

Green Nanoparticle-Based Biopesticides for Sustainable Management of Citrus Canker Disease

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Abstract

Citrus canker, caused by the Gram-negative bacterium *Xanthomonas citri* subsp. *citri* (Xcc), remains a major threat to global citrus production, inducing severe yield losses (up to 50%) and trade restrictions due to its aggressive virulence, environmental resilience, and rapid resistance development to copper-based fungicides. Traditional control strategies copper sprays, windbreaks, and eradication face limitations in sustainability, phytotoxicity, and regulatory scrutiny. This review explores green nanoparticle-based biopesticides as eco-friendly alternatives, synthesized via biogenic routes using plant extracts (*Azadirachta indica*, *Aloe vera*), microorganisms (bacteria, fungi, algae), and biopolymers to produce metal/metal oxide NPs (Ag, CuO, ZnO, Fe₃O₄) with enhanced antimicrobial efficacy. Mechanisms include ROS generation, membrane disruption, efflux pump inhibition, and biofilm prevention, achieving 70–100% in vitro inhibition and 50–80% field symptom reduction at low doses (10–100 ppm). Field trials demonstrate superior performance over conventional treatments, with reduced environmental persistence and minimal phytotoxicity. Challenges such as scalability, regulatory approval, and long-term ecosystem impacts are addressed, emphasizing the potential of green NPs for integrated pest management (IPM) in sustainable citrus cultivation amid climate change.

Keywords: Citrus Canker, *Xanthomonas Citri*, Green Synthesis, Nanoparticles, Biopesticides, Antimicrobial Mechanisms, Phytotoxicity, Sustainable Agriculture, Integrated Pest Management, Metal Oxide NPs

1. Epidemiological Dynamics and the Evolutionary Resilience of *Xanthomonas citri*

The global citrus industry stands at a precarious juncture, threatened by the pervasive spread of Asiatic citrus canker (ACC), a devastating bacterial disease that undermines both the productivity and marketability of citrus fruits. The causal agent, *Xanthomonas citri* subsp. *citri* (hereafter Xcc), is a Gram-negative bacterium characterized by its high virulence and ability to colonize a wide

range of rutaceous hosts (Ahmed et al., 2020; Alsamhary, 2024). Historically, the pathogen was identified in Asia in the early nineteenth century before disseminating across major citrus-producing regions worldwide, including the Americas, Africa, and the Arabian Peninsula (Ali et al., 2026). The economic ramifications of Xcc are multifaceted, involving direct yield losses through fruit drop and shoot dieback, as well as indirect losses resulting from international quarantine regulations that restrict the trade of fresh fruit from infected zones (Behlau et al., 2023). Xcc is traditionally described as a genetically monomorphic pathogen, indicating a low level of genetic variability when analyzed through standard housekeeping genes. This monomorphic nature suggests a limited adaptive potential; however, the pathogen's continued success in diverse environmental conditions and its ability to develop resistance to chemical control agents reveal a more complex evolutionary reality (Guo et al., 2025). Recent advancements in high-throughput sequencing and whole-genome comparative analysis have uncovered polymorphisms and adaptive mechanisms, such as transcription activator-like effectors (TALEs), plasmid-mediated horizontal gene transfer, and transposons, which facilitate its pathogenicity and environmental resilience (Huang et al., 2021).

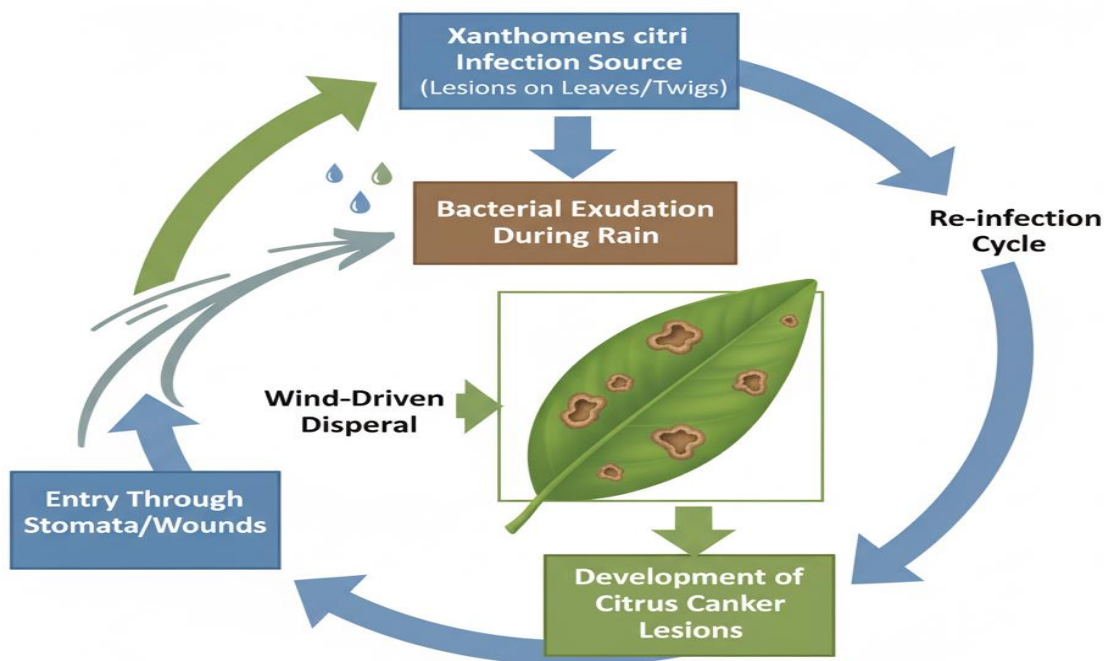
The classification of Xcc into pathotypes A, A-star, and A-w further illustrates the pathogen's specialization. Pathotype A represents the most significant threat due to its broad host range across nearly all Citrus species (Boondech et al., 2025). In contrast, pathotypes A-star and A-w exhibit a narrow host range, primarily localized to Mexican lime (*Citrus x aurantiifolia*), often mediated by specific type III effectors like *avrGfl* which limit their ability to infect other cultivars (Arce-Leal et al., 2020).

Table 1. Characteristics of *Xanthomonas citri* Pathotypes

Pathotype	Host Range	Primary Genetic Determinant	Geographic Prevalence
A (Generalist)	Broad (Most Citrus spp.)	TALE-rich genome	Global
A-star (Specialist)	Narrow (Mexican Lime)	Specific T3SS effectors	SE Asia, Arabian Peninsula
A-w (Specialist)	Narrow (Mexican Lime)	<i>avrGfl</i> effector	Florida, SE Asia

The persistence of Xcc in the field is facilitated by its ability to survive in lesions on infected twigs and leaves, providing a continuous source of inoculum for subsequent growing seasons (Behlau et al., 2011). Under conducive environmental conditions, such as high humidity and wind-blown rain, the bacteria are exuded from lesions and dispersed to healthy tissues, entering through natural openings like stomata or through mechanical wounds (Din et al., 2026). This cycle of infection necessitates frequent intervention, which has historically relied on the heavy application of copper-based bactericides and antibiotics (Graham et al., 2026). The infection and dissemination of *Xanthomonas citri* follow a cyclic epidemiological pattern influenced by environmental conditions. The disease cycle and transmission pathways are illustrated in Figure 1.

Figure 1: Global Epidemiology of Infection Cycle of Citrus Canker



2. The Crisis of Conventional Bactericides and Resistance Mechanisms

The management of citrus canker has reached a critical threshold due to the widespread emergence of resistance to traditional chemical treatments. For over two decades, copper sprays have been the primary defense against endemic citrus canker (Babu et al., 2025). Copper-based compounds, such as copper oxychloride and copper hydroxide, act as protective surface films that inhibit bacterial colonization (Ali et al., 2023). However, the efficacy of these sprays is often compromised by wind-driven rain, which bypasses the surface film by forcing bacteria directly into the leaf intercellular spaces through the stomata (Shahbaz et al., 2023).

Prolonged and intensive use of copper has led to the development of copper-resistant (Cu-r) strains of *Xcc*. This resistance is frequently mediated by plasmid-borne gene clusters, most notably the *cop* operon, which includes *copL*, *copA*, and *copB* (Delgado et al., 2026). The molecular mechanism of resistance involves the sequestration of copper ions in the periplasm by copper-binding proteins, thereby preventing toxic concentrations of free Cu^{2+} from reaching the cytoplasm (Giachino & Waldron, 2020). Genetic clustering within bacterial populations in groves suggests that these Cu-r strains can coexist with susceptible populations and may exhibit increased transmission rates, complicating the long-term sustainability of copper-based management (Kang et al., 2020).

Simultaneously, the use of antibiotics like streptomycin, which has been a staple in bacterial disease control since 1955, is failing. In certain regions, such as Jeju Island, Republic of Korea, up to 72% of *Xcc* isolates have demonstrated resistance to streptomycin (Sebbane & Lemaître, 2021). This high prevalence of resistance is attributed to excessive and prolonged application, which imposes a strong selective pressure on the bacterial population (Lewis et al., 2024).

Beyond the issue of resistance, the environmental and physiological impacts of conventional chemicals are significant. Excessive copper accumulation in the soil can lead to toxicity in citrus roots, impairing nutrient uptake and overall tree vigor (Santra, 2013). Furthermore, high rates of copper application can cause phytotoxicity, commonly known as "copper burn," on the fruit rinds, which reduces the market value of the crop even if disease control is achieved (Mir et al., 2021).

The urgent need for alternatives that are effective against resistant strains and environmentally benign has propelled nanotechnology to the forefront of agricultural research (Kumari et al., 2023).

3. Principles of Green Nanotechnology in Agriculture

Nanotechnology involves the design and application of materials with dimensions between 1 and 100 nanometers. At this scale, materials exhibit unique physicochemical properties such as increased surface-area-to-volume ratios, enhanced reactivity, and specific optical characteristics that differ significantly from their bulk counterparts (Pramanik et al., 2020). In agriculture, these properties can be harnessed to develop high-potency biopesticides, smart delivery systems, and nano-sensors for disease detection (Murthy et al., 2023).

The synthesis of nanoparticles is traditionally categorized into physical and chemical methods. Physical methods require significant energy inputs, while chemical methods often involve toxic reducing agents and hazardous solvents (Sonaba, 2019). These traditional approaches present sustainability challenges, particularly regarding the generation of toxic waste and the high cost of production (Srivastava & Bhargava, 2022).

"Green" or biogenic synthesis has emerged as a transformative paradigm, utilizing biological entities plants, bacteria, fungi, and algae to synthesize nanoparticles under ambient conditions without the use of hazardous chemicals (Shah et al., 2025). In green synthesis, biological molecules serve as both reducing agents and capping agents, stabilizing the nanoparticles to prevent aggregation (Devi et al., 2024).

Table 2. Comparison of Nanoparticle Synthesis Methods

Synthesis Method	Core Mechanism	Environmental Profile	Scalability
Physical	Energy-driven (Laser/Heat)	Energy-intensive	Moderate
Chemical	Reductant-driven (Toxic chemicals)	Hazardous waste risk	High
Green (Biogenic)	Phytochemical-driven (Natural extracts)	Sustainable/Eco-friendly	High

Green synthesis offers several advantages for the citrus industry. By utilizing agricultural byproducts, such as citrus fruit peels, the process aligns with the principles of a circular bioeconomy, converting waste into high-value biopesticides (Ahmed et al., 2016). Furthermore, biogenic nanoparticles often exhibit enhanced biocompatibility and stability, as the natural capping layer provides a protective shield that can improve adherence to plant surfaces (Mittal et al., 2013).

4. Plant-Mediated Synthesis and the Role of Citrus Waste Valorization

The use of plant extracts for nanoparticle synthesis is perhaps the most promising green technology for citrus canker management. Plant extracts are rich in secondary metabolites including flavonoids, polyphenols, terpenoids, and proteins that possess high antioxidant and antimicrobial activities. These compounds facilitate the reduction of metal salts into nanoparticles through a series of redox reactions (Iravani, 2011). For example, the synthesis of silver nanoparticles (AgNPs) using leaf extracts involves the reduction of silver nitrate (AgNO_3):

$\text{Ag}^+ + \text{Phytochemicals} \rightarrow \text{Ag}^0 (\text{Nanoparticle}) + \text{Oxidized Products}$ (Song & Kim, 2009).

The global citrus industry generates millions of tons of citrus processing waste (CPW) annually, consisting primarily of peels, pulp, and seeds. This byproduct is a valuable feedstock for green nanotechnology, as it contains high concentrations of bioactive flavonoids like hesperidin and naringin. Extracts from lemon, orange, and grapefruit peels have been successfully used to synthesize ultra-small metal nanoparticles that can easily penetrate bacterial cell walls (Soto et al., 2019).

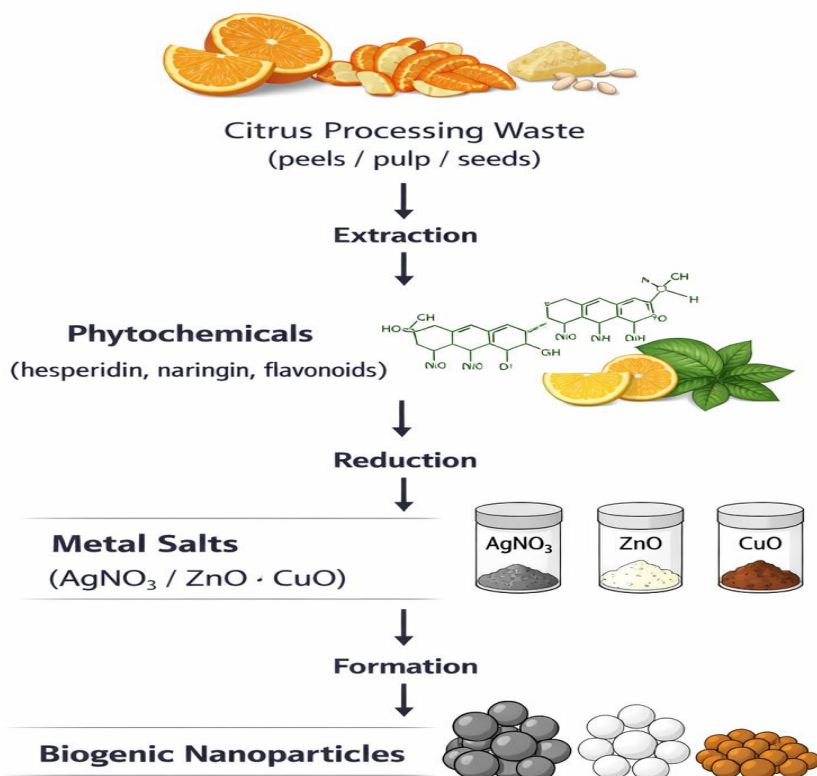
The specific phytochemical composition of the extract significantly influences the morphology and stability of the resulting nanoparticles (Singh et al., 2018). Hesperidin, the predominant flavonoid in orange peel, and its aglycone form, hesperetin, have been used to functionalize biogenic silver nanoparticles. Structural characterization through Fourier Transform Infrared (FTIR) spectroscopy has confirmed that these molecules interact with the silver core through their hydroxyl groups, providing a robust biological coating that enhances the antimicrobial efficacy of the particles (Khan et al., 2021).

Table 3. Phytochemical Sources and Resulting Nanoparticles

Source Material	Dominant Phytochemicals	Resulting Nanoparticles	Mean Size (nm)
Orange Peel (C. sinensis)	Hesperidin, Polyphenols	AgNPs, SPIONs	20–32
Lemon Peel (C. limon)	Eriocitrin, Essential Oils	AgNPs, CuONPs	59.7
Grapefruit Peel (C. paradisi)	Naringin, Narirutin	AgNPs, ZnO	Ultra-small
Neem Leaf (A. indica)	Azadirachtin, Terpenoids	AgNPs	15–20

Research has demonstrated that the type of solvent used during extraction can also alter the morphology of the nanoparticles. For instance, synthesis using copper (II) nitrate as a precursor and citrus peel extracts in distilled water often yields rod-like shapes, whereas acetone extracts tend to produce spherical particles (Velmurugan et al., 2015; Khalil et al., 2014). Spherical nanoparticles are generally preferred for agricultural sprays due to their high surface-area-to-volume ratio and uniform dispersal properties (Makarov et al., 2014). Citrus processing waste provides a rich source of phytochemicals capable of reducing metal salts into stable nanoparticles. The valorization pathway for citrus waste-derived nanoparticles is illustrated in Figure 2.

Figure 2: Plant-Mediated Nanoparticle Synthesis Using Citrus Waste



5. Fungal and Microbial Synthesis: Exploiting Antagonistic Synergies

Beyond plants, microorganisms such as fungi and bacteria serve as effective "nano-factories." Mycosynthesis, or fungus-mediated synthesis, is particularly attractive due to the large-scale production of extracellular enzymes and secondary metabolites by fungal mycelia, which act as efficient reducing and capping agents (Sharma et al., 2021).

Trichoderma species, widely used in agriculture as biological control agents (BCAs), have shown significant potential for the synthesis of zinc oxide (ZnO) and silver nanoparticles. These fungi produce a suite of extracellular metabolites that facilitate their survival under environmental stress, and these same metabolites can be harnessed to reduce metal ions into non-toxic metallic nanoparticles (Singh & Verma, 2020). Interestingly, co-cultivation of different Trichoderma strains can stimulate the synthesis of novel secondary metabolites, leading to enhanced antimicrobial activity against pathogens like *Xanthomonas oryzae* and Xcc (Chatterjee et al., 2019).

Bacteria also play a crucial role in biogenic synthesis. *Bacillus* species, such as *Bacillus cereus* SZT1 and *Bacillus thuringiensis* (Btk-2), have been used to synthesize AgNPs (Das et al., 2020). These biogenic AgNPs, stabilized with proteins and alcoholic groups from the bacterial culture supernatant, exhibit strong *in vitro* and *in vivo* antibacterial activity. The use of beneficial bacteria for synthesis provides a dual advantage: the resulting nanoparticles can be combined with the live bacterial isolates in an integrated pest management (IPM) strategy, where the bacteria provide long-term protection through competition and the induction of systemic acquired resistance (SAR) in the host plant (Mehta et al., 2022).

The mycosynthesis process generally involves exposing the fungal mycelium to a metal salt solution. The fungus responds by producing enzymes, such as reductases, which catalyze the reduction of toxic metal ions into solid nanoparticles (Shankar et al., 2020). These biosynthesized nanoparticles have been found to be less harmful to beneficial soil microflora and the plants themselves compared to their chemically synthesized counterparts (Prasad et al., 2018).

6. Mechanisms of Action: Multi-Target Antimicrobial Efficacy

The primary advantage of green nanoparticles over traditional antibiotics and copper bactericides lies in their multi-target mechanism of action. This complexity makes it exceedingly difficult for pathogens like Xcc to develop resistance, as it would require simultaneous mutations across multiple pathways (Timilsina et al., 2020).

6.1 Physical Disruption and Membrane Potential

The initial stage of nanoparticle-mediated death involves the physical interaction between the nanoparticle and the bacterial cell wall. Due to their extremely small size and high surface energy, nanoparticles accumulate on the cell membrane, altering its permeability and dissipating the transmembrane proton motive force (PMF) (Pal et al., 2007; Li et al., 2019). This disruption leads to the leakage of intracellular biomolecules and the eventual collapse of the cell's physical integrity (Choi & Hu, 2008).

6.2 Generation of Reactive Oxygen Species (ROS)

One of the most potent mechanisms of green nanoparticles is the induction of oxidative stress through the generation of Reactive Oxygen Species (ROS). ROS include highly reactive molecules such as hydroxyl radicals (.OH), superoxide anions (O₂⁻), and hydrogen peroxide (H₂O₂) (Rai et al., 2012). These molecules cause irreversible damage to major cellular components:

Lipids: Through peroxidation, ROS destroy the integrity of the plasma membrane.

Proteins: Oxidation or denaturation of enzymes leads to the arrest of cellular respiration.

Nucleic Acids: ROS induce strand breaks or base modifications in DNA, blocking replication and

transcription (Durán et al., 2016).

6.3 Inhibition of Virulence Proteins and Biofilms

Advanced green nanoparticles do not merely kill the pathogen; they also neutralize its virulence. Xcc relies on several key proteins for host infection and survival, including Ferredoxin-NADP⁺ reductase (FAD-FNR) and the type-III effector protein XopAI (Zhao et al., 2022). Molecular docking studies have shown that specific phytochemicals found in green nanoparticle coatings, such as nirurinetin, bind to the active site pockets of these proteins with high binding energies (e.g., -10.32 kcal/mol), effectively inhibiting their function and reducing the pathogen's ability to maintain an infection (Gupta et al., 2021).

Furthermore, green nanoparticles are highly effective at inhibiting biofilm formation. Biofilms are complex bacterial communities embedded in a matrix of exopolysaccharides (EPS) that shield the bacteria from antibiotics and host immune responses. By preventing the initial adhesion of Xcc and reducing EPS secretion, nanoparticles make the pathogen more vulnerable to the plant's natural defenses (Kaur et al., 2021).

7. Advanced Nano-Delivery Systems: Vesicles and Chitosan

The delivery of biopesticides to the target site, whether the leaf surface or the internal tissues where Xcc resides, is a critical challenge in citrus groves. Innovative nanocarriers are being developed to enhance the stability, adherence, and controlled release of active ingredients (Zhang et al., 2020).

7.1 Apoplastic Extracellular Vesicles (EVs)

An emerging strategy in "phyto-nano-drug delivery" involves the use of plant-derived extracellular vesicles (EVs). These are naturally occurring nanovesicles (30–1000 nm) found in the apoplastic fluid of leaves (Yang et al., 2021). When green-synthesized AgNPs are encapsulated within these EVs, they form a hybrid delivery system that is significantly more effective than the nanoparticles alone. The EV-encapsulated particles show enhanced antimicrobial activity against *Xanthomonas* species, likely because the biological membrane of the vesicle protects the silver core from environmental degradation and improves its interaction with the bacterial target (Cai et al., 2020).

7.2 Chitosan Nanofibers and Nanocapsules

Chitosan is perhaps the most suitable polymer for agricultural encapsulation due to its natural abundance, biodegradability, and intrinsic antibacterial properties. Chitosan's free amino groups allow for easy functionalization and the formation of various nanostructures, including nanogels, nanofibers, and nanocoatings (Rashid et al., 2021).

Research has demonstrated that chitosan/polyvinyl alcohol (PVA) hybrid nanofibers can effectively encapsulate citrus essential oils such as those from lemon, lime, and grapefruit with efficiencies as high as 88% (Oliveira et al., 2020). These nanofibers preserve the bioactive properties of the oils, providing sustained antioxidant and antibacterial activity. Similarly, chitosan-coated ZnO nanoparticles synthesized from *Pinus roxburghii* needles have shown higher antibacterial activity against Xcc than non-encapsulated particles (Patel et al., 2022).

Table 4. Advanced Delivery Matrices for Citrus Protection

Delivery Matrix	Active Component	Key Advantage	Application Method
Apoplastic EVs	Biogenic AgNPs	Protection of bioactive core	Systemic Spray
Chitosan (Nanofiber)	Essential Oils	Sustained release/Adherence	Protective Coating

Chitosan (Coating)	ZnO Nanoparticles	Enhanced uptake/Mechanical strength	Foliar Spray
MOF (ZIF-8)	Fungicides	pH-responsive release	Targeted Delivery

The development of stimuli-responsive nanocarriers represents the future of smart agriculture. PH-responsive nanoparticles, such as those based on Metal-Organic Frameworks (MOFs), can be engineered to release their payload specifically under the acidic conditions characteristic of many pathogen-infected microenvironments (Delgado et al., 2026).

8. Metabolomics and the Metabolic Reprogramming of Xcc

To gain a nuanced understanding of how biogenic nanoparticles overcome Xcc, researchers have employed NMR-based metabolomics to track the pathogen's metabolic shifts upon exposure. These studies have revealed that the antimicrobial action of functionalized nanoparticles involves a complete reprogramming of the bacterial metabolism (Carrola et al., 2018).

A comparative study between AgNPs coated with hesperidin (AgNP@HSD) and hesperetin (AgNP@HST) illustrated this phenomenon. While both formulations were effective, their metabolic impacts were distinct (Alkhulaifi et al., 2020).

- AgNP@HST Response: This aglycone-functionalized particle triggered targeted oxidative stress, leading to a significant depletion of energy and redox metabolites, resulting in a collapse of cellular homeostasis (Raja et al., 2023).
- AgNP@HSD Response: The hesperidin-coated particles induced a broad stress response characterized by elevated amino acid levels. The bacteria attempted a metabolic adaptation strategy, prioritizing the maintenance of intracellular ATP and NAD(P)H levels as a survival mechanism (Jarak et al., 2017).

This metabolic evidence underscores the structural influence of the biological coating on the nanoparticle's efficacy (Babele, 2019).

9. Field Efficacy and Comparative Trials: From Lab to Orchard

The ultimate test of any biopesticide is its performance under field conditions, where environmental variables can drastically reduce the efficacy of conventional treatments (Din et al., 2026).

9.1 Zinkicide vs. Conventional Copper

Zinkicide, a nano-zinc oxide formulation, has been evaluated in extensive field trials in Florida. Results from 2014 and 2015 trials demonstrated that Zinkicide reduction of grapefruit canker incidence exceeded that of cuprous oxide bactericides (Graham et al., 2016). One of the unique attributes of Zinkicide is its locally systemic nature; it forms a protective film while also penetrating the leaf tissue, providing a level of control that exceeds traditional preventative copper sprays (Kah et al., 2018). Critically, Zinkicide provided this superior control without the phytotoxicity often associated with copper applications (Adisa et al., 2019).

9.2 Integrated Ag/Cu Nanoparticles

Comparative studies using silver and copper nanoparticles synthesized from *Eucalyptus globulus* leaves have also shown promising results (El-Rafie et al., 2010;). Under laboratory conditions, a combination of silver and copper nanoparticles produced the highest inhibition zone (21.06 mm) against Xcc (Prabhu & Poulouse, 2012). In field evaluations, the combination treatment resulted in the minimum disease incidence (35.23%) and minimum disease severity (23.23%) compared to either nanoparticle used alone (Franci et al., 2015).

9.3 Adherence and Longevity

The adherence of nano-formulations to the plant surface is vital for protecting expanding fruit over the 90-to-120-day development period. Studies using copper-loaded silica nanogel (CuSiNG) technology have shown that high surface area significantly improves adherence to citrus leaves (Iftikhar & Mubeen, 2024).

10. Ecotoxicological Implications and Soil Health

As the adoption of nano-biopesticides increases, it is imperative to evaluate their impact on the broader agricultural ecosystem, particularly the soil microbial community (Kumari et al., 2023).

10.1 Impact on Soil Microbiota

Soil microorganisms are sensitive bioindicators that respond rapidly to environmental changes. Studies on ZnO nanoparticles have indicated that low concentrations (up to 250 mg/kg in soil) can have a stimulating effect on both plants and soil microbiota, enhancing mineralization and plant growth-promoting activities (Ge et al., 2011; Raliya et al., 2017).

For instance, low concentrations of FeO and ZnO nanoparticles have been shown to increase the diversity and richness of soil microbes compared to control groups (Dimkpa et al., 2012; Servin et al., 2015). However, toxic effects emerge at higher concentrations. High doses of ZnO or Ag nanoparticles can inhibit seedling growth and decrease the abundance of beneficial phyla such as Proteobacteria, Actinobacteria, and Planctomycetes (Doğaroğlu & Köleli, 2017). The degree of toxicity is governed by particle size, coating, and soil characteristics (Simonin & Richaume, 2015).

6.2 Phytotoxicity and Nutrient Accumulation

Phytotoxicity studies have shown that high concentrations of Ag nanoparticles can inhibit seed germination and reduce root and shoot lengths in various terrestrial plants. In citrus orchards, nano-formulations like Zinkicide appear to reduce these risks, as zinc has a much lower risk of phytotoxicity than copper and can improve carbohydrate and chlorophyll production (Timilsina et al., 2020).

11. Techno-Economic Assessment and Regulatory Pathways

The commercialization of green nano-biopesticides requires a balance between technological efficacy, economic feasibility, and regulatory compliance (Yang et al., 2021).

11.1 Industrial Feasibility

Economic evaluations of industrial-scale biosynthetic production of copper nanoparticles using *Citrus medica* Linn. extract have demonstrated strong financial feasibility (Din et al., 2026). A projected facility producing 24,000 kg per year was analyzed:

- Initial Capital Cost: 272,640.00 USD.
- Annual Operating Profit: 698,655.90 USD.
- Payback Period: Achieved within the 3rd year of operation.

Compared to physical and chemical synthesis, the green approach is significantly more cost-effective as it utilizes aqueous plant extracts rather than expensive chemical precursors (Sindhushree et al., 2025).

11.2 Global Regulatory Frameworks

The regulatory landscapes in the USA and the EU present different hurdles for nano-biopesticides. In the United States, the EPA's dedicated biopesticide division utilizes a risk-based approach under

the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). This system has generally allowed for the faster authorization of biocontrol products compared to Europe (Durán et al., 2016).

In the European Union, the regulatory environment is undergoing a transition. Current legislation (Regulation EC No. 1107/2009) was designed with chemical pesticides in mind, leading to lengthy approval processes (Iravani, 2011). However, the EU Bioeconomy Strategy 2025 and the forthcoming EU Biotech Acts are designed to streamline requirements and introduce tools like "regulatory sandboxes" (Kaur et al., 2025).

Table 5. Global Regulatory Models for Nano-biopesticides

Region	Primary Regulation	Governance Model	Future Outlook
USA	FIFRA (EPA)	Risk-based Assessment	Expanding biocontrol availability
EU	EC No 1107/2009	Hazard-based/Precautionary	Reform under Bioeconomy Strategy 2025

12. Conclusion

Green nanoparticle-based biopesticides represent a paradigm shift in citrus canker management, offering potent, environmentally benign alternatives to copper-reliant strategies that align with global sustainability goals and regulatory demands for reduced chemical residues. By leveraging biogenic synthesis for tailored Ag, CuO, ZnO, and hybrid NPs, these formulations achieve superior bactericidal action through multifaceted mechanisms oxidative stress, cellular disruption, and resistance evasion while minimizing phytotoxicity and ecological risks at optimized doses. Field evaluations confirm their efficacy in diverse citrus agroecosystems, integrating seamlessly into IPM frameworks with cultural and biological controls. However, realizing full potential requires addressing scalability barriers, standardizing synthesis protocols, conducting multi-year phytotoxicity studies, and navigating biosafety regulations. As climate change exacerbates pathogen pressures, adopting green NPs could safeguard citrus productivity, enhance farmer livelihoods, and promote resilient, low-input agriculture, ultimately contributing to global food security and environmental health.

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