

## Green-Synthesized Metallic Nanoparticles for Targeted Antimicrobial Applications in Agriculture

Komal Ambreen<sup>1</sup>, Shazia Gul<sup>2</sup>, Moeen Ullah<sup>3</sup>, Mohammad Maaz Tajamul<sup>4</sup>, Osama Akbar<sup>5</sup>, Abdul Wasay<sup>6</sup>, Muhammad Brahamdag Shabir Kashani<sup>7</sup>

<sup>1</sup> Department of Plant Pathology, College of Agriculture University of Sargodha.  
ambreenkomal@yahoo.com

<sup>2</sup> Department of Microbiology, Hazara university Mansehra. gshazmicro@gmail.com

<sup>3</sup> Department of Horticulture, The University of Agriculture Peshawar. moinbrc000@gmail.com

<sup>4</sup> Department of Horticulture, The University of Agriculture Peshawar.  
maazkakakhel39@gmail.com

<sup>5</sup> Department of Plant Breeding and Genetics, University of Agriculture Faisalabad.  
osamashawani0@gmail.com

<sup>6</sup> Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.  
wasay2060@gmail.com

<sup>7</sup> Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.  
brahamdagkashani@gmail.com

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### Abstract

The escalating global food demand, projected to reach 10 billion people by mid-century, is currently threatened by the rise of antimicrobial resistance (AMR) and the environmental degradation caused by traditional synthetic agrochemicals. Green-synthesized metallic nanoparticles (MNPs) have emerged as a transformative solution, utilizing biological agents such as plant extracts, fungi, and bacteria to produce sustainable, cost-effective, and biocompatible alternatives for crop protection. Unlike conventional physical or chemical synthesis, green nanotechnology adheres to the principles of green chemistry by employing biogenic capping agents that enhance nanoparticle stability and facilitate targeted interaction with pathogens. These nanoparticles exert antimicrobial action through multi-targeted mechanisms, including cell membrane disruption, the generation of reactive oxygen species (ROS), and genomic interference, which significantly reduce the likelihood of developing resistance. Furthermore, advancements in stimuli-responsive delivery systems triggered by pH, temperature, enzymes, or light allow for precision application that maximizes efficacy while minimizing phytotoxicity and off-target effects. While green MNPs demonstrate significant benefits in seed nano-priming, foliar protection, and post-harvest preservation, their integration into large-scale agriculture faces hurdles regarding standardization and evolving global regulatory frameworks, such as the 2025 EFSA updates. This review underscores the potential of green MNPs as a cornerstone of sustainable precision agriculture, provided that their environmental fate and trophic transfer are rigorously monitored.

**Keywords:** Multi-Omics Integration, Precision Oncology, Systems Biology, Genomics, Epigenetics, Transcriptomics, Proteomics, Metabolomics, Tumor Microenvironment, Spatial

## 1. Introduction

The contemporary agricultural sector is currently navigated through a complex intersection of burgeoning global food demands and the catastrophic emergence of antimicrobial resistance (AMR) (Tasker et al., 2025). As the global population projections trend toward nearly 10 billion by mid-century, the pressure on agricultural systems to increase yield and reduce loss has never been more acute (Graham et al., 2025). Historically, the primary defense against phytopathogens including bacteria, fungi, and viruses has relied upon the intensive application of synthetic agrochemicals (Jangid et al., 2025). The indiscriminate use of traditional pesticides and antibiotics has triggered a cascade of unintended consequences, ranging from the degradation of soil microbial health and the contamination of aquatic ecosystems to the selection of multidrug-resistant (MDR) microbial strains that threaten both food security and human health (Fatima et al., 2023).

In response to these challenges, nanotechnology has emerged as a disruptive and transformative frontier. By manipulating matter at the atomic or molecular scale, specifically between 1 and 100 nanometers, researchers have developed materials with unique physicochemical, optical, and biological properties that differ significantly from their bulk counterparts (Cardoso et al., 2025). Among these, metallic nanoparticles (MNPs) including silver (Ag), gold (Au), copper (Cu), and zinc oxide (ZnO) have demonstrated exceptional antimicrobial efficacy (Ifedinezi et al., 2024). However, the conventional synthesis of these particles via physical or chemical means often involves high-energy consumption and the use of hazardous reagents, necessitating a shift toward "green nanotechnology" (Madani et al., 2022). Green-synthesized metallic nanoparticles, produced using biological agents such as plant extracts, fungi, and bacteria, offer a sustainable, cost-effective, and biocompatible alternative tailored for targeted antimicrobial applications in agriculture (Ijaz et al., 2020).

## 2. Theoretical Framework and Synthesis Paradigms

The development of MNPs typically follows two broad trajectories: top-down and bottom-up approaches. Top-down synthesis involves the physical disintegration of bulk materials into nanoscale units through techniques such as mechanical milling, grinding, and physical vapor deposition (Palagati et al., 2024). While effective for producing large quantities, these methods often suffer from energy inefficiency and lack of precise control over particle morphology. Bottom-up synthesis, conversely, relies on the chemical reduction of metallic ions to atomic clusters that eventually grow into nanoparticles (Tharaud et al., 2022). Green synthesis represents the most refined iteration of the bottom-up approach, substituting toxic reducing agents like sodium borohydride (NaBH<sub>4</sub>) or hydrazine (N<sub>2</sub>H<sub>4</sub>) with biological metabolites (Gao et al., 2020).

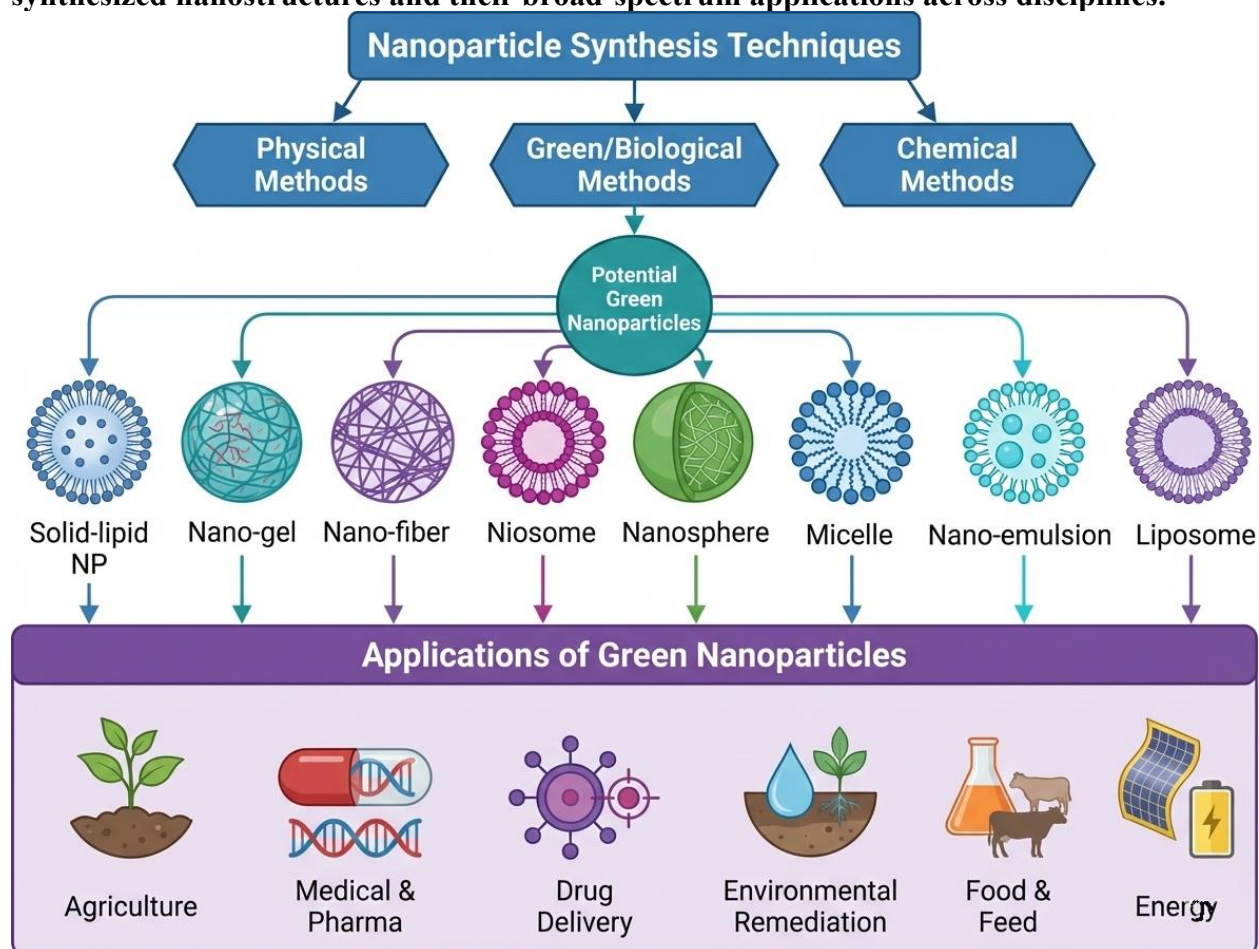
**Table 1: Comparison of Physical, Chemical, and Green Synthesis Paradigms**

Feature	Physical Synthesis	Chemical Synthesis	Green Synthesis
Mechanism	Mechanical disintegration	Chemical reduction	Bioreduction/Phytochemical mediation
Reagents	None (physical forces)	Hazardous reductants (e.g., hydrazine)	Biological extracts (plant/microbial)

<b>Energy Usage</b>	High (milling, laser ablation)	High (reflux, high pressure)	Low (ambient temperature, microwave)
<b>Biocompatibility</b>	Low	Low (residual chemical traces)	High (biogenic capping agents)
<b>Byproducts</b>	Minimal	Toxic chemical waste	Biodegradable residues

The transition toward biogenic synthesis is not merely an environmental preference but a strategic enhancement of nanoparticle functionality. Green synthesis adheres to the twelve principles of green chemistry, aiming to minimize hazardous waste and maximize atom economy (Kirubakaran et al., 2026). Furthermore, the biological entities used in synthesis serve a dual purpose: they act as reducing agents and as capping or stabilizing agents. This biogenic capping layer, often referred to as a "protein corona" or "phytochemical corona," prevents the aggregation of nanoparticles, enhances their colloidal stability, and facilitates better interaction with the cellular membranes of target pathogens (Samuel et al., 2022).

**Figure 1. Classification of nanoparticle synthesis paradigms, illustrating diverse green-synthesized nanostructures and their broad-spectrum applications across disciplines.**

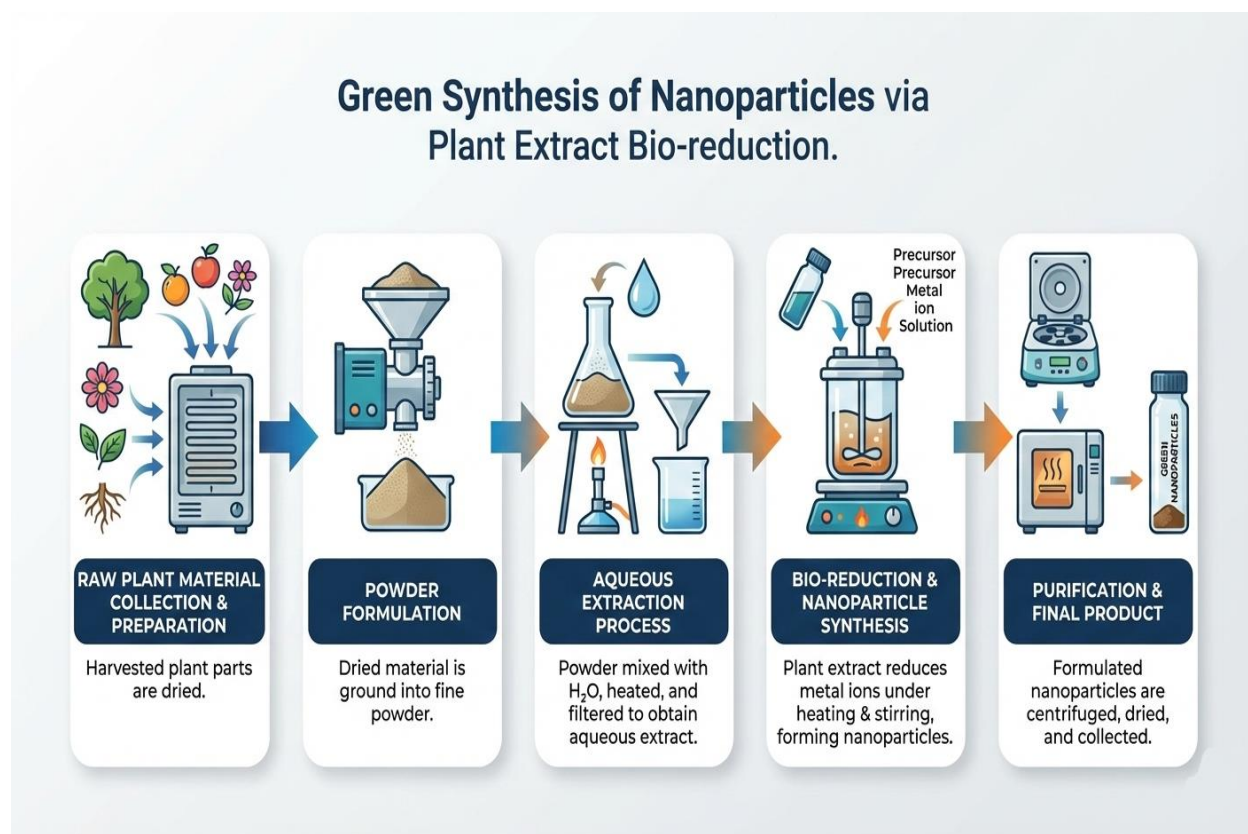


### 3. Phytochemical Repositories and Bioreduction Mechanisms

Plants are regarded as the most viable biological factories for large-scale MNP production due to their abundant phytochemical diversity and relative ease of handling compared to microbial cultures (Shah et al., 2025). The extracts derived from roots, stems, leaves, flowers, and fruits are

rich in primary and secondary metabolites, including polyphenols, flavonoids, terpenoids, alkaloids, and proteins, which facilitate the conversion of metal salts into zero-valent nanoparticles (Al-Qurashi et al., 2025).

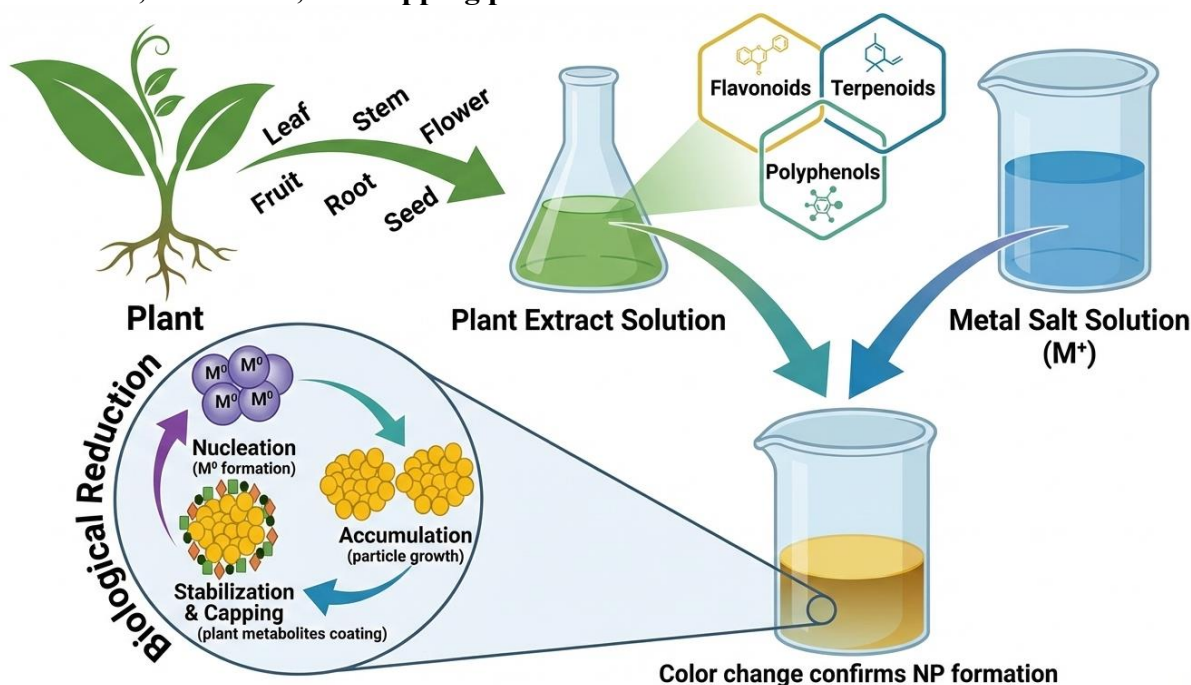
**Figure 1. Schematic illustration of the experimental workflow for the green synthesis of metallic nanoparticles via plant extract bio-reduction.**



### 3.1. Molecular Mechanisms of Bioreduction

The bioreduction process involves a series of complex oxidation-reduction reactions. Functional groups such as hydroxyl (-OH), carboxyl (-COOH), and amino (-NH<sub>2</sub>) groups donate electrons to metal ions, such as Ag<sup>+</sup> or Au<sup>3+</sup>, reducing them to their elemental form (Ag<sup>0</sup> or Au<sup>0</sup>) (Ayele et al., 2021). For example, as described flavonoids often undergo keto-enol tautomerism during the synthesis of silver nanoparticles. The keto form transforms into an unstable enol form, releasing reactive hydrogen atoms that drive the reduction of silver ions (Rai et al., 2020). Concomitantly, the presence of various phenolic acids ensures that the resulting particles remain stable in aqueous media, resisting oxidative degradation (Gebre, 2023).

**Figure 2. Mechanistic pathway of phyto-mediated nanoparticle synthesis, highlighting the critical roles of secondary metabolites (flavonoids, terpenoids, and polyphenols) in the bioreduction, nucleation, and capping phases.**



Terpenoids also contribute significantly by oxidizing aldehydic groups into carboxylic acids. This process is highly sensitive to the pH of the reaction medium; higher pH levels generally shift the equilibrium toward the right, enhancing the rate of metal reduction and influencing the final particle size and shape (Cardoso et al., 2025). Proteins and enzymes, such as nitrate reductase, are particularly important in microorganism-mediated synthesis but also play a critical role in plant-based synthesis by providing structural support and preventing nanoparticle agglomeration (Banerjee et al., 2022).

**Table 2: Plant Species and Phytochemicals used for Green Synthesis of Metallic Nanoparticles**

Plant Species	Part Used	Metal Nanoparticle	Primary Phytochemicals	Antimicrobial Activity
<i>Vitex negundo</i>	Leaf	Silver (Ag)	Flavonoids, Alkaloids, Tannins	Broad-spectrum antibacterial
<i>Carica papaya</i>	Leaf	Silver (Ag)	Saponins, Cardiac glycosides	Antiviral activity
<i>Moringa oleifera</i>	Leaf	Silver (Ag)	Quercetin, Kaempferol	Antibacterial response
<i>Punica granatum</i>	Fruit	Gold (Au)	Phenolic acids, Tannins	Antibacterial activity
<i>Azadirachta indica</i>	Leaf	Silver (Ag)	Bioactive compounds	Antifungal against <i>Penicillium</i>
<i>Cucumber leaves</i>	Leaf	Silver (Ag)	Leaf extracts	<i>E. coli</i> inhibition
<i>Rice husk</i>	Husk	Silver (Ag)	Agricultural waste	Long-term antibacterial

### 3.2. Microbial Biosynthesis and Bioprocess Optimization

While plant-mediated synthesis is often preferred for its simplicity, microbes including bacteria, fungi, and algae offer highly specialized intracellular and extracellular synthesis pathways. Bacteria such as *Escherichia coli* or *Bacillus subtilis* reduce metal ions primarily through enzymes like nitrate reductase or sulfite reductase (Bokolia et al., 2025). Extracellular synthesis is the industrially favored route as it simplifies purification by secreting the nanoparticles into the surrounding broth, thereby eliminating the need for complex cellular lysis (Ali et al., 2020).

Fungi, or "mycosynthesis," utilize their high biomass and metabolic diversity to produce larger quantities of MNPs. Enzymes like laccase and reductase, secreted by fungi such as *Trichoderma viride* or *Aspergillus niger*, act as potent reductants and stabilizers. Algae also contribute to this green ecosystem by using pigments like chlorophylls and carotenoids as bio-reductants (Rana et al., 2023).

### 4. Mechanistic Foundations of Targeted Antimicrobial Action

The efficacy of green-synthesized MNPs as antimicrobial agents stems from their ability to simultaneously attack multiple cellular targets, a characteristic that minimizes the likelihood of pathogens developing resistance (Mayegowda et al., 2023).

#### 4.1. Disruption of Cell Membranes and Structural Integrity

The primary mode of action for metallic nanoparticles, particularly silver and copper, involves physical interaction with the microbial cell wall. The high surface-area-to-volume ratio of MNPs facilitates an intensive electrostatic attraction between the positively charged nanoparticles (or their released ions) and the negatively charged bacterial membranes (Fatehbasharзад et al., 2021). This interaction leads to the formation of "pits" or pores, causing morphological changes such as cytoplasm shrinkage and membrane detachment. For Gram-negative bacteria, this process is particularly lethal as it disrupts the outer lipopolysaccharide layer, leading to the leakage of intracellular materials like ATP, proteins, and reducing sugars (Ahmad et al., 2022).

#### 4.2. Generation of Reactive Oxygen Species (ROS)

A central pillar of MNP toxicity is the induction of oxidative stress. Metallic nanoparticles act as catalysts for the generation of reactive oxygen species (ROS), including hydroxyl radicals (OH), superoxide ions (O<sub>2</sub><sup>-</sup>), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Min et al., 2023). These ROS molecules induce lipid peroxidation in the cell membrane, damaging fatty acids and compromising permeability. Furthermore, ROS-induced oxidative damage extends to essential intracellular enzymes and the genetic material of the pathogen, effectively shutting down its metabolic core (Canaparo et al., 2020).

#### 4.3. Genomic and Proteomic Interference

Silver and copper ions released from nanoparticles have a profound affinity for sulfur and phosphorus atoms, which are key components of proteins and DNA. Upon entry into the microbial cell, these ions bind to the thiol (-SH) groups of essential enzymes, rendering them inactive and halting cellular respiration (Ramos-Zúñiga et al., 2023). In the nucleus, ions interact with DNA bases, disrupting replication and transcription processes. This multi-targeted approach ensures that even if a pathogen develops a defense against one mechanism (such as an efflux pump), it remains vulnerable to membrane rupture or genomic failure (Jangid et al., 2025).

### 5. Stimuli-Responsive and Targeted Delivery Systems

The "targeted" aspect of these nanoparticles in agriculture is achieved through advanced surface

functionalization and the design of stimuli-responsive systems. As described by (Camara et al., 2019), these smart nano-agrochemicals are engineered to release their active payload only in response to specific environmental or biological triggers, thereby maximizing bioefficacy and reducing off-target toxicity (Lahlali et al., 2025).

### 5.1. pH and Temperature-Triggered Release

Agricultural environments frequently exhibit pH fluctuations due to plant metabolic processes, soil composition, or the presence of pathogens. pH-responsive systems often utilize biopolymers like alginate or chitosan to encapsulate MNPs (Lee et al., 202). In acidic environments which are characteristic of many fungal infections the chitosan matrix becomes protonated, leading to swelling and the controlled release of the antimicrobial nanoparticles (Azeem, 2025). Temperature-responsive systems leverage thermodynamic changes to modulate release kinetics; for example, increased ambient temperature can expand the pores of a carrier matrix, facilitating the diffusion of active ingredients during peak pest activity periods (Pavithran et al., 2019).

### 5.2. Enzyme and Photo-Responsive Systems

Internal stimuli from pests, such as enzymes found in the digestive tracts of insects or larvae (e.g., cellulase or alpha-amylase), can be used to trigger the degradation of a nanoparticle carrier (Chamani et al., 2025). A notable example involves the use of silica-based systems where the active ingredient is conjugated via urea bonds. In the presence of the enzyme urease, these systems show a targeted cumulative release of up to 81.94% after 30 hours, compared to only 10% in the absence of the enzyme (Dong et al., 2024).

Photo-responsive systems represent another innovative avenue, utilizing light-sensitive molecules like azobenzene. These carriers undergo structural transformations (trans-cis isomerization) when exposed to specific wavelengths of light, such as UV or sunlight, effectively "opening" the nanogate to release pesticides or antimicrobial agents (Floean, 2020). Such precision ensures that the concentration of MNPs remains below the threshold of phytotoxicity for the host plant while exceeding the lethal dose for the target pathogen (Di Martino et al., 2023).

**Table 3: Stimuli-Responsive Mechanisms for Targeted Agrochemical Delivery**

Stimulus	Mechanism of Action	Carrier/System Example	Agricultural Result
<b>pH</b>	Ionization/Swelling of polymers	Chitosan/Alginate	Targeted release in acidic fungal infection sites
<b>Enzyme</b>	Cleavage of carrier walls	Silica/Carboxymethylcellulose	Release in the presence of insect gut enzymes
<b>Light</b>	Trans-cis isomerization	Azobenzene/Biochar	93.7% weed control efficacy under UV-Vis light
<b>Temperature</b>	Thermodynamic movement	Chitosan-based coatings	Increased diffusion at mosquito developmental stages

## 6. Strategic Applications in Agriculture

The deployment of green MNPs in agriculture spans the entire production cycle, from seed treatment and nutrient delivery to post-harvest preservation (Shahid et al., 2024).

### 6.1. Seed Nano-Priming and Coating

Nano-priming involves soaking seeds in nanoparticle suspensions for a specific duration to enhance germination rates and stress tolerance. Green-synthesized AgNPs, fabricated using *Azadirachta indica* (neem) leaf extract, have demonstrated superior efficacy over chemically synthesized particles (Singh et al., 2024). For instance, green AgNPs increased potato seed germination by 19% over chemical AgNPs and 50% over hydroprimed controls. This improvement is attributed to the smaller crystallite size and higher colloidal stability of the green particles, which allow them to penetrate the seed coat more effectively and activate essential water channel proteins (Durgadevi et al., 2025). Seed coating with MNPs provides a localized protective barrier against soil-borne pathogens like *Pythium* and *Phytophthora*. Nanoparticles like silver and copper oxide possess inherent antimicrobial properties that reduce the risk of seedling diseases while simultaneously delivering essential micronutrients like zinc and iron during early growth (Afzal et al., 2020).

### 6.2. Post-Harvest Preservation and Green Mold Management

The application of green nanotechnology in post-harvest management is a critical strategy for reducing the 30% to 40% of global fruit and vegetable waste (Damodar et al., 2026). Green mold disease, caused by the fungus *Penicillium digitatum*, is a major threat to the citrus industry. Green-synthesized AgNPs, often integrated into edible chitosan-based coatings, effectively inhibit mycelial growth and spore germination (Zhang et al., 2025).

**Table 4: Agricultural Applications of Metallic Nanoparticles in Enhanced Crop Production**

Application	Target Crop	Nanoparticle Type	Concentration/Mode	Key Result
Seed Priming	Rice	Silver (Ag)	20 mg/L	Increased germination and catalase activity
Foliar Spray	Tomato	Alumina (Al <sub>2</sub> O <sub>3</sub> )	400 mg/L	Control of <i>Fusarium</i> root rot
Soil Mix	Lettuce	Copper (CuO)	200 mg/kg	Improved photosynthesis and biomass
Fruit Spray	Citrus	Silver (Ag)	Post-harvest application	Mitigation of green mold disease
Coating	Strawberry	Iron Oxide (Fe <sub>3</sub> O <sub>4</sub> )	3% IO + 1.5% Chitosan	Extended shelf life, weight loss reduced to 6%

In the case of strawberries, the application of iron oxide nanoparticles in a chitosan matrix significantly reduced weight loss from 21.6% (in control samples) to just 6%. These nano-coatings maintain the fruit's firmness and titratable acidity, preserving nutritional quality during transport and storage (Velloso et al., 2025).

## 7. Environmental Fate, Soil Health, and Food Chain Transfer

While green MNPs are inherently more biocompatible than chemical ones, their environmental fate must be rigorously evaluated. Soil acts as the primary sink for agricultural nanoparticles, and their presence can alter the soil microbial community composition and structure (Barman, 2024).

### 7.1. Impacts on Soil Microbiota

At low concentrations (typically up to 500 mg/L), metallic nanoparticles like ZnO can actually

stimulate the growth and biochemical activity of plant growth-promoting rhizobacteria (PGPR). Excessive accumulation can lead to the alteration of agriculturally significant phyla, such as Proteobacteria and Actinobacteria (Vasant et al., 2024). Studies have shown that silver nanoparticles can decrease the total soil microbial count while potentially increasing microbial biomass carbon, suggesting a selective pressure on the microbial population (Strekalovskaya et al., 2024).

## 7.2. Trophic Transfer and Bioaccumulation

The movement of nanoparticles through the terrestrial food chain is a burgeoning area of ecotoxicological research. Nanoparticles like La<sub>2</sub>O<sub>3</sub> and CuO have been shown to transfer from tomato leaves to *Helicoverpa armigera* caterpillars, with particulate trophic transfer factors (TTF) of 1.47 and 0.99, respectively (Isibor et al., 2024). Such transfer can destroy larval intestinal immunity by increasing pathogenic bacteria and decreasing probiotic populations. Similarly, snails consuming AgNP-exposed lettuce leaves exhibit biomagnification, with titanium dioxide nanoparticles potentially inhibiting the trophic transfer of silver while enhancing its retention in the organism's tissues (Jampilek et al., 2025). These findings underscore the necessity of "nanoeotoxicology" in making comprehensive regulations for nanosafety (Borgatta et al., 2023).

## 8. Comparative Safety and Phytotoxicity Analysis

A critical advantage of green synthesis is the mitigation of phytotoxicity. Conventional chemical nanoparticles often trigger an overproduction of ROS in plants, leading to oxidative damage, reduced chlorophyll content, and stunted growth (Shah et al., 2025). In contrast, green-synthesized silver and gold nanoparticles frequently exhibit positive or "attenuated" effects on plant physiology (Tharaud et al., 2022).

For example, green-synthesized AgNPs from cucumber extracts have been shown to increase protein contents and stimulate the upregulation of essential minerals like manganese (Mn) while downregulating toxic ones like aluminum (Al) (Ifedinezi et al., 2024). This phenomenon is attributed to the protective biogenic capping layer, which prevents the rapid, uncontrolled release of metal ions that characterizes chemical particles (Graham et al., 2025).

**Table 5: Comparison of Phytotoxicity Between Chemical and Green-Synthesized Silver Nanoparticles**

Parameter	Chemical AgNPs	Green AgNPs
ROS Production	High (triggers defense systems)	Low (negligible free radicals)
Plant Growth	Toxic at moderate doses	Stimulates growth/germination
Photosynthesis	Decline in chlorophyll	Promotes chlorophyll synthesis
Membrane Damage	High lipid peroxidation (MDA)	Minimal lipid peroxidation
Antimicrobial Effect	Rapid, but potentially toxic	Long-term, sustainable efficacy

## 9. Commercialization Hurdles and Global Regulatory Perspectives

The transition from academic research to industrial application for green MNPs is constrained by challenges in standardization and reproducibility (Tasker et al., 2025). The variability in plant biomass composition, seasonal differences in phytochemical levels, and poorly defined reaction conditions can lead to inconsistencies in particle size and morphology (Samuel et al., 2022).

### 9.1. Regulatory Frameworks (2025 Update)

As of 2025, regulatory agencies have introduced stringent guidelines for the use of nanomaterials in the food and feed chain. The European Food Safety Authority (EFSA) has updated its novel

food guidance (effective February 2025), requiring exhaustive characterization and tiered toxicological data for any engineered particle below 100 nm (Ijaz et al., 2020).

**Toxicology Requirements:** Tiered testing starting with *in vitro* screening and escalating to *in vivo* studies. Mandatory tests for genotoxicity, 90-day repeated doses, and specific assays for neurotoxicity and immunotoxicity are required if initial results show concerns (Rai et al., 2020). In the United States, the EPA regulates MNPs under the Toxic Substances Control Act (TSCA) and FIFRA, requiring companies to provide detailed information on the health and environmental effects of any nano-based agri-product (Stretz et al., 2025). These heightened regulatory barriers reflect a global effort to balance the benefits of nanotechnology with the imperative of environmental and consumer safety (Al-Qurashi et al., 2025).

## 10. Conclusion

Green-synthesized metallic nanoparticles represent a critical shift toward a more sustainable and resilient agricultural paradigm. By leveraging the natural bioreduction potential of phytochemicals and microbial metabolites, researchers can produce high-performance antimicrobial agents that bypass the environmental and energy costs of traditional synthesis. The multi-targeted antimicrobial mechanisms of these biogenic particles ranging from membrane perforation to the disruption of cellular respiration provide a robust defense against multidrug-resistant phytopathogens that threaten global food security. Strategic applications, such as nano-priming seeds to increase germination by up to 19% or utilizing iron oxide nano-coatings to drastically reduce post-harvest weight loss in fruits, demonstrate the tangible economic and nutritional benefits of this technology. However, the path to commercialization is governed by the necessity for stringent safety evaluations. While green MNPs exhibit significantly lower phytotoxicity compared to chemical counterparts, their long-term impact on soil microbiota and potential for bioaccumulation through the food chain remain vital areas of ongoing research. As global regulatory agencies establish more rigorous standards for nanomaterial characterization and toxicological screening, the focus of future developments must remain on balancing innovative efficacy with ecological and consumer safety.

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