicology of AgNPs

Albeit, AgNPs applications are advantageous in controlling fungal growth and mycotoxin contaminations in the agricultural sector. Their potential toxic effects cannot be overlooked; thus, rigorous nanotoxicological studies are consistently required, especially AgNPs products deemed for agricultural application to mandate their overall safety to ecology (Ferdous and Nemmar, 2020). The growing utilization of AgNPs and Ag formulations in food packaging and antifungal agents already in the market has prompted concerns on the integrity, possible migration, and toxicity of AgNPs (Bumbudsanpharoke et al., 2018; Bumbudsanpharoke and Ko, 2015; Iavicoli et al., 2017; Tortella et al., 2020). Commercial NanoAg low-density polyethylene (LDPE) bags from Sunriver Industrial Co., Ltd., was evaluated in migration studies according to the Chinese standard GB/T 5009.60-2003 using ultrapure water, 4% acetic acid, 95% ethanol, and hexane as simulating solutions at different temperatures for 15 days. The fresh NanoAg bag contained 100 μg Ag per 1 g LDPE, and atomic absorption spectroscopy (AAS) analysis revealed that the amount of Ag migration increased as a function of time and temperature. This was attributed to the AgNPs on the surface layer leaching out and diffusion of simulants and change the overall crystalline state with simultaneous oxidation of AgNPs.

To extensively investigate the ecotoxicology and phytotoxicology of AgNPs concerning the vast majority of increased fabrications of these nanoparticles in the agriculture sector for controlling microbial manifestation such as fungal contamination with subsequent production of mycotoxins. It is of paramount to carry out such ecotoxicological studies in multispecies model organisms (e.g., live terrestrial or aquatic organisms: vertebrates, invertebrates, plants, algae, and microorganisms as biological markers) and also include phytotoxicity investigated, especially for in-field application of AgNPs exposure to crops (Tortella et al., 2020; Yin et al., 2018). Rodrigues et al. (2020) studied the effects of silver nanomaterials (AgNM) against standard ecotoxicological model organism—*Enchytraeus crypticus*, via water and soil exposure to four different AgNPs [polyvinylpyrrolidone (PVP) coated (PVP-AgNM), noncoated (NC-AgNM), JRC reference Ag NM300K, and silver nitrate ($AgNO_3$)]. They tested all life stages (cocoons, juveniles, and adults) evaluating five endpoints, including hatching success, survival, reproduction, avoidance, and gene expression of the animals upon the exposure to the four tested materials. Acute and chronic exposure from 1 to 21 days was investigated, and the main impact observed was that acute exposure via water to cocoons caused longer-term effects on survival and reproduction. On the other hand, chronic exposure to cocoons from 11 to 17 days, hatch delay, and impairment were observed. Also, juveniles were more sensitive than adults concerning survival. Generally, the toxicity profile rankings of the tested materials were as follows: $AgNO_3 \geq$ Ag NM300K $\gg$ NC-AgNM $\geq$ PVP-AgNM. Table 23.2 shows some of the AgNPs formulations investigated for their antifungal and ecotoxicological imprint against fungi and their mycotoxins/phytotoxins.

Nanoencapsulation of fungicides presents less cytotoxicity and genotoxicity to plants than the conventional pristine formulation, suggestive of the benefit of nanomaterials in providing crop protection with no crop damage and improved crop production (Nuruzzaman et al., 2016; Shang et al., 2019). The stability of the AgNPs is very important as it also contributes to the overall efficiency and toxicity of the AgNPs. Most importantly, a study by Jo et al. (2009) revealed that the treatment of *Oryza sativa* L. leaves with electrochemical Ag and AgNPs was effective against plant-pathogenic fungi, *B. sorokiniana* and *M. grisea*, and did not present any phytotoxicity to *O. sativa* L. leaves, which is ideal for practical agricultural use. Likewise, any use of AgNPs and/or formulation and products containing AgNPs must adhere to local, national, and international standards. In the United States, the US Environmental Protection Agency (EPA) in coalition with the US Conference of Governmental Industrial Hygienists established metallic Ag limit at 0.1 ppm, soluble compounds of Ag at 0.01 ppm. Besides, the US National Institute for Occupational Safety and Health (NIOSH) set limits of all forms of Ag at 0.01 ppm (EPA, 2012). In the European Union, AgNPs are regulated through the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) program.

The US Center for Disease Control (CDC) with the Department of Health and Human Services and NIOSH has suggested recommended subchronic exposure limits for AgNPs sized 15–20 nm. The bulletin contains a physiologically based pharmacokinetic (PBPK) model for estimating exposures to AgNPs for occupational monitoring that would not induce adverse lung or liver effects. Estimates ranged from 0.19 to 195 $\mu g/m^3$ exposed for 8 h time-weighted average (TWA) airborne concentration, assuming biologically relevant tissue dose (soluble or total silver), particle size, shape and charge, PBPK model parameters, and laboratory rat effect level estimate (NIOSH, 2015). Human beings who would have developed argyria were estimated to be exposed to 47–253 $\mu g/m^3$ of AgNPs for 45 years. The current NIOSH limit for metallic dust of Ag and soluble compounds containing AgNPs is 10 $\mu g/m^3$ as an 8 h TWA concentration (European Chemicals Agency, 2020; NIOSH, 2015). WHO has advised that 10 g of Ag lifetime should present No Observed Adverse Effect Levels (NOAELS), and on the other hand, the US EPA derived an oral reference dose (RfD), which represents an ingested daily dose of Ag for a lifetime NOAELS in human beings to be approximately 0.0056 mg/kg/day based on the minimal dose that can induce the onset of argyria (i.e., 1 g of metallic Ag) in 7.8% of individuals

Table 23.2 Antifungal effects and ecotoxicology profile of AgNPs formulations against fungi.

AgNP formulation product	Capping/ stabilizing ligand	Fungi	Mycotoxins/phytotoxins	Ecotoxicity profile	Ref
AgNPs (18.9 nm)	Biosynthesis by *Serratia* spp.	*Bipolaris sorokiniana*	Prehelminthosporol, dihydroprehelminthosporol, victoxinine, and prehelminthosporolactone	***Soil invertebrates:*** Concentration/dose: 0.05, 0.1 and 0.5 ppm Exposure time: 24–72 h Main effects: Reproduction potential and expression of the *sod-3* and *daf-12* genes decreased at 0.1 and 0.5 ppm	Mishra et al. (2014)
AgNPs (20–30 nm)	Not coated	*B. sorokiniana* and *Magnaporthe grisea*	Prehelminthosporol, dihydroprehelminthosporol, victoxinine, prehelminthosporolactone, and tenuazonic acid	***Soil invertebrates:*** Concentration/dose: 0.1, 0.5 and 1 ppm Exposure time: 24 h Main effects: AgNPs caused decreases in ROS formation, expression of PMK-1, p38 MAPK, and hypoxia-inducible factor, GST enzyme activity, and reproductive potential in wild type, unlike the pmk-1 mutant 48 h LC_{50}: Adult zebrafish: 7.07 (6.04–8.28) ppm Juvenile zebrafish: 7.20 (5.9–8.6) ppm 48 h LC_{50}: ***Aquatic invertebrates:*** *Daphnia pulex* adults: 0.040 (0.030–0.050) ppm *Ceriodaphnia dubia* neonates: 0.067 ppm *Pseudokirchneriella subcapitata*: 0.19 ppm	Yun et al. (2015); Courtois et al. (2019)

AgNPs (10 nm)	Trisodium citrate dehydrate, polyvinyl pyrrolidone, sodium tetrahydridoborate	*Alternaria alternata, P. digitatum,* and *Alternaria citri*	Tenuazonic acid, alternariol, alternariol monomethyl ether, altenuene, altertoxin I, citrinin, cyclopiazonic acid, ochratoxin A, patulin, penicillic acid, penitrem A, frequentin, palitantin, mycophenolic acid, viomellein, gliotoxin, citreoviridin, and rubratoxin B	***Soil invertebrates:*** Concentration/dose: 500 ppm Exposure time: 5 weeks Main effects: Accumulation of Ag in organisms (more with AgNPs than with $AgNO_3$) and a decrease in the unsaturated degree of fatty acids $LC50_{8\text{-}h}$ in *Daphnia manga* 5.04 ± 0.84 ppm	Courtois et al. (2019)
AgNPs (15.5 nm)	Biosynthesis by the fungus *A. fulvum*	*Candida* spp., *Aspergillus* spp., *Fusarium* spp.	Deoxynivalenol, 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, nivalenol, fusarenon X, T-2 toxin, HT-2 toxin, neosolaniol, diacetoxyscirpenol, zearalenone, fumonisin B_1, fumonisin B_2, and fusaric acid	***Soil invertebrates:*** Concentration/dose: 0.05, 0.1 and 0.5 ppm Exposure time: 24–72 h Main effects: Reproduction potential and expression of the *sod-3* and *daf-12* genes decreased at 0.1 and 0.5 ppm	Xue et al. (2016); Courtois et al. (2019)

daf-12, abnormal dauer formation protein 12; GST, glutathione S-transferase; LC_{50}, mean lethal dose of AgNPs/AgNPs formulation that is lethal for 50% of tested animal model used within the dose group; p38 MAPK, p38 mitogen-activated protein kinases; PMK-1, mitogen-activated protein kinase pmk-1; ROS, reactive oxygen species; sod-3, superoxide dismutase 3.

after intravenous medical therapy for 2–9.75 year. When this dosage is converted into oral administration of 10 g advised by WHO accounting for oral absorption (i.e., $1\,g \div 0.1 = 10\,g$) translates to a lifetime dose as follows:

$$\begin{aligned} 10\text{ g over a lifetime} &= 10{,}000\text{ mg} \div 70\text{ years} \div 365\text{ days / year} \div 70\text{ kg body weight} \\ &= 0.0056\text{ mg / kg / day} \end{aligned}$$

Although short- or long-term dietary investigations with AgNPs are still not available, a 28–90 day gavage dosing studies with AgNPs showed NOAELS for traditional experimental animal model toxicological endpoints range from 0.5 to ~500 mg Ag/kg/day (EFSA ANS Panel, 2016; European Chemicals Agency, 2020). Wang et al. (2020) investigated the anti-biofilm and fungicidal activities of positively charged AgNPs synthesized and stabilized with trimethylchitosan nitrate (TMCN) against *C. albicans* SC5314-virulent strain with strong biofilm activity, as well as other reference strains: *C. albicans* ATCC 76615, *C. tropicalis* ATCC 750, and *C. glabrata* ATCC 15545. Cytotoxicity was monitored using L929 fibroblast cell lines and embryotoxicity evaluated using *Danio rerio* (zebrafish). Results revealed that TMCN-AgNPs exhibited the best antifungal potential at 6.2 and 49.4 ppm for *C. tropicalis* ATCC 750 and both *C. albicans* ATCC 76615 and *C. glabrata* ATCC 15545, respectively. Furthermore, cytotoxicity data showed no significant toxicity toward L929 cells even at 31.7 ppm; embryotoxicity assay revealed that zebrafish eggs preinfected with *C. albicans* SC5314 and then treated with TMCN-AgNPs (24.8 ppm) greatly diminished the amount of biofilm formation on eggs and no damage was observed, thereby rescuing embryos survival by 70%. Therefore, dosage > 24.8 ppm induced some deaths affecting heart and organ development. This work established toxicity limits for TMCN-AgNP which is paramount for mandating ecotoxicology and phytotoxicology limits to regulate the use of AgNPs in agriculture for controlling fungal growth, mycotoxin contamination, and other related pathogens/diseases.

A study by Yin et al. (2011) monitored AgNP uptake and their effect on grass (*Lolium multiflorum*), and it was reported that AgNPs < 6 nm strongly affected *L. multiflorum* growth and induced morphological changes; this was not observed with similar concentrations of $AgNO_3$ and AgNPs > 25 nm, signifying the role that size contributes to toxicity. There is an alarming concern regarding the interaction and effects of AgNPs on plants and soil microorganism's homeostasis (Montes de Oca-Vásquez et al., 2020). AgNPs can influence the growth of plants and rhizospheric microbial communities with symbiotism, such as arbuscular mycorrhizal fungi, which play an important role in plant development. It is also imperative to evaluate the environmental impact of biosynthetic AgNPs; AgNPs produced using *A. tubingensis* were investigated against aerobic heterotrophs soil microorganisms, rice seeds (*O. sativa*), and zebrafish (*D. rerio*). AgNPs had a low effect on soil microbiota compared to $AgNO_3$, however; they influenced the germination of rice seeds and subsequent growth development had a dose-dependent inhibitory effect, and AgNPs at 7.1 ppm did not induce mortality of the *D. rerio*.

A study by Cao et al. (2017) investigated growth responses of maize (*Zea mays* L.) and rhizospheric soils with mycorrhizal fungal colonies treated with different

AgNPs concentrations (0.025, 0.25, and 2.5 ppm) and Ag. The results demonstrated that 2.5 ppm of AgNPs significantly reduced plant root biomass attributed by Ag accumulation in plant tissues, resulting in increased antioxidant enzyme activity that causes oxidative stress and decreased photosynthetic carbon for fungi, as well as affecting the diversity and composition of the soil colonies. Furthermore, this simultaneously changed ecological functions, thereby weakening the phosphorus (P) cycle in the soil as a result of decreased soil alkaline phosphatase activity, thus limiting P content availability in plants for development. The authors also emphasized the importance of evaluating the effects of AgNPs on agricultural ecosystems, especially signaling responses between fungi and symbiotic microorganism.

According to food safety regulations, AgNPs in polymeric matrices utilized for active packaging material to increase the shelf-life of food and provide protection against fungal growth with subsequent production of mycotoxins warrant rigorous in vivo cytotoxicity investigations in multispecies animal models to reflect true real-life simulations of their ecotoxicity (Bahrami et al., 2020; Liu et al., 2020). A recent study by Maziero et al. (2020) highlighted the importance of multispecies in vitro and in vivo ecotoxicological analysis of tri-alanine-phosphine peptide ("Katti Peptide") stabilized gum arabic protein AgNPs (AgNP-GP) in different experimental models as follows: (i) *Daphnia similis* for the acute ecotoxicity (EC_{50}) tests; (ii) *D. rerio* for the evaluation of acute embryotoxicity (LC_{50}) tests; and (iii) Sprague Dawley rats for toxicity behavioral investigations. Their results revealed that AgNP-GP exhibited EC_{50} in *D. similis* was 4.40 ppm, LC_{50} in *D. rerio* was 177 ppm, and oral administration of AgNP-GP in Sprague Dawley rats in both male and female animals for 28 days showed no adverse effects in doses of up to 10.0 mg/kg body weight. Such investigations are appropriately tailored to assess the overall real-life systemic toxicity of AgNPs through utility of relevant animal species, for testing the in vivo toxicity of AgNPs, which assumes a paramount role in gaining long-term toxicity information. Toxicity studies in animals with similar receptor/epitope distribution as human beings provide vital information on in vivo toxicity, ultimately translating the data obtained in assessing tissue cross-reactivity profile in human tissues. This will help in defining AgNPs toxicity limits for various formulations in a myriad of applications such as antifungal agents in agriculture.

23.4 Life-cycle analysis of AgNPs for controlling mycotoxins in the agricultural sector

Among the nanomaterials with commercial applications, AgNPs are one of the most used forms of metallic nanoparticles. Europe Union, in 2014, there was a total production of approximately 50 tons of Ag, which eventually led to the accumulation of AgNPs released into the environment and contaminates the soil. Full life cycle assessments of AgNPs have sparked considerable interest in many researchers considering their applications in several sectors, including agriculture and food. Pourzahedi et al. (2017) and Elamawi et al. (2018) emphasized the importance of establishing rigorous full life cycle assessments to extensively observe and quantify all environmental burdens from upstream to beyond ecotoxic effects, thereby considering the benefits provided by these products during and after utilization (Iavicoli et al., 2017). Also, design detailed ecotoxicological studies that are much longer and carried out in multispecies experimental models to determine the toxic effect of AgNPs to potential toxicity in human beings before mass production and use of agricultural applications are imperative.

Pourzahedi et al. (2017) evaluated all these aspects of several commercial products containing AgNPs, including a food container and a plastic bag for food storage, and estimate cradle-to-gate environmental impacts. They concluded that the main environmental impact caused by AgNPs was related to the electricity use and emissions from Ag mining. The concentration of AgNPs found in these products was between 0.005% and 3%, which means that the contribution of Ag was very much dependent on the composition of the product. Therefore, impacts related to nonnano emissions upstream, like the production of the plastic matrix, may offer much more burden to the environment than the AgNPs themselves. In addition, studying AgNPs release profiles are imperative to define the minimum concentration of AgNPs, thus balancing product performance and reducing the impacts on the environment.

Another important point to consider on full life cycle assessment of AgNP is their route of synthesis which can dictate their overall toxicity profile in addition to their capping/stabilizing agents used during synthesis (Iavicoli et al., 2017). Pourzahedi et al. (2017) compared seven different routes observing the magnitude and patterns of impacts considering different environmental categories and presented a cradle-to-gate life cycle inventory. The differences in antimicrobial activity of the nanoparticles according to their route of synthesis were considered during the assessment. The seven methods were as follows: chemical reductions using trisodium citrate, sodium borohydride ethylene glycol and soluble starch from potatoes, and physical techniques, flame sprays pyrolysis, arc plasma, and reactive magnetron sputtering. The authors observed that, albeit the chemical reduction using starch is considered a bio-based reduction method, it presented the highest impacts for ozone depletion, acidification, eutrophication, noncarcinogenic, and ecotoxicity. This showed the importance of analyzing the whole life cycle impacts before adopting a synthetic route. Moreover, it is also important to mention that the researchers emphasize that for all routes, the greatest contribution of the process is related to Ag extraction because it involves mining, refining, and transport. Hence, recovering Ag from spent solutions may be a way to minimize these effects (Pourzahedi and Eckelman, 2015). This further emphasizes the consistent need for continued innovation for raw material acquisition (upstream processing) and end-of-life (downstream) stages, which is ubiquitous across several industrial sectors, as shown in Fig. 23.3.

Furthermore, it is of paramount to study the effects of the accumulation and major interactions of AgNPs with soil, soil biota, sediment, water, biota in water bodies, plants, and animals/human beings to fully comprehend the ecotoxicology in multispecies associated with their use in agriculture to control fungal growth and mycotoxin contamination (Maziero et al., 2020; Mishra and Singh, 2015). These

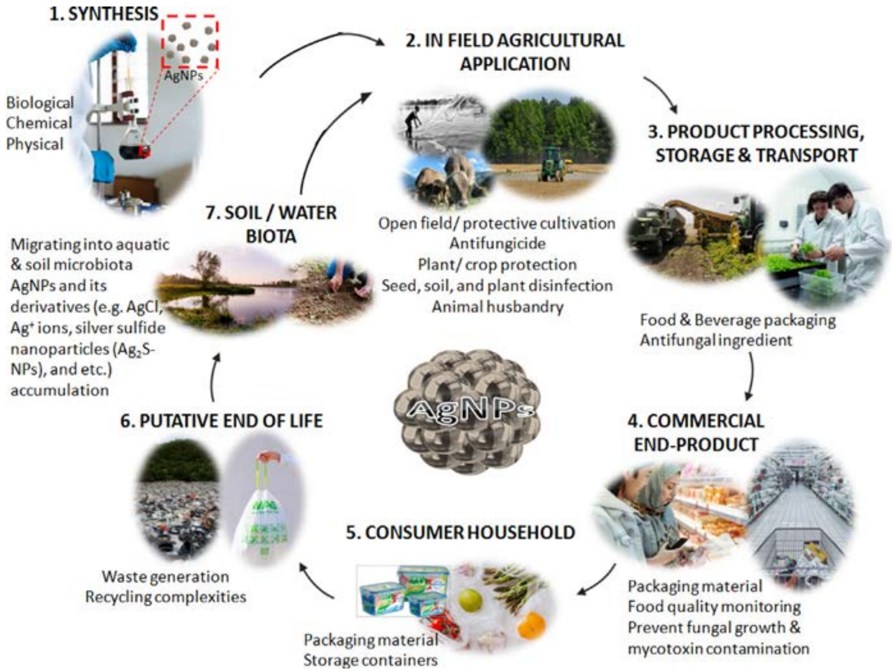

FIG. 23.3

Life-cycle assessments of AgNPs for quality assurance and quality control measures from various synthetic routes to possible migration to the soil/water microbiota. A cascade of pathways that include agricultural application in controlling fungal growth and mycotoxin contamination, product integrity maintenance through antifungal storage containment against fungal manifestation, end-product to consumer mycotoxins-free, active antifungal packaging material, end-of-life can facilitate a feedback loop of migration to the environment.

nanoparticles may enter agriculture through plant sludges that are applied to fields during crop cultivation. AgNPs tend to oxidize or dissolve releasing Ag^+ ions when they are in the soil after application. Eventually, Ag^+ may get subsidized and become less toxic to microorganisms. The half-life of this new substance varies from some years to over a century depending on its redox conditions (Dale et al., 2013; Riebeling and Kneuer, 2014). When exposed to organisms, the contact to body fluids containing proteins, lipids, and polysaccharides may form a corona around the nanoparticle, and this corona will influence the cellular uptake (Riebeling and Kneuer, 2014). As there is a growing number of AgNP incorporated packaging materials, it is imperative to consider the added complexity and potential exposure routes in minimizing AgNPs concentration per realized benefit from upstream embedded energy, downstream handling, and economic perspective to comply with regulatory entity standards (Bahrami et al., 2020). Moreover, the rate of recycling has increased over the years; therefore, it is important to consider packaging recycling when incorporating

AgNPs as this generates complexities in the recycling process, which changes and influences the physical, chemical, and biological reactivity of the adjacent environment, including plants, animals, and human beings through the discharge of AgNPs and/or Ag^+ ions into terrestrial or aquatic environments, which may increase the probability of ecotoxicity (European Union, 2018; Tortella et al., 2020).

The disposal and migrating of AgNPs into wastewater leads to AgNPs and its derivatives (e.g., AgCl, Ag^+ ions, silver sulfide nanoparticles (Ag_2S-NPs), etc.), resulting from their chemical biotransformation to the predominant Ag species—Ag_2S, which has a longer shelf-life over several years/decades, in sewage sludge. Part of the sewage sludge is rediverted to utilization in agricultural irrigation, the soil being a recipient of contamination by AgNPs and derivatives (Montes de Oca-Vásquez et al., 2020). In the first review published by Courtois et al. (2019), a primary study was carried out on the potential impact of Ag species introduced to the soil via sewage sludge, from ecologically important microorganisms, fungi, algae, terrestrial plants, and soil invertebrates, all-important in soil ecosystem stability and sustainability.

AgNPs and their chemical biotransformation products can enter cells through biological membranes causing physiological, biochemical, and molecular effects of organisms and microorganisms. In soils, exposure to AgNPs can modify biomass and microbial diversity, retard the growth of plants, and inhibit the reproduction of invertebrates. Generally, Ag_2S has been reported to have low bioavailability, therefore less toxic to plants as compared to Ag^+ ions. Some studies have demonstrated that the ecotoxicological profile hierarchy of Ag as follows: $AgNO_3 >$ free Ag^+ ions $>$ AgNPs $> Ag_2S$ (Yan and Chen, 2019) and smaller size particles exhibit high toxicity than larger particles. However, the evaluation of the toxicity of AgNPs and Ag-derived species is complex, and there is much to be studied about the ecotoxicology of Ag species in soils, such as the possibility of translocation along the trophic chain due to the bioaccumulation in plant and animal tissues for better risk assessment. A recent study by Montes de Oca-Vásquez et al. (2020) investigated the effects of environmental realistic AgNPs concentration on soil microbial diversity exposed for 7–60 days. Their results revealed that after 7 days postincubation (pi), microbial biomass increased, and after 60 days pi, a decrease in biomass (mainly Gram + and actinobacteria) was observed, including relative abundance of the fungal community. However, little effects were observed on the overall microbial diversity and soil biogeochemical properties and cycles mediated by extracellular enzyme activities.

The tracking analysis of AgNPs exposure and bioaccumulation in the environment is difficult and remains unknown as to the levels that could affect the overall food chain. This is exacerbated by the limited knowledge of a comprehensive understanding of the ecotoxicological behavior of AgNPs, possible interactions with agrosystem coformulants, regulatory and legislative frameworks for AgNP nanotoxicity that govern occupational safety practices and policies in food contact materials that can yield first-in-human (FIH) translational ecotoxicological interpretation (Iavicoli et al., 2017; Maziero et al., 2020). Aid in the development of regulatory consensus, and nanospecific risk assessment analysis on the application of AgNPs in agriculture with emphasis to the long-standing principles of "*prevention through design*" and "*safety by design,*" this can mitigate unintended consequences. It is important to acknowledge that various factors such as organic matter content, pH, presence of HA, ionic strength, AgNPs concentration, size, or the presence and nature of capping/stabilizing ligands in combination, which can influence the fate, transformation/translocation (such as aggregation, chlorination, dissolution, oxidation, and sulfidation) and migration of AgNPs, which ultimately dictates the realistic evaluation of their ecotoxicological profile (Tortella et al., 2020).

23.5 Prospective ecotoxicological framework

An alternative strategic framework for the development of Green nanoenabled agricultural products is of paramount for evaluating both ecotoxicity and phytotoxicity dynamics through sustainable ecological practices (SEP) model for smart agricultural use of AgNPs. This SEP model would enhance agricultural efficiency and sustainability by elucidating ways to maximize the safety of AgNPs utilization in both up- and downstream of food production, processing, and use in controlling fungal and mycotoxin contamination, thereby ensuring a safe sustainable food supply chain. It is critical to fully understand the mechanistic insight of the ecotoxicology, phytotoxicity, nanospecific risk assessment, management, and governance of AgNPs designs and applications in agriculture for controlling fungal growth and mycotoxin contamination. This contributes to the realistic continued innovation in product design for food/feed protection from fungal and mycotoxins contamination, thus allowing for rapid advancements that promote agricultural sustainability through the SEP model, as shown in Fig. 23.4.

Detail information on the following is mandated to be disclosed:

(i) AgNPs concentration range and crop type(s) used for exposure studies
(ii) Metrics for benefit quantification (e.g., mycotoxins monitoring) for application studies
(iii) Ecotoxicology and phytotoxicology results between studies must be compared

All these critical information is necessary to quantify trade-offs of nanoenabling agriculture early in the design process to preclude future unintended consequences. The SEP model, in addition to the abovementioned criteria, aims to provide a platform for a synergistic next-generation AgNPs design that protects plants from fungal manifestation and mycotoxins contamination with the added armament of enhancing plant growth through improving nitrogen-fixation capability and photosynthesis in roots and leaves to encourage conversion efficiency and the energy utilization for a sustainable agricultural standard, which will be the driving force in modern agriculture and agrifood nanobiotechnology. The SEP model would help not only create but monitor ongoing nano-bio industrial products toward a concerted understanding of the complex interplay between agroecosystems, nanoparticles, and their level of exposure to human/animal health.

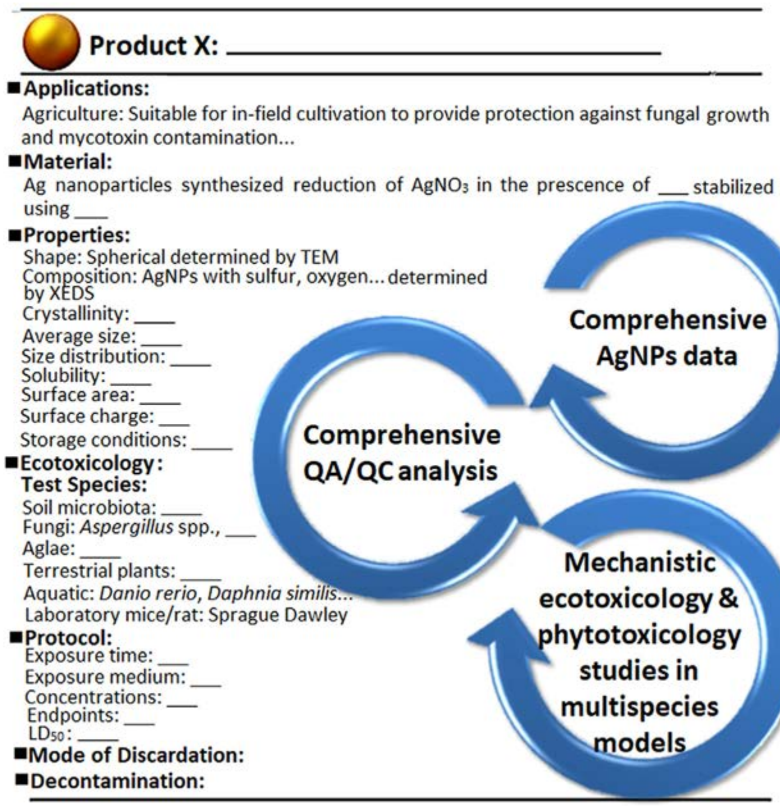

FIG. 23.4

Sustainable ecological practices (SEP) model framework for AgNPs products destined for application in agriculture to provide comprehensive data reporting, specifically as it relates to the active ingredient used. The mechanism (proposed and when possible, empirically supported) underlying ecotoxicology and phytotoxicology reports which govern the desired application and outcome, thus informing the continued sustainable design of promising solutions in accordance to set environmental limits.

23.6 Conclusion

The increased utilization of AgNPs and their derivatives in agriculture has prompted concerns on the scarcity of ecotoxicology and phytotoxicology multispecies studies on AgNPs already in the market because they demonstrate/provide optimal production yields and protection from fungi and mycotoxin contamination. These sidelines, the potential environmental and human/animal/plant health secondary collateral consequences, are often neglected from the initial AgNPs-enabled product design objectives. The green nanotechnological synthetic routes of AgNPs as new fungicides are explored with the aid to limit and generate no toxic byproducts to the environment.

However, further investigations into life cycle assessments of AgNPs with their ecotoxicology and phytotoxicology profiles in multispecies experimental models to yield realistic FIH translational ecotoxicological interpretation to AgNPs exposure must be mandatory to establish regulatory limit consensus on AgNPs. Finally, the suggested SEP model framework for AgNPs products/formulations would be the driving force in modern agriculture and agrifood nanobiotechnology governing safety and sustainability.

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