



Review article

Green-synthesized iron oxide nanoparticles for bacterial wilt control: Comprehensive mechanistic and efficacy insights

Vanshika Kardam, Gyanika Shukla*

Department of Biotechnology, Chaudhary Charan Singh University, Meerut 250004, India

Article Info

Article history:

Received 10 December 2025

Revised 28 February 2026

Accepted 3 April 2026

Available online 17 April 2026

Keywords:

Antibacterial mechanisms,
Bacterial wilt,
Green synthesis,
Iron oxide nanoparticles,
Plant disease management,
Ralstonia solanacearum,
Sustainable agriculture

Abstract

Importance of the Work: Bacterial wilt, caused by *Ralstonia solanacearum* (International Society for Plant Pathology; www.isppweb.org) is a devastating plant disease that is difficult to control with conventional methods. Green-synthesized iron oxide nanoparticles (IONPs) offer a sustainable, eco-friendly and biocompatible alternative. Their biogenic production uses natural extracts, reduces toxic inputs and energy consumption, and provides strong antibacterial activity.

Objectives: To review the biogenic synthesis of IONPs from plant and microbial sources, and to evaluate their mechanisms, characterization and potential for bacterial wilt control.

Materials and Methods: The synthesis process involved three stages: reduction of metal salts by biomolecules, nucleation and growth into nanoscale clusters, and stabilization/capping to prevent aggregation. Characterization of these IONPs was performed using a suite of techniques: ultraviolet-visible Spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), Dynamic light scattering (DLS) and atomic force spectroscopy (AFM).

Results: The reviewed studies showed that green-synthesized IONPs exhibited strong antibacterial efficacy against *R. solanacearum* both *in vitro* and in greenhouse trials. In tomato plants, root-zone treatments and foliar applications significantly reduced disease incidence dropping as low as 13.09% compared to 98.01% in untreated controls in some reports. Furthermore, IONPs improved plant growth parameters, including biomass, shoot length and chlorophyll content, while enhancing soil health through increased enzyme activity and nutrient availability.

Main Findings: The primary finding was that IONPs combat bacterial wilt through a multifaceted mechanism involving: disrupting bacterial cell membranes via electrostatic interactions; generating reactive oxygen species that damage DNA and proteins; and inhibiting the formation of protective biofilms by interfering with quorum sensing. These findings suggest that integrating IONPs into disease management strategies could substantially reduce reliance on chemical pesticides and enhance agricultural resilience.

* Corresponding author.

E-mail address: gyanikashukla@gmail.com (G. Shukla)

online 2452-316X print 2468-1458/Copyright © 2026. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), production and hosting by Kasetsart University Research and Development Institute on behalf of Kasetsart University.

<https://doi.org/10.34044/j.anres.2026.60.2.11>

Introduction

At the nano scale, materials can be engineered to display novel quantum, physical and chemical behaviors (Newberry, 2022). Such advances allow the creation of structures from atomic to submicron levels that can be integrated into larger systems. These nanomaterials show great promise in areas such as healthcare, diagnostics and agriculture (Hossain et al., 2025). In agriculture, nanotechnology offers groundbreaking solutions to improve productivity, sustainability and resource efficiency by engineering materials at the nanoscale. Nano-fertilizers and nano-pesticides revolutionize nutrient and pest management through controlled-release mechanisms that deliver essential nutrients or active agents directly to plants. This precision reduces waste and environmental damage compared to conventional methods, where often, the latter lead to runoff and ecological harm (Mim et al., 2015). Furthermore, nanosensors advance precision agriculture by monitoring soil moisture, nutrient levels and plant health in real time, enabling farmers to optimize resources and boost crop yields sustainably (Zain et al., 2023). Nanomaterials, such as carbon-based nanocomposites and nano-clays, are used in soil remediation to adsorb heavy metals and pesticide residues, thus preserving soil fertility and preventing water contamination (Dhanapal et al., 2024). However, nanotechnology in agriculture faces challenges such as toxicity, bioaccumulation and impacts on

soil microbes, alongside regulatory and risk assessment barriers (Tran et al., 2024). Further research is vital to ensure the safe use of nanoparticles (NPs). This review explored the antibacterial efficacy of green-synthesized iron oxide nanoparticles (IONPs) and their potential to enhance crop productivity by mitigating plant diseases with special reference to bacterial wilt (*Ralstonia solanacearum*, naming authority- International Society for Plant Pathology; www.isppweb.org, providing a comprehensive understanding of their role in sustainable agriculture.

NPs have revolutionized plant disease management by offering a sustainable approach as their unique properties enable targeted control of a wide range of plant pathogens (Izuafa et al., 2015). Metal NPs have antimicrobial activity through various mechanisms (Table 1). Additionally, NPs can enhance plant defense by inducing systemic acquired resistance and delivering antifungal or antibacterial agents efficiently (Ruffatto et al., 2025). Green-synthesized NPs, produced using biological extracts and processes, are particularly promising due to their biocompatibility and reduced environmental impact (Singaravelu et al., 2025). For example, IONPs synthesized from *Moringa oleifera* or *Azadirachta indica* extracts effectively controlled fungal bacterial pathogens (Ayub et al., 2025). Similarly, NPs of silver and copper have shown efficacy against *fungal pathogens* (Sharma et al., 2025). In addition, these NPs improve soil health and plant growth by enhancing nutrient availability and antioxidant enzyme activity (Gupta and Saharan, 2026).

Table 1 Overview of NP types, modes of action, target plant pathogens and references.

Type of metal nanoparticle	Mode of action	Target plant pathogen	References
Silver (Ag)	Produces reactive oxygen species (ROS), denatures proteins, damages cell membranes	<i>Fusarium</i> spp., <i>Pseudomonas</i> spp.	Tariq et al. (2022)
Ag	Oxidative stress through the ROS	<i>Sclerotinia sclerotiorum</i> , <i>Colletotrichum falcatum</i> , <i>Colletotrichum gloeosporioides</i> and <i>Rhizoctonia solani</i>	Singh et al. (2024a)
Ag	Oxidative stress through the ROS	<i>Holotrichia</i> spp.	Shukla et al. (2022)
Ag	-	<i>Phytophthora infestans</i>	Singh et al. (2021)
Copper (Cu)	Releases Cu ions, produces ROS, stops enzymes from working	<i>P. infestans</i> , <i>Xanthomonas</i> spp.	Rai et al. (2018)
Zinc (Zn)	Causes oxidative stress, disrupts enzyme functions	<i>Rhizoctonia solani</i>	Mahmoud et al. (2024)
Zn	-	<i>Alternaria solani</i>	Singh et al. (2022a)
Iron oxide nanoparticles	Interacts with proteins and DNA of microorganisms	Soil-borne fungal pathogens	Panwar et al. (2025)
Silicon dioxide (SiO ₂) NPs	Interferes with spore germination, strengthens plant defenses	<i>Fusarium brachygibbosum</i>	Belhedi et al. (2025)
Graphene oxide	Destroys membranes, absorbs germs, eliminates them	Bacteria	Sengupta et al. (2019)
Chitosan NPs	Induces systemic resistance in plants exposed to antimicrobials	Various fungi	El-Mohamedya et al. (2019)

Table 1 Continued

Type of metal nanoparticle	Mode of action	Target plant pathogen	References
Alginate NPs	Acts as a vehicle for gradual delivery of antifungal drugs	Various fungi	Khan et al. (2025a)
Ag-Chitosan NPs	Combines immune-boosting properties of chitosan with antibacterial silver	Bacteria	Malak et al. (2022)
ZnO-Chitosan NPs	Slow nutrient release combined with antimicrobial action	Bacterial and fungal pathogens	Fatima et al. (2025)

Green synthesis of IONPs for agricultural applications

IONPs have emerged as valuable agricultural tools due to their nanoscale size, high surface-to-volume ratio, magnetic properties and biocompatibility, enabling applications in plant disease control, nutrient delivery and soil improvement (Chand and Vasquez-Guardado, 2025). Green synthesis using plant or microbial extracts yields eco-friendly IONPs that avoid using toxic chemicals and reduce energy inputs (Haider et al., 2024), supporting sustainable alternatives to conventional agrochemicals (Table 2). Their strong antimicrobial activity-mediated through reactive oxygen species (ROS) generation, membrane disruption, protein denaturation and DNA damage, effectively suppresses pathogens such as *Fusarium* spp. and *A. solani*, with *Spinacia*

oleracea-derived IONPs reducing *Fusarium* wilt in tomatoes by nearly 48% (Lu et al., 2023; Ashraf et al., 2022a; Anwaar et al., 2024). In addition, IONPs trigger Systemic acquired resistance, enhancing defense gene expression and antioxidant enzyme activity (Noman et al., 2024). As nutrient carriers, they improve micronutrient uptake, exemplified by *Rhododendron arboreum*-synthesized IONPs increasing wheat iron absorption and grain yield by up to 12% (Singh et al., 2025a), while enhancing photosynthesis, biomass production and stress tolerance (Alam et al., 2025; Ashraf et al., 2022a; Singh et al., 2022b; Mukhtiar et al., 2024). In soils, IONPs enhance microbial activity organic carbon and nutrient cycling such as increased dehydrogenase activity in legumes (Koleva et al., 2022), while their biodegradable, non-toxic nature reduces environmental pollution, supporting long-term sustainable soil management (Sharma and Thakur, 2025).

Table 2 Green-synthesized IONPs: overview of synthesis approaches, particle properties, biological targets and crop performance impacts.

IONP synthesis method	Particle size (nm)	Target pest or pathogen and/or effect on growth and yield of plant	Reference(s)
Green synthesis using <i>Spinacia oleracea</i> extract	4	Targeting <i>Fusarium</i> wilt in tomatoes, increasing lycopene, vitamin C, total flavonoids and protein levels	Ashraf et al. (2022a)
Microbial synthesis using fungal metabolites	71	Targeting <i>Ralstonia solanacearum</i> , increasing chlorophyll levels, antioxidant activity, dehydrogenase activity, nutrient availability and iron content in tomato	Naik et al., (2025)
Green synthesis using <i>Psidium guajava</i> extract	29-41	Targeting <i>Alternaria solani</i> , enhancing plant growth, micronutrient increase and biofortification in potato	Anwaar et al. (2024)
Green synthesis using <i>Aloe vera</i> extract	20-48	Targeting fungi	Talebi (2024)
Green synthesis using <i>Ocimum sanctum</i> extract	30-70	Targeting <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	Ahmad et al. (2017)
Green synthesis using <i>Lantana camara</i> , <i>Ocimum basilicum</i> and <i>Thymus decussatus</i> extract	48	Targeting <i>Botrytis cinerea</i> , significantly increasing vitamin and mineral content in strawberry plants	Mogazy et al. (2022)
Microbial synthesis using mushroom extract	~17	Targeting <i>Aspergillus niger</i>	Kamal et al. (2025)
Green synthesis using <i>Platanus orientalis</i> extract	~80	Targeting <i>Aspergillus niger</i> and <i>Mucor piriformis</i>	Devi et al. (2019)
Green synthesis using <i>Polygonum paronychioides</i> extract	~88	Targeting <i>Pseudomonas syringae</i> and <i>Clavibacter michiganensis</i>	Mushtaq et al. (2025)

Table 2 Continued

IONP synthesis method	Particle size (nm)	Target pest or pathogen and/or effect on growth and yield of plant	Reference(s)
Microbial synthesis using <i>Streptomyces</i> spp.	80–180	Targeting <i>Fusarium oxysporum</i> , <i>P. aeruginosa</i>	Attea et al. (2024)
Green synthesis using <i>Laurus nobilis</i> extract	~9	Targeting <i>Listeria monocytogenes</i> , <i>Aspergillus flavus</i> and <i>Penicillium spinulosum</i>	Jamzad and Kamari Bidkorpeh (2020)
Green synthesis using <i>Syzygium aromaticum</i> extract	-	Targeting <i>Bacteria</i>	Gaminda et al. (2024)
Green synthesis using <i>Bacillus aryabhatai</i>	86	Targeting <i>Xanthomonas oryzae</i> , improving photosynthetic efficiency, chlorophyll content, carotenoid content and nutrient content in rice	Ahmed et al. (2022)
Green synthesis using <i>Pseudomonas fluorescens</i>	20-25	Targeting fungi	Ashrafi-Saiedlou et al. (2025)
Green synthesis using <i>Salvinia molesta</i> extract	82	Increased seed germination in tomato	Madusanka et al. (2023)
Green synthesis using <i>Prosopis cineraria</i> extract	~43	Enhanced growth and yield, improving resilience to drought stress in wheat	Singh et al. (2022b)
Various plant extract and black coffee	~20	Improved plant growth and reduced Fusarium wilt in tomato	Ashraf et al. (2022b)
Green synthesis using pomegranate peel extract	12	Boosted growth and yield under salinity stress in maize	Abdulfatah et al. (2025)
Green synthesis using <i>Cannabis sativa</i> extract	164	Many-folds increase in chlorophyll content, antioxidants and polyphenols in soybean	Pavlicevic et al. (2025)
Green synthesis using <i>Chenopodium album</i> and <i>Fumaria indica</i> extract	2-7.5	Improved callus growth, enhanced plant morphological growth and reduced microbial contamination in rice	Ullah et al. (2024)
Green synthesis using <i>Psidium guajava</i> extract	29-41	Improved resilience to biotic stress and enhanced growth in potato	Anwaar et al. (2024)
Green synthesis using onion extract	~8	Enhanced growth and yield in cucumber	Al-Hamdani et al. (2024)
Green synthesis using orange peel extract	13	Enhanced root and shoot growth; increased biomass in tomato	Rojo et al. (2024)
Green synthesis using <i>Emblica officinalis</i> extract	30-70	Increased chlorophyll content and metabolic activity in rice	Singh et al. (2024b)
Microbial synthesis using <i>Bacillus subtilis</i>	-	Enhanced growth and boosted stress resilience against arsenic stress	Khan et al. (2022)
Green synthesis using <i>Aspalathus linearis</i> extract	~18	Increased growth and boosted stress defence response against drought in sorghum	Ndou et al. (2023)
Microbial synthesis using <i>Trichoderma harzianum</i>	207	Boosted seedling growth in soybean	Guilger-Casagrande et al. (2022)

~ = approximately.

Green synthesis is an eco-friendly and sustainable approach to producing NPs by harnessing biological systems, such as plants, bacteria, fungi, algae and enzymes, to reduce metal ions into nanoscale particles (Fig. 1). Unlike traditional physical and chemical synthesis methods, which often rely on toxic chemicals, high energy inputs and harsh conditions, biogenic synthesis offers a greener alternative by utilizing natural biochemical processes that minimize environmental impact and eliminate the need for hazardous substances (Pechyen et al., 2024). This method leverages the inherent reducing and stabilizing capabilities of biomolecules found in biological entities, making it a promising strategy for producing NPs with applications in agriculture, medicine and environmental remediation. By avoiding harmful byproducts and reducing

energy consumption, biogenic synthesis aligns with the growing demand for sustainable nanotechnology, providing a safer and more environment-responsible pathway for NP production (Cardoso et al., 2025). Typically, chemical synthesis techniques, such as co-precipitation or hydrothermal methods, require toxic reducing agents (such as hydrazine) and solvents (such as chloroform) leading to environmental hazards and high production costs. Physical methods, including laser ablation or ball milling, demand major energy inputs and specialized equipment, often resulting in polydisperse NPs with poor biocompatibility. In contrast, green synthesis operates under ambient conditions, leveraging natural reductants for efficient, scalable production (Nyabadza et al., 2023). However, challenges in green methods include variability

in the NP yield due to biological source fluctuations. Overall, green approaches reduce energy use by up to 90% and eliminate hazardous byproducts, making them preferable for agricultural applications (Osman et al., 2024).

As shown in Fig. 1, plant-derived extracts from leaves, stems, flowers, fruits and roots act as natural reducing and stabilizing agents for NP synthesis, while similarly, microbial sources, such as bacteria, fungi, algae and microbial extracts contribute enzymatic and metabolic activity to facilitate NP formation. The process involves the reduction of precursor ions into atoms, followed by nucleation into clusters and subsequent growth and stabilization into IONPs. The use of biological systems ensures a cost-effective, non-toxic and environmentally sustainable approach compared to conventional chemical and physical methods.

Many diverse biological entities serve as sources for biogenic NP synthesis due to their rich content of biomolecules with reducing and stabilizing properties. Plants such as *Ocimum sanctum* (holy basil) and *Azadirachta indica* (neem) are used widely because their leaves, stems or roots contain phytochemicals, including alkaloids, flavonoids and terpenoids, that facilitate NP formation (Khan et al., 2025b). Bacteria, including *E. coli* and *B. subtilis*, are used for their ability to produce enzymes and proteins that reduce metal ions into NPs (Iravani et al., 2014). Fungi, such as *Penicillium* spp. and *Aspergillus niger*, are valued for their high biomass and extracellular enzyme production, which enhance the efficiency of NPNP synthesis (Rami et al., 2024).

Algae, such as *Sargassum wightii*, contribute bioactive compounds that aid in the reduction process, while actinomycetes and yeasts offer additional microbial pathways for NPNP (El-Sheekh et al., 2022). These organisms contain biomolecules, such as proteins, enzymes, phenolics and polysaccharides, that act as reducing agents to convert metal salts into NPs and as capping agents to stabilize them, ensuring controlled size and shape.

Plant extracts from leaves, roots, fruits or seeds are popular due to their high content of bioactive compounds, including polyphenols, flavonoids and alkaloids, that facilitate the reduction of FeCl_3 to iron oxide forms. For example, aqueous extracts of *Egeria densa* have been used to synthesize IONPs, confirmed based on ultraviolet-visible spectroscopy (UV-Vis) showing a color change indicative of NP formation (Yekeen et al., 2025). Date seed extracts from varieties such as Ambara and Sukhri have produced IONPs with biomedical potential, demonstrating antioxidant and antimicrobial properties (Kathiresan and Durairaj, 2025). These plant-based methods are scalable and utilize agricultural waste, promoting so-called circular economy principles to keep materials and products in circulation for as long as possible and thus minimizing waste and resource use. Plant-mediated NP synthesis is a widely adopted biogenic approach due to its simplicity, cost-effectiveness and abundance of plant-derived biomolecules (Osman et al., 2025). The phytochemicals in the extract interact with the NPs, stabilizing them by capping their surfaces to prevent aggregation and to maintain their nanoscale properties (Hosseinzadeh et al., 2023).

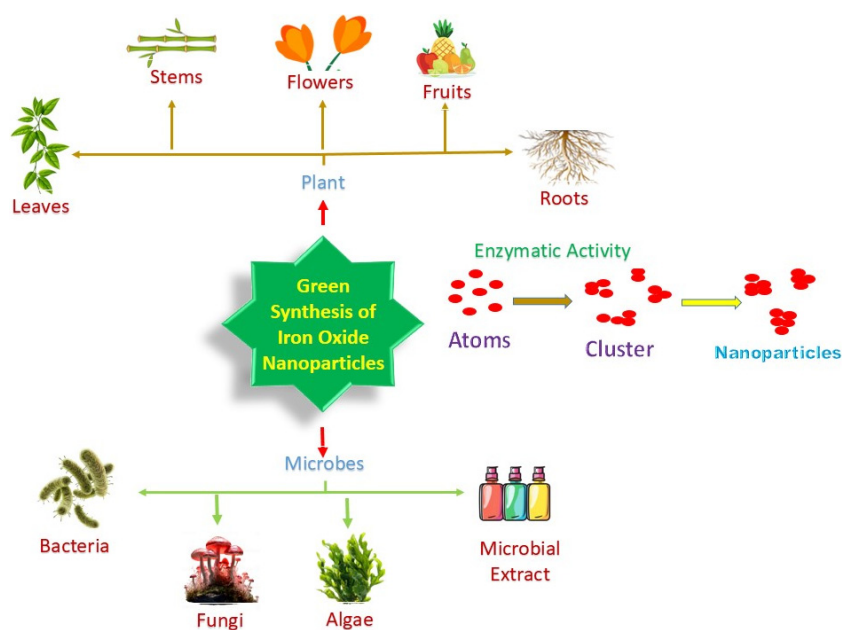


Fig. 1 Green synthesis of IONPs using plants and microbes, illustrating eco-friendly synthesis pathway of IONPs through biological sources.

Microbial synthesis harnesses bacteria, fungi or yeast to produce IONPs through enzymatic reduction and stabilization. *Bacillus sp.* GMS10 has been used to biosynthesize IONPs with potent antibacterial effects (Fatih et al., 2024). *Pseudomonas fluorescens* cell-free supernatant facilitated IONP formation, characterized for biomedical applications (Ashrafi-Saiedlou et al., 2023). In addition, marine green algae, such as *Caulerpa antennina*, have served as sources for NPs with antimicrobial properties (Yosri et al., 2021). These microbial routes offer advantages in controlled environments but may require optimization for yield. Recent advancements include fungal-mediated synthesis for scalable production. For example, *Sargassum muticum* algal extracts have been noted for sulfated polysaccharides that reduce ferric salts effectively (Flores-Contreras et al., 2023). Biomolecules, such as chitosan or enzymes from natural sources, enhance morphology control, with bioflocculants from microbial sources being used for IONP synthesis, emphasizing eco-compatibility (Mohammed et al., 2025). These sources expand the versatility of green synthesis, with applications in magnetic hyperthermia and imaging.

Biogenic synthesis of NPs offers a range of compelling advantages over conventional physical and chemical methods, making it a preferred choice for researchers seeking sustainable, biocompatible and cost-effective alternatives. One of the primary benefits is its sustainability and environmental friendliness, as it minimizes the reliance on hazardous chemicals, toxic solvents and energy-intensive processes commonly associated with traditional synthesis techniques. For example, often, chemical methods use harmful reducing agents, such as hydrazine or sodium borohydride, which pose risks to both human health and the environment. In contrast, biogenic synthesis utilizes natural biological systems, such as plants, bacteria or fungi, aligning with green chemistry principles and substantially reducing environmental impact (Ayub et al., 2025). Another key advantage is cost-effectiveness, as biogenic synthesis leverages readily available and inexpensive raw materials, such as plant extracts or microbial cultures, eliminating the need for expensive equipment or reagents. Additionally, the process often occurs under ambient conditions, further lowering energy costs compared to energy-intensive physical methods such as laser ablation or high-temperature annealing (Abuzeid et al., 2023). Furthermore, the simplicity and scalability of biogenic synthesis make it accessible for use in resource-limited settings, as it requires minimal equipment and can be optimized for large-scale production with appropriate biological conditions

(Shah et al., 2025). Notably, biogenic NPs often exhibit enhanced functionalization, as the biomolecules involved in their synthesis provide additional functional groups that improve their activity and enable conjugation for specific applications in biomedicine or environmental remediation (Sharma et al., 2019). Finally, biogenic synthesis allows for shape and size control, offering precise tunability of NP morphology by adjusting parameters such as the pH, temperature and extract concentration. This results in greater particle homogeneity compared to chemical synthesis, which often struggles with inconsistent particle sizes and shapes (Vinukonda et al., 2025).

Typically, the biogenic synthesis process involves three key stages: reduction, nucleation and growth and stabilization. In the first stage, biomolecules in biological extracts, such as alkaloids, terpenoids, flavonoids or proteins, reduce metal salts to their zero-valent metallic forms, initiating NP formation. The second stage (nucleation and growth) involves the aggregation of these reduced metal atoms into nanoscale particles, with their size, shape and structure influenced by factors such as the type of biological extract, reaction temperature and pH of the medium. Finally, stabilization or capping occurs when biomolecules adsorb onto the surface of the newly formed NPs, preventing agglomeration and controlling their morphology (Shah et al., 2021). Phytochemicals, such as polyphenols and flavonoids in plant extracts, act as reducing agents by donating electrons to Fe^{3+} ions, while also capping the NPs to prevent aggregation. Enzymes in microbial systems, such as reductases, facilitate intracellular or extracellular reduction via electrostatic binding to metal ions, while biomolecules, such as proteins and terpenoids, from plant sources contribute to stabilization. These mechanisms ensure biocompatibility and functional group attachment (Edo et al., 2025).

Several factors critically influence the efficiency and characteristics of NPs produced through biogenic synthesis. By carefully optimizing these parameters, researchers can tailor NPs to meet specific application requirements, enhancing their efficacy in fields including agriculture, medicine and environmental remediation. The pH of the reaction medium is a pivotal factor in biogenic synthesis, as it directly affects the activity of biomolecules and the kinetics of metal ion reduction. Acidic or alkaline conditions influence the ionization state of functional groups in biological extracts, which serve as reducing and capping agents. Extreme pH values can denature biomolecules, reducing their efficacy and resulting in larger or less stable particles. The concentration of the metal

precursor can greatly impact the yield and size distribution of IONPs. Generally, higher precursor concentrations increase the availability of metal ions, leading to higher yields but often resulting in larger particles due to enhanced nucleation and growth rates. Similarly, temperature and reaction time are critical in controlling the kinetics of IONP formation. Higher temperatures accelerate the reduction process by enhancing the activity of reducing agents in biological extracts; however in addition, they can alter particle morphology (Ebrahimezhad et al., 2018). The choice of biological extract is paramount, as different plants, microorganisms or biomolecules contain varying levels of reducing and capping agents such as polyphenols, flavonoids and proteins. These agents not only reduce metal ions but also stabilize the NPs by preventing agglomeration (Zanbili and Poursattar Marjani, 2025).

Characterization techniques for green-synthesized IONPs

Characterization is essential to confirm the formation, structural integrity, physicochemical properties, stability and functionality of green-synthesized IONPs. Since plant-mediated synthesis involves diverse bioactive compounds,

detailed characterization is required to validate NP formation and determine their size, morphology, crystallinity, elemental composition and surface properties. Key parameters influencing antimicrobial activity in agricultural applications include small and uniform particle size, narrow size distribution, high surface area, stable surface charge and optimal dispersibility, which enhance microbial interaction, ROS generation and controlled release of active species (Zúñiga-Miranda et al., 2023). Collectively, spectroscopic, microscopic and surface characterization techniques provide a comprehensive profile of IONPs, ensuring reliability, reproducibility and suitability for agricultural and biomedical applications. Techniques, such as UV-Vis spectroscopy, confirm NP formation, Fourier-transform infrared spectroscopy (FTIR) identifies functional groups responsible for reduction and stabilization, X-ray diffraction (XRD) determines crystallinity and phase composition, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) reveal morphology and size, energy-dispersive X-ray spectroscopy (EDX) verifies elemental composition and atomic force spectroscopy (AFM) and dynamic light scattering (DLS) evaluate surface topology, hydrodynamic size and dispersion stability (Table 3 and 4).

Table 3 Analytical techniques for characterization and validation of green synthesized metal-based NPs

Technique	Purpose	Information obtained	Advantages	Limitations	What it reveals	References
Ultraviolet -visible Spectroscopy	Detection and monitoring of NP formation	Surface plasmon resonance, reaction kinetics	Rapid, simple, non-destructive	Cannot provide size or morphology details	Confirms NP formation; monitors reduction efficiency of plant extracts	Nizamov et al. (2022)
FTIR	Identification of functional groups	Surface chemistry, biomolecule-NP interactions	Identifies capping/ stabilizing phytochemicals	Limited structural resolution; requires interpretation	Reveals biomolecules responsible for reduction and stabilization in green synthesis	Pasieczna-Patkowska et al. (2025)
XRD	Crystallographic structure determination	Phase identification, crystallite size	Precise crystal structure data	Amorphous materials difficult to analyze; peak broadening issues	Determines crystalline nature of NPs synthesized by plant/biomaterial extracts	Ali et al. (2023)
TEM	High-resolution imaging	Size, morphology, lattice fringes, aggregation state	Nanometer-scale detail	Expensive; sample prep artifacts possible	Shows size and shape control achieved by green reducing agents	Li et al. (2023)
SEM	Surface morphology	Particle shape, surface texture, aggregation	Wide field of view; easy imaging	Lower resolution than TEM	Validates surface morphology affected by natural capping agents	Pechyen et al. (2024)
EDX	Elemental analysis	Composition, purity	Integrated with SEM/TEM	Cannot detect light elements well	Confirms elemental iron; reveals presence of bimolecular residues	Sivalingam, 2025
DLS	Particle size distribution in colloids	Hydrodynamic size, polydispersity index	Quick, good for colloidal systems	Sensitive to aggregation; gives larger “hydrated” size	Shows colloidal stability imparted by green capping molecules	Németh et al. (2022)

Table 3 Continued

Technique	Purpose	Information obtained	Advantages	Limitations	What it reveals	References
Raman spectroscopy	Vibrational analysis	Surface molecular signatures	Non-destructive	Fluorescence interference common	Identifies biomolecular interactions on FeNP surfaces	Zhang et al. (2023)
AFM	Nanoscale surface topology	3D morphology, roughness	High-resolution 3D mapping	Small scan area; slow	Reveals surface modifications due to plant/polymer capping	Gulati and Adachi, 2023
ICPMS and AAS	Quantitative elemental analysis	Iron concentration, purity	Highly sensitive	Destructive; sample digestion needed	Determines iron yield and residual precursor ions	Cabr�� et al. (2023); Fu et al. (2021)

Table 4 Characterization of green-synthesized IONPs

Source	Characterization Techniques	Key result/Value	Reference(s)
<i>Coriandrum sativum</i> leaf extract	UV-Vis, TEM, DLS, VSM, DSC	- DLS mean size ~163 nm, PDI = 0.091; - Zeta potential ~ -13.8 mV; - VSM magnetization = 3.483 emu/g; - DSC: endothermic peak at ~ 176.9 ��C	Singh et al. (2022c)
<i>Vitex leucoxydon</i> leaf extract	UV-Vis, FTIR, SEM, EDX, XRD	- UV-Vis absorption maximum at 395 nm; - SEM: size ~ 45-100 nm; - XRD: average diameter ~ 136 nm; - FTIR: disappearance/shift of peaks at ~ 923.96 and 1709.89 cm⁻¹ (carboxylic acids/unsaturated aldehyde/ketone groups) indicating reduction/capping	Nahari et al. (2022)
<i>Myristica fragrans</i> (leaf) extract	UV-Vis, FTIR, SEM, XRD, Zeta potential	- UV-Vis peak at 292 nm; - FTIR suggests involvement of phenolic & hydroxyl groups in reduction/stabilization; - SEM: polydisperse, aggregated & irregular morphology; - XRD: broad peak at 2�� ~ 24.1��, indicating amorphous structure; - Zeta potential: -65.2 mV (good colloidal stability)	Salim et al. (2025)
<i>Piper betle</i> (Green betel) leaf extract	UV-Vis, XRD, FTIR, SEM/HR-TEM, EDS, VSM	- UV-Vis: continuous absorption 300–800 nm (no sharp SPR), band-gap energy ~ 1.87 eV; - XRD: crystal planes consistent with magnetite (2�� ~ 30.3��, 35.5��, 43.2��, 57.0��, 62.8��) (JCPDS 19-0629); - TEM/SEM: spherical + needle-like morphology; size ~ 29.8 nm; - FTIR: Fe-O bands at 482 and 567 cm⁻¹; - VSM: saturation magnetization Ms ~ 9.18 emu/g, remanence Mr ~ 0.49 emu/g, coercivity Hc ~ 78.22 Oe; - EDS: Fe ~ 73.35 wt%, O ~ 20.88 wt%	Khoirotnin et al. (2023)
<i>Pseudomonas fluorescens</i> (cell-free supernatant)	UV-Vis, XRD, FTIR, FE-SEM, EDX, TEM, BET, VSM	- SEM size ~ 20 nm; TEM size ~ 24; - Iron content ~ 90% (EDX); - XRD: rhombohedral Fe₂O₃, crystal size ~ 23 nm (Scherrer) - UV-Vis SPR peak ~ 365 nm, - Functional groups (OH, NH, etc.) from bacterial supernatant observed by FTIR	Ashrafi-Saiedlou et al. (2025)
<i>Aspergillus flavus</i> (filtrate, extracellular)	UV-Vis, FTIR, TEM	- TEM particle size: 28–33 nm; - FTIR indicated presence of protein / bioactive molecules on NP surface (capping)	Sidkey (2020)

Oe = Oersted; ~ = approximately;    = almost equal to.

UV-Vis spectroscopy provides rapid and non-destructive confirmation of NP synthesis by detecting characteristic surface plasmon resonance (SPR) or ligand-to-metal charge transfer bands, enabling monitoring of precursor reduction, nucleation and NP stability (Nizamov et al., 2022). Typically, green-synthesized IONPs have absorption peaks in the range 240–300 nm. In our preliminary study, IONPs synthesized using *Gardenia jasminoides* (International Plant Names Index; www.ipni.org leaf extract showed a characteristic peak (Fig. 2). Roy et al. (2022) reported a peak at 240 nm for IONPs synthesized using *Catharanthus roseus*, while Shabbir et al. (2023) observed a peak at 310 nm using *Madhuca indica*. Similarly, Şahin et al. (2024) reported a peak at 268 nm for *Prunus avium* and Yekeen et al. (2025) observed a peak at 290 nm using *Egeria densa*, confirming successful IONPs formation.

FTIR spectroscopy identifies functional groups involved in the reduction and stabilization of NPs by analyzing vibrational signatures of phytochemicals. In our study, the FTIR analysis showed a broad peak at 3201 cm^{-1} corresponding to O-H stretching of phenolic and alcoholic compounds, peaks at 1557 cm^{-1} and 1490 cm^{-1} indicating aromatic C=C and amide groups and a peak at 1047 cm^{-1} corresponding to C-O stretching,

confirming phytochemical involvement in NP stabilization. The Fe-O peak at 523 cm^{-1} confirmed IONPs formation (Fig. 3). Kouhbanani et al. (2019) reported Fe-O stretching at 609.5 cm^{-1} , O-H stretching at 2067.5 cm^{-1} and C=C stretching at 1635.5 cm^{-1} in *Teucrium polium*-mediated IONPs. Nahari et al. (2022) observed peaks at 923.96 cm^{-1} and 1709.89 cm^{-1} in plant extract but not in synthesized IONPs, indicating the involvement of these functional groups in NP formation. Additionally, Rauf et al. detected O-H, C-H, C=O and N-H functional groups in *Thevetia peruviana*-mediated IONPs, confirming their role in reduction and stabilization.

XRD provides precise information on crystallinity, phase composition and crystallite size by analyzing the diffraction patterns of atomic planes (Kumar et al., 2022). Nahari et al. (2022) confirmed crystalline IONPs with an average diameter of 136 nm. Ravi et al. (2025) identified characteristic Miller indices confirming NP formation. Nughwal et al. (2025) and Salim et al. (2025) reported amorphous IONPs based on broad diffraction peaks, including IONPs synthesized using *Myristica fragrans*. Similarly, Tyagi et al. (2021) confirmed amorphous and non-crystalline structures in green-synthesized IONPs.

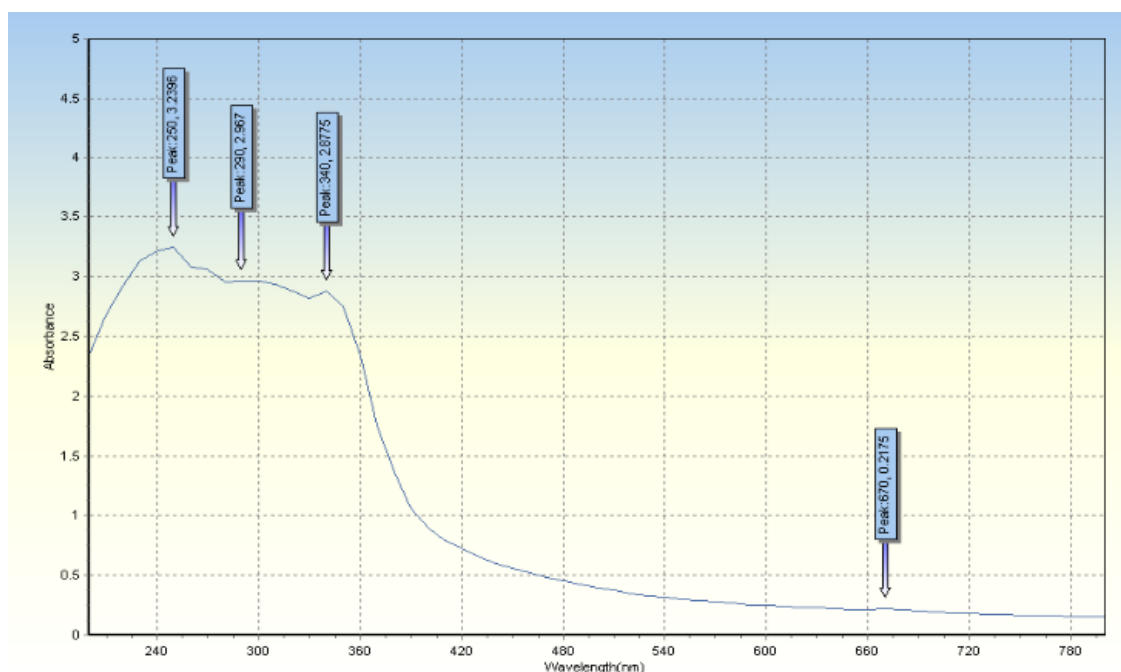


Fig. 2 Ultraviolet-visible spectral report of IONPs synthesized using *Gardenia jasminoides* leaf extract, revealing distinct absorption features indicative of successful NP formation, with spectrum showing prominent peaks at approximately 263 nm, 291 nm and 340 nm, correspond to ligand-to-metal charge transfer transitions typically associated with Fe-O bonding in iron oxide nanostructures.

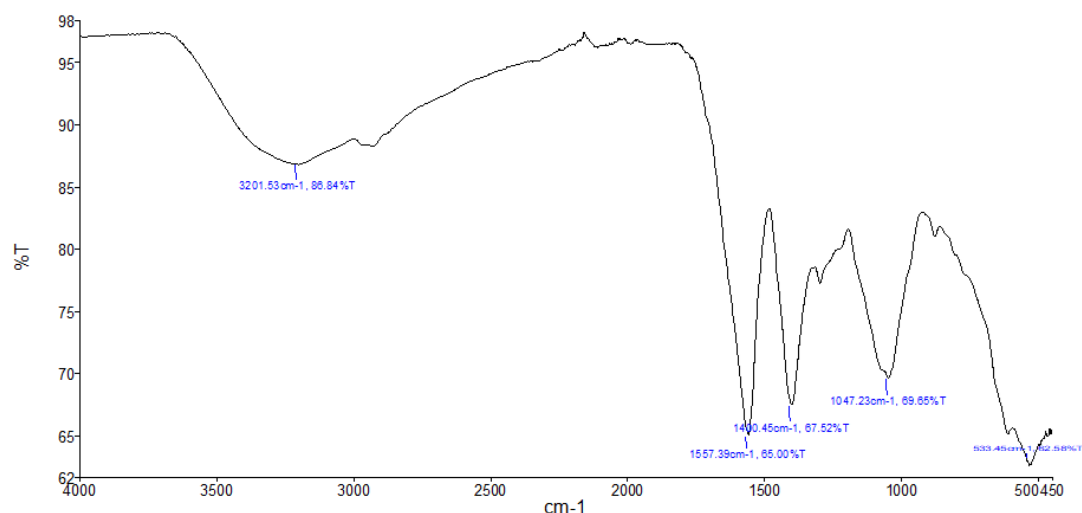


Fig. 3 Fourier-transform infrared spectrum of green-synthesized IONPs, where broad band around 3201 cm^{-1} corresponds to O-H stretching vibrations (indicating phenolic and alcoholic groups commonly found in plant metabolites, likely serving as reducing agents during NP synthesis), with peaks at 1557 cm^{-1} and 1490 cm^{-1} attributable to C=C stretching of aromatic rings and possible N-H bending from amide groups (suggesting the participation of proteins or polyphenolic compounds in capping and stabilizing the NP surface), absorption at 1047 cm^{-1} corresponding to C-O stretching (reflecting presence of polysaccharides or alcohol groups possibly contributing to NP stabilization) and notably, characteristic Fe-O stretching band at 523 cm^{-1} , confirming the successful formation of IONPs.

Electron microscopy techniques, including SEM and TEM, provide detailed visualization of NP morphology, size and structural features (Fig. 4). SEM reveals surface morphology, aggregation and topography, while TEM provides high-resolution internal structural information and precise particle size measurements. Hao et al. (2021) reported particle sizes in the range 50–80 nm using SEM and TEM. Saleem

and Khan (2023) observed approximately 33 nm IONPs synthesized using *Syzygium cumini*. Aldossari et al. (2023) reported spherical IONPs with sizes of 30–70 nm. Mutar et al. (2025) observed irregularly shaped particles averaging 54 nm. Ravi et al. (2025) reported heteromorphic particles in the range 25–55 nm synthesized using *Cinnamomum verum*, with aggregation attributed to magnetic behavior.

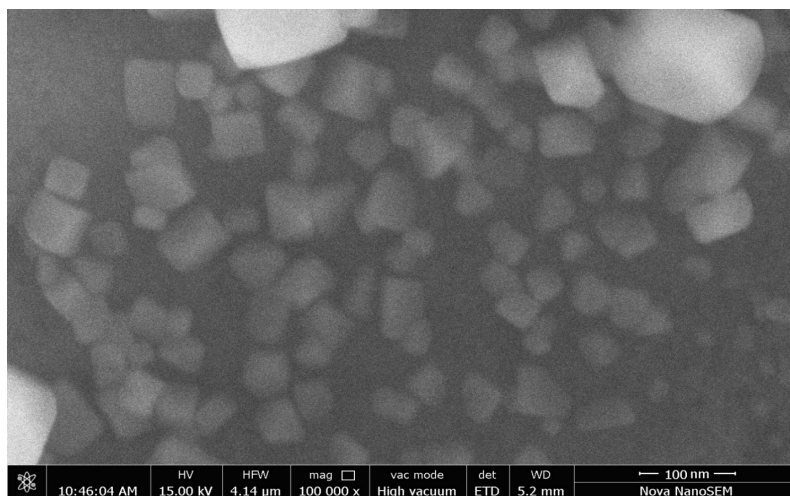


Fig. 4 Scanning electron microscopy image of green-synthesized IONPs, illustrating well-defined, predominantly polygonal and irregularly shaped particles that are uniformly distributed with limited aggregation, indicating effective stabilization by plant-derived phytochemicals, where NP dimensions estimated in range of approximately 40–90 nm, confirming successful formation of nanoscale iron oxide structures, with clear surface features and distinct particle boundaries further validating efficient nucleation and growth during green synthesis, magnification = $100,000\times$ and scale bar = 100 nm.

EDX analysis confirms elemental composition and purity by detecting the characteristic X-ray emissions of constituent elements (Raval et al., 2019). In our study, EDX confirmed the presence of iron-based nanomaterials (Fig. 5). Kiwumulo et al. (2022) detected iron peaks in the range 0.66–7 keV, confirming a high iron content, along with Na, Al and Cl peaks that indicated capping agents. Ekwumengbo et al. (2023) reported atomic percentages of Fe (77%), O (20%) and C (3%). Yekeen et al. (2025) observed iron peaks at 6.5–7 keV and Fe and O proportions of 60% and 40%, respectively.

AFM provides nanoscale surface characterization by measuring topography, roughness and particle morphology, offering insights into surface stability and functional performance (Bellotti et al., 2022). Mohammed et al. (2020)

reported IONPs with diameters of approximately 50–60 nm, consistent with XRD results. García et al. (2023) measured surface roughness of 58 pm and confirmed quasi-spherical morphology. Hasan et al. (2024) observed well-dispersed spherical NPs with good stability.

DLS measures hydrodynamic size, polydispersity index and dispersion stability by analyzing Brownian motion (Jia et al., 2023). In our study, IONPs had a size distribution in the range 90–100 nm (Fig. 6). Roy et al. (2022) reported an average diameter of 290 nm using *Catharanthus roseus*, while Corrêa et al. (2024) reported NP sizes of 419 nm and Ravi et al. (2025) recorded a minimum particle size of 99 nm under optimized conditions. In addition, Nughwal et al. (2025) reported an average diameter of 437 nm for IONPs synthesized using *Argemone mexicana*.

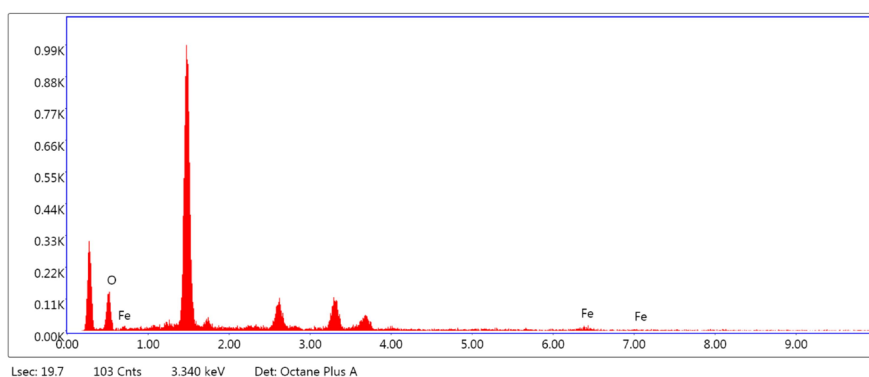


Fig. 5 The EDX analysis graph of IONPs. The spectrum displayed clear signals corresponding to oxygen (O) and iron (Fe), verifying the formation of iron oxide. Quantitative data showed a dominant oxygen content of 97.23 wt% and 99.19 at%, alongside an iron content of 2.77 wt% and 0.81 at%. The high oxygen proportion may be attributed to strong surface oxidation and the presence of phytochemical residues from the plant extract, which often contribute additional oxygen-rich functional groups on the NP surface.

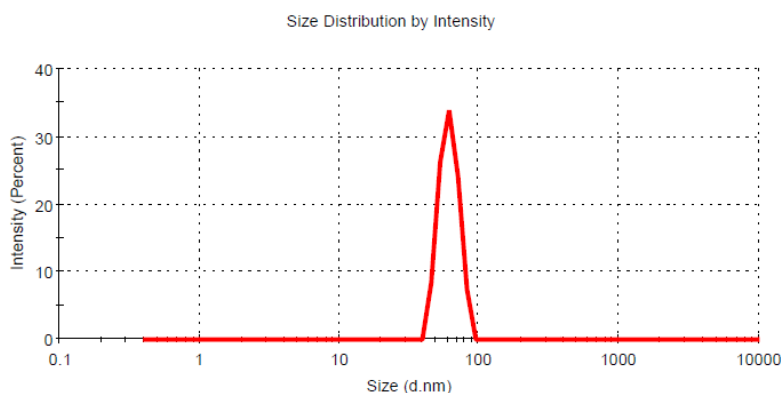


Fig. 6 Size distribution by intensity from DLS report of green-synthesized IONPs, revealing narrow and well-defined size distribution, indicating good colloidal stability, with prominent peak centered around 90–110 nm, confirming synthesized NPs had hydrodynamic diameter within this nanoscale range, peak sharpness suggesting relatively low degree of aggregation and uniform particle population, likely due to effective stabilization by phytochemicals present in plant extract.

Together, these complementary characterization techniques provide comprehensive validation of NP formation, structural integrity, physicochemical properties and stability, ensuring the effectiveness and reliability of green-synthesized IONPs for antimicrobial applications in agriculture.

Bacterial wilt pathogen, *R. solanacearum*: Host range and agricultural importance

R. solanacearum is a soil-borne, Gram-negative bacterium that causes bacterial wilt in major crops such as banana, tomato, potato and eggplant (Wang et al., 2023). It enters through roots, spreads in the xylem and causes rapid wilting, with control complicated by its high genetic and phenotypic diversity, broad host range and ability to survive in soil, water, plant debris and latent infections (Chachar et al., 2025). The pathogen is aerobic, non-spore-forming, highly variable and classified into phylotypes, with an approximately 5.8 Mb genome divided between a chromosome and megaplasmid that enhances adaptability and virulence (Chachar et al., 2025). It is rod-shaped, motile and capable of persisting for years or decades in water often entering a viable but non-culturable (VBNC) state, while virulence relies on T3SS effectors, extracellular polymeric substance (EPS)-mediated vascular blockage, quorum sensing and natural transformation (Hu et al., 2025). Infecting over 450 plant species across approximately 50 families, it causes vascular obstruction, wilting and tissue breakdown through chemotaxis-mediated root entry, rapid xylem colonization and secretion of EPSs and cell wall-degrading enzymes such as cellulases and pectinases (Ababa, 2024).

Bacterial wilt of tomato

Bacterial wilt in tomato appears as sudden wilting without yellowing, followed by vascular browning and bacterial ooze from cut stems, with severe infections leading to stunting, collapse and up to 90% yield loss, especially in tropical and subtropical regions (Seleim et al., 2014). The pathogen spreads via contaminated soil, water, tools and alternate hosts, with race 1 biovar 3 widespread in the USA. Resistance in cultivars, such as Hawaii 7996, may fail under high inoculum or new strains. Metabolomic studies have shown increased putrescine in xylem sap, accelerating wilt (Phiri et al., 2024).

Bacterial wilt of potato

Also called brown rot, the disease causes stunting, leaf wilting and brown vascular rings in stems and tubers, often with bacterial ooze that reduce yield and tuber quality, triggering export restrictions and major economic losses ((Khairy et al., 2021)). Spread occurs through infected seed tubers, irrigation water and equipment, with the pathogen surviving long-term in moist soils. Race 3 biovar 2 is especially virulent in highland tropics and guidelines stress avoiding surface water to limit outbreaks (Khairy et al., 2021).

Bacterial wilt of eggplant

In eggplant, the disease causes gradual wilting, chlorosis and vascular browning, sometimes affecting only one side of the plant due to uneven colonization (Reference). Bacterial ooze may appear in advanced stages and spread through contaminated soil, water or root wounds is common (Bamaniya et al., 2025). Crop rotation is ineffective when alternate hosts exist and diagnosis requires streaming tests or molecular assays (Bamaniya et al., 2025).

Moko disease of banana

Moko disease causes leaf wilting, yellowing, internal pseudostem discoloration and fruit rot, with bacterial ooze visible from cut tissues, which can result in entire plantations being devastated, with losses reaching 100% in unmanaged cases (Blomme et al., 2017). The pathogen spreads via insects, tools and infected planting material, with race 2 strains causing severe outbreaks in Cavendish bananas across Asia and Latin America (Blomme et al., 2017).

Challenges in management of bacterial wilt using conventional approaches

The *R. solanacearum* species complex has extensive genetic diversity, with multiple races, biovars and four phylotypes, driven by a 5.8 Mb genome with high recombination rates, making it challenging to develop universally effective resistant cultivars or chemical controls (Ghorai et al., 2022). For example, resistant tomato varieties, such as Hawaii 7996, often fail against novel strains, such as race 3 biovar 2 in cooler climates, due to strain-specific virulence (Siddique et al., 2024). In contrast, green-synthesized IONPs offer broad-spectrum antibacterial activity by targeting multiple

cellular processes achieving up to 80% inhibition of diverse *R. solanacearum* strains *in vitro* (Alam et al., 2019).

Conventional biocontrol agents, such as *Pseudomonas fluorescens* and chemical pesticides, often exhibit inconsistent efficacy in field conditions due to soil microbial competition, abiotic stressors and the pathogen's biofilm formation within xylem vessels (Müller and Behrendt et al., 2021). Biofilms, composed of EPSs, shield *R. solanacearum* from antibiotics and biocontrols, while chemical pesticides contribute to environmental pollution and resistance development (Heredia-Ponce et al., 2021). NPs outperform these methods by disrupting biofilms through quorum sensing inhibition and physical penetration of EPS matrices, reducing biofilm formation (Sahli et al., 2022). Furthermore, green-synthesized IONPs, derived from eco-friendly sources, minimize environmental harm and exhibit sustained antibacterial activity, unlike chemical treatments that degrade rapidly or harm soil ecosystems (Suppiah et al., 2023).

Traditional diagnostic methods, such as culturing or PCR-based assays, are slow, costly and lack field portability, delaying detection of *R. solanacearum* in asymptomatic plants, soil or water, which allows undetected spread via contaminated irrigation or tools. Techniques, such as loop-mediated isothermal amplification, are promising but limited by cost and expertise requirements (Dittapongpich and Surat, 2003). Nanotechnology-based solutions integrated with magnetic or optical nanosensors, enable rapid, sensitive and field-deployable detection of pathogens, with Narware et al. (2025) demonstrating real-time monitoring capabilities that reduced the outbreak response many times compared to conventional assays. This precision enhances early intervention, providing a critical advantage over traditional methods.

R. solanacearum's ability to form biofilms and enter a VBNC state enables it to evade conventional treatments and persist in soil or asymptomatic hosts for years, rendering cultural practices such as crop rotation ineffective (Grey and Steck, 2001). IONPs address these challenges by disrupting biofilm integrity and inhibiting quorum sensing (Algadi et al., 2024).

Conventional methods struggle to translate laboratory successes to field conditions due to variability in soil types, climate and pathogen strains, with resistant cultivars and biocontrols often failing under high inoculum pressure. IONPs demonstrate superior field performance, reducing wilt severity by 65–85% in crops, such as tomatoes, through targeted antimicrobial action and plant defense induction (Ashraf et al., 2022). Their controlled-release formulations

and biodegradability minimize environmental degradation, offering a practical and sustainable alternative to conventional approaches that require frequent reapplication.

Antibacterial mechanisms of IONPs against R. solanacearum and impact on plant growth

IONPs exhibit potent antibacterial effects through multiple mechanisms, making them highly effective against Gram-negative bacteria such as *R. solanacearum*. These mechanisms target critical bacterial structures and processes, disrupting their survival and proliferation (Fig. 7). Primarily, IONPs act by disrupting bacterial membranes through electrostatic interactions, causing destabilization, leakage of intracellular contents and cell lysis (Shoudho et al., 2024). Additionally, these NPs stimulate the generation of ROS, such as hydroxyl radicals, which induce oxidative stress, damaging bacterial DNA, proteins and lipids, thereby impairing essential cellular functions (Mammari et al., 2022). Another key mechanism is the release of Fe^{3+} ions from IONPs in aqueous environments,

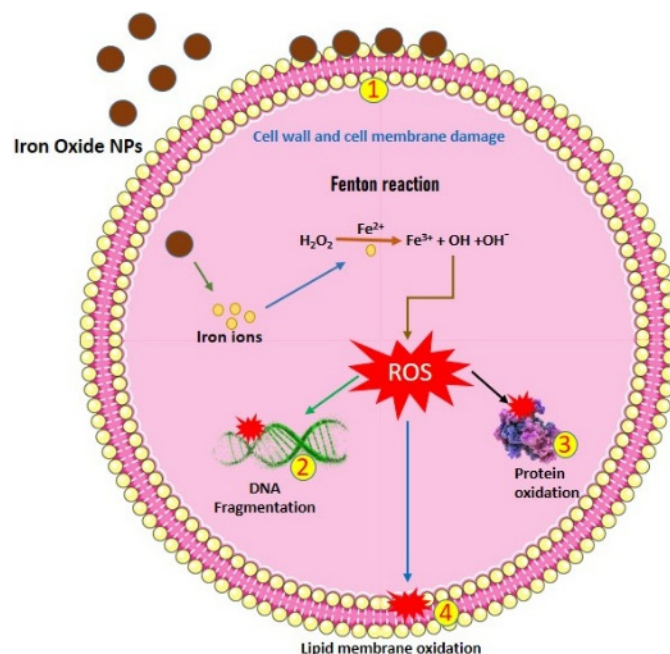


Fig. 7 Schematic representation of antimicrobial mechanism of IONPs, represented by brown spheres interacting with the microbial cell membrane, causing (1) cell wall and membrane damage that allows internalization and release of iron ions (Fe^{2+}). Inside the cell, Fe^{2+} catalyzes the Fenton reaction with H_2O_2 , generating highly reactive hydroxyl radicals ($\cdot\text{OH}$) and other reactive oxygen species (ROS). Then, ROS accumulation leads to multiple cellular damage, including (2) DNA fragmentation, (3) protein oxidation and (4) lipid membrane oxidation, ultimately resulting in cell death. This illustrates the oxidative stress-mediated toxicity pathway of IONPs against microbial cells.

which interfere with bacterial metabolism and enzyme activity by disrupting critical biochemical pathways, further contributing to bacterial death. NPs play a crucial role in inhibiting biofilm formation, as biofilms shield bacteria from antibiotics and host defenses (Godoy-Gallardo et al., 2021). Collectively, these multifaceted antibacterial actions (membrane disruption, ROS generation, ion release, biofilm inhibition) make IONPs a promising tool for combating bacterial wilt caused by *R. solanacearum*, offering potential applications in agriculture and disease management (Alam et al., 2019). However, most of the reported research has only evaluated the efficacy of IONPs under *in vitro* or controlled conditions. Alam et al. (2019) reported that IONPs showed strong antibacterial activity, with 6 mg/mL completely inhibiting bacterial growth *in vitro* and causing severe structural damage to bacterial cells and when applied to pot-grown tomato plants, a 6% w/v root-zone treatment significantly reduced disease severity and improved plant growth, including shoot length, root development and biomass. El-Lakkany et al. (2025) developed IONPs and AgNPs. Both NPs showed strong antibacterial activity against *R. solanacearum* in laboratory and greenhouse tests, with the AgNPs and IONPs reducing disease severity in tomatoes by 67% and 27%, respectively and improving plant growth and chlorophyll content. Furthermore, their TEM analysis confirmed that NPs damaged bacterial cell membranes. Naik et al. (2025) reported that IONPs derived from fungal extracts significantly suppressed *R. solanacearum* by inducing the generation of ROS, which compromised bacterial cell membrane integrity. Specifically, IONPs significantly improved the growth of tomato plants under controlled conditions, enhancing biomass, chlorophyll levels and antioxidant activity when applied at 100 parts per million. Furthermore, disease incidence dropped sharply to 13.09% compared to 98.01% in untreated infected plants. In addition, soil health improved, with increased enzyme activity, nutrient availability and iron content.

One of the most important mechanisms of IONPs is their ability to generate ROS that induce oxidative stress, which damages bacterial DNA, proteins and lipids, impairing critical cellular functions (Singh et al., 2025b). Pereira et al. (2012) reported that IONPs catalyzed the Fenton reaction, where $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions facilitated the production of highly reactive hydroxyl radicals in the presence of hydrogen peroxide. These ions can disrupt bacterial metabolism by interfering with enzyme activity and essential biochemical pathways. For example, Fe^{3+} ions can bind to bacterial enzymes, altering their active sites and inhibiting processes such as respiration

and nutrient uptake (Sun et al., 2011). More recent research has demonstrated that the rate of ion release is influenced by environmental factors, such as pH and temperature, with acidic conditions accelerating ion dissolution and enhancing antibacterial effects (Ezealigo et al., 2021).

Biofilms are complex bacterial communities encased in extracellular polymeric substances, which confer resistance to antibiotics and host immune responses. Recent studies have indicated that NPs disrupt quorum sensing, a communication mechanism that regulates biofilm development, by interfering with signaling molecules like acyl-homoserine lactones (AHLs) (Juszczuk-Kubiak et al., 2024). Additionally, NPs physically penetrate the EPS matrix, weakening its structural integrity and exposing embedded bacteria to external stressors (Guo et al., 2023). Surface-functionalized NPs with antimicrobial peptides have been shown to enhance biofilm disruption, offering a synergistic approach to combat bacterial resistance (Campos et al., 2025).

Challenges and future prospects

Although green-synthesized IONPs have shown strong potential against *R. solanacearum*, their agricultural application still faces several challenges, including variability in their physicochemical properties, limited scalability, environmental and safety concerns, weak regulatory frameworks and strain-specific pathogen responses. Addressing these barriers requires standardized synthesis methods, comprehensive toxicity assessments, clear application guidelines and their integration into broader disease-management strategies.

From an agronomic perspective, the timing and purpose of IONP application are critical. Current evidence indicates that IONPs can function both as a preventive and curative tool, but with differing efficiencies. When applied preventively, such as through soil drenching or seed/seedling treatments before the onset of infection, IONPs can reduce pathogen establishment in the rhizosphere, strengthen plant physiological responses and prime defense mechanisms. Preventive application is beneficial, especially in fields with a known history of bacterial wilt. As a treatment, IONPs can suppress ongoing infections by damaging bacterial cells, reducing pathogen load and improving plant vigor; however, their curative effect may be less consistent than preventive use, particularly in severely infected plants. Therefore, an integrated application strategy is recommended for maximum effectiveness, combining early-season preventive use with targeted treatment during disease onset (Akhter et al., 2025).

Additionally, the cost of IONPs influences their practicality in large-scale agriculture. While green synthesis using plant extracts is generally more affordable than chemical or physical methods, production costs remain higher than conventional pesticides due to purification, stabilization and characterization requirements. However, the cost of IONPs is expected to decline as synthesis protocols become more standardized and scalable and industrial production expands. Ultimately, initial production expenses may be offset by long-term economic benefits such as reduced yield losses, lower pesticide dependency and improved soil health (Osman et al., 2024).

The environmental impacts of residual IONPs in agricultural soils require careful evaluation. Often, although green-synthesized NPs are considered safer due to their biocompatible coatings, IONP residues may still alter soil microbial communities, affect nutrient cycling or accumulate in plant tissues (Kamran et al., 2020). Fortunately, iron-based NPs have a natural advantage as they can gradually oxidize into iron ions that integrate into the soil nutrient pool, potentially enriching iron availability for crops. Nevertheless, high or repeated doses may pose risks such as NP aggregation, changes in soil enzyme activity or unintended toxicity to beneficial organisms. Long-term field studies and ecotoxicological assessments are needed to determine safe concentration thresholds and degradation behavior under real agricultural conditions (Shirsat and K, 2024).

Despite these challenges, the prospects are highly promising for green-synthesized IONPs in plant disease management. Advances in nanotechnology may lead to multifunctional IONPs engineered for controlled antimicrobial release or smart delivery systems, such as magnetic targeting, to precisely affect infected sites while minimizing off-target effects. Hybrid synthesis approaches combining plant extracts with microbial enzymes or functional nanomaterials, such as graphene, may produce NPs with enhanced stability, biocompatibility and antibacterial potency. Furthermore, machine learning and AI-driven optimization could refine synthesis parameters, enabling consistent and high-quality production on a commercial scale (Hanna et al., 2025).

Eventually, in agriculture, IONPs may become integrated into precision-farming platforms, serving as nanosensors for real-time pathogen detection or as nano-fertilizers that simultaneously provide nutrients and combat disease, supporting sustainable practices in the face of climate change. Continued investigation of molecular interactions between IONPs and *R. solanacearum* will likely reveal new antibacterial pathways and inform the development of formulations that

are more effective in inducing plant resistance or disrupting pathogen biofilms (Yadav et al., 2023).

Ultimately, collaborative efforts among researchers, industry partners and policymakers are essential to establish safety guidelines, conduct multi-location field trials and accelerate the commercialization of these eco-friendly solutions. With proper regulation and optimized use, IONPs hold considerable promise for reducing reliance on chemical pesticides, enhancing plant resilience and contributing to global food security.

Conclusion

The green synthesis of IONPs represents a transformative approach in nanotechnology for agriculture, offering eco-friendly and biocompatible alternatives to traditional chemical methods for synthesizing nanomaterials with potent antibacterial properties against devastating pathogens such as *R. solanacearum*. There are diverse biogenic sources, mechanisms of synthesis, characterization techniques and applications of IONPs in plant disease management, demonstrating their efficacy in disrupting bacterial membranes, generating ROS, inhibiting biofilms and enhancing crop yields, while promoting soil health and sustainability. By leveraging natural reducing agents from plants and microbes, green synthesis minimizes environmental hazards and aligns with the principles of circular economy, addressing key challenges in modern farming such as nutrient deficiencies and pathogen resistance. Although there are still limitations, such as the variability in NP properties, along with regulatory hurdles, the integration of IONPs into integrated disease management strategies holds immense promise for reducing economic losses from bacterial wilt and supporting resilient agricultural systems. Future progress will require interdisciplinary research to standardize synthesis protocols, conduct rigorous toxicity and environmental impact assessments and develop field-ready formulations. With such advancements, green-synthesized IONPs could become integral components of integrated disease management strategies, reducing economic losses from bacterial wilt and contributing significantly to resilient, sustainable and environment-responsible agricultural systems.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgement

Professor Jitender Singh, Coordinator of Department of Biotechnology, Chaudhary Charan Singh University, Meerut, UP, India provided essential support.

References

- Ababa, G. 2024. Pathogenic diversity, ecology, epidemiology and management practices of the potato bacterial wilt (*Ralstonia solanacearum*) disease. *Cogent Food Agric.* 10: 2407953.
- Abdulfatah, H.F., Abdulrahman, M.F., Naji, E.F. 2025. Green synthesis of iron nanoparticles to promote seed germination of *Zea mays* under salinity condition. *Heliyon* 11: e41823.
- Abuzeid, H.M., Julien, C.M., Zhu, L., Hashem, A.M. 2023. Green synthesis of nanoparticles and their energy storage, environmental and biomedical applications. *Crystals* 13: 1576.
- Ahmad, T., Phul, R., Khatoon, N., Sardar, M. 2017. Antibacterial efficacy of *Ocimum sanctum* leaf extract-treated iron oxide nanoparticles. *New J. Chem.* 41: 20552061.
- Ahmed, T., Noman, M., Jiang, H., et al. 2022. Bioengineered chitosan-iron nanocomposite controls bacterial leaf blight disease by modulating plant defense response and nutritional status of rice (*Oryza sativa* L.). *Nano Today* 45: 101547.
- Akhter, T., Rather, R.A., Mishra, N.S., John, R. 2025. nanoparticles in plant systems: Omics-based perspectives on stress adaptation and toxicological implications. *Plant Nano Biol.* 13: 100181.
- Alam, T., Jalil, S., Jilani, G., et al. 2025. Enhancing crop resilience to water stress through iron nanoparticles: A critical review of applications and implications. *Plant Stress* 16: 100905.
- Alam, T., Khan, R.A.A., Ali, A., Sher, H., Ullah, Z., Ali, M. 2019. Biogenic synthesis of iron oxide nanoparticles via *Skimmia laureola* and their antibacterial efficacy against bacterial wilt pathogen *Ralstonia solanacearum*. *Mater. Sci. Eng. C* 98: 101–108.
- Aldossari, S.M., Rehman, L.U., Ahmad, I., et al. 2023. Phytosynthesized iron oxide nanoparticles using aqueous extract of *Saccharum arundinaceum*, their characterizations, antiglycation and cytotoxic activities. *ACS Omega* 8: 41214–41222.
- Algadi, H., Alhoot, M.A., Al-Maleki, A.R., Purwitasari, N. 2024. Effects of metal and metal oxide NPs against biofilm-forming bacteria: A systematic review. *J. Microbiol. Biotechnol.* 34: 1748.
- Al-Hamdani, H., Ahmed, S.H., Al-Newani, H.R.H., Salem, K.F. 2024. Effect of iron nanoparticles prepared from onion extract on cucumber plant growth and production. *South Asian Res. J. Bio. Appl. Biosci.* 6: 159–168.
- Ali, M.H., Azad, M.A.K., Khan, K.A., Rahman, M.O., Chakma, U., Kumer, A. 2023. Analysis of crystallographic structures and properties of silver nanoparticles synthesized using PKL extract. *ACS Omega* 8: 28133–28142.
- Anwaar, S., Ijaz, D.E.S., Anwar, T., Qureshi, H., Nazish, M., Alrefaei, A.F., Almutairi, M.H., Alharbi, S.N. 2024. Boosting *Solanum tuberosum* resistance to *Alternaria solani* through green synthesized ferric oxide NPs. *Sci. Rep.* 14: 2375.
- Ashraf, H., Anjum, T., Riaz, S., Batool, T., Naseem, S., Li, G. 2022a. Sustainable synthesis of microwave-assisted IONPs using *Spinacia oleracea* L. for control of fungal wilt. *J. Nanobiotechnol.* 20: 8.
- Ashraf, H., Batool, T., Anjum, T., Illyas, A., Li, G., Naseem, S., Riaz, S. 2022b. Antifungal potential of green synthesized magnetite. *Front. Microbiol.* 13: 754292.
- Ashrafi-Saiedlou, S., Rasouli-Sadaghiani, M., Fattahi, M., Ghosta, Y. 2025. Biosynthesis and characterization of iron oxide nanoparticles fabricated using cell-free supernatant of *Pseudomonas fluorescens*. *Sci. Rep.* 15: 1018.
- Attea, S.A., Ghareeb, M.A., Kelany, A.K., Elhakim, H.K., Allemailem, K.S., Bukhari, S.I., Rashidi, F.B., Hamed, A.A. 2024. Biosynthesis of iron oxide nanoparticles by marine *Streptomyces* sp. *Molecules* 29: 4784.
- Ayub, A., Wani, A.K., Malik, S.M., Ayub, M., Singh, R., Chopra, C., Malik, T. 2025. Green nanoscience for healthcare. *Biotechnol. Rep.* e00913.
- Bamaniya, B.S., Das, T., Sherpa, T.L., Tare, K., Rana, M., Bag, N. 2025. Bacterial wilt caused by *Ralstonia solanacearum*. *Natl. Acad. Sci. Lett.* 48: 213–216.
- Belhedi, M., Sghaier-Hammami, B., Masiello, M., et al. 2025. Silicon dioxide nanoparticles affect morphology and pathogenicity of *Fusarium brachygibbosum*. *Front. Nanotechnol.* 7: 1569453.
- Bellotti, R., Picotto, G.B., Ribotta, L. 2022. AFM measurements and tip characterization of nanoparticles. *Nanomanuf. Metrol.* 5: 127–138.
- Blomme, G., Dita, M., Jacobsen, K.S., Pérez Vicente, L., Molina, A., Ocimati, W., Poussier, S., Prior, P. 2017. Bacterial diseases of bananas and enset. *Front. Plant Sci.* 8: 1290.
- Cabr , M., Fern ndez, G., Gonz lez, E., Abell , J., Verdaguer, A. 2024. Single particle ICP-MS characterization of gold nanoparticles. *J. Anal. At. Spectrom.* 39: 2508–2513.
- Campos, J.V., Pontes, J.T.C., Canales, C.S.C., Roque-Borda, C.A., Pavan, F.R. 2025. Targeting biofilm-forming bacteria with antimicrobial peptides. *BME Front.* 6: 0104.
- Cardoso, B., Nobrega, G., Afonso, I.S., Ribeiro, J.E., Lima, R.A. 2025. Sustainable green synthesis of metallic nanoparticles. *J. Environ. Chem. Eng.* 13: 116921.
- Chachar, Z., Xue, X., Fang, J., et al. 2025. Key mechanisms of plant-*Ralstonia solanacearum* interaction in bacterial wilt pathogenesis. *Front. Microbiol.* 16: 1521422.
- Chand, K., Vasquez-Guardado, E.S. 2025. Magnetic nanoparticles: synthesis and applications in life sciences. *ChemistryOpen* 2500214.
- Corr a, C.R.R., de Siqueira, A.B., Matos Lopes, P.R., Ambrosio, J.A.R., Simioni, A.R., de Vasconcelos, L.G., de Moraes, E.B. 2024. Green synthesis of iron nanoparticles from the baru (*Dipteryx alata*) endocarp extract for the efficient removal of rhodamine B and caffeine from water through the heterogeneous Fenton process. *AQUA* 73: 771–789.
- Devi, H.S., Boda, M.A., Shah, M.A., Parveen, S., Wani, A.H. 2019. Green synthesis of iron oxide nanoparticles using *Platanus orientalis* leaf extract for antifungal activity. *Green Process. Synth.* 8: 38–45.
- Dhanapal, A.R., Thiruvengadam, M., Vairavanathan, J., Venkidasamy, B., Easwaran, M., Ghorbanpour, M. 2024. Nanotechnology approaches for the remediation of agricultural polluted soils. *ACS Omega* 9: 13522–13533.
- Dittapongpitch, V., Surat, S. 2003. Detection of *Ralstonia solanacearum* in soil and weeds from commercial tomato fields using immunocapture and the polymerase chain reaction. *J. Phytopathol.* 151: 239–246.

- Ebrahiminezhad, A., Zare-Hoseinabadi, A., Sarmah, A.K., Taghizadeh, S., Ghasemi, Y., Berenjian, A. 2018. Plant-mediated synthesis and applications of iron nanoparticles. *Mol. Biotechnol.* 60: 154–168.
- Edo, G.I., Mafe, A.N., Ali, A.B., et al. 2025. Eco-friendly nanoparticles phytosynthesis via plant extracts: mechanistic insights, recent advances and multifaceted uses. *Nano TransMed* 100080.
- Ekwumemgbo, P.A., Shallangwa, G.A., Okon, I.E., Awodi, I. 2023. Green synthesis and characterization of iron oxide nanoparticles using *Prosopis africana* leaf extract. *Comm. Phys. Sci.* 9: 125–136.
- El-Lakkany, A.A., Balabel, N.M., Rageh, M.M., Hanafy, M.S., Nasr, N.F., Moselhy, M.A. 2025. Antibacterial efficacy of iron oxide and silver nanoparticles against bacterial wilt pathogen *Ralstonia solanacearum*. *Sci. Rep.* 15: 32666.
- El-Mohamedya, R.S.R., Abd El-Aziz, M.E., Kamel, S. 2019. Antifungal activity of chitosan nanoparticles against some plant pathogenic fungi in vitro. *Agric. Eng. Int. CIGR J.* 21: 201–209.
- El-Sheekh, M.M., Morsi, H.H., Hassan, L.H., Ali, S.S. 2022. The efficient role of algae as green factories for nanotechnology and their vital applications. *Microbiol. Res.* 263: 127111.
- Ezealigo, U.S., Ezealigo, B.N., Aisida, S.O., Ezema, F.I. 2021. Iron oxide nanoparticles in biological systems: antibacterial and toxicology perspective. *JCIS Open* 4: 100027.
- Fatih, H.J., Ashengroph, M., Sharifi, A., Zorab, M.M. 2024. Green-synthesized α -Fe₂O₃-nanoparticles as potent antibacterial, anti-biofilm and anti-virulence agent against pathogenic bacteria. *BMC Microbiol.* 24: 535.
- Fatima, U., Shoaib, A., Fatima, Q., Alsahli, A.A., Ahmad, P. 2025. Zinc-chitosan nanocomposites as guardians against the dreaded phytopathogenic fungus *Macrophomina phaseolina* in *Vigna radiata* L. *Plant Stress* 15: 100710.
- Flores-Contreras, E.A., Araújo, R.G., Rodríguez-Aguayo, A.A., et al. 2023. Polysaccharides from the Sargassum and brown algae genus: extraction, purification and their potential therapeutic applications. *Plants* 12: 2445.
- Fu, Y., Wan, Q., Qin, Z., Li, S., Li, S., Wang, J., Zhang, W. 2021. Concentration determination of gold nanoparticles by flame atomic absorption spectrophotometry. *Acta Geochim.* 40: 498–506.
- Gaminda, K.A.P., Thomas, I.B.K., Lakmauri, P., Abeyasinghe, T., Jayasinghe, C., Senthilnithy, R. 2024. Green synthesis of iron nanoparticles using *Syzygium aromaticum* extracts and their applications: nitrate removal, malachite green degradation and antibacterial activity. *Environ. Nanotechnol. Monit. Manag.* 21: 100925.
- García, D.G., Garzón-Romero, C., Salazar, M.A., Lagos, K.J., Campaña, K.O., Debut, A., Vizuete, K., Rivera, M.R., Niebieskikwiat, D., Benitez, M.J., Romero, M.P. 2023. Bioinspired synthesis of magnetic nanoparticles based on iron oxides using orange waste and their application as photo-activated antibacterial agents. *Int. J. Mol. Sci.* 24: 4770.
- Ghorai, A.K., Dutta, S., Roy Barman, A. 2022. Genetic diversity of *Ralstonia solanacearum* causing vascular bacterial wilt under different agro-climatic regions of West Bengal, India. *PLoS One* 17: e0274780.
- Godoy-Gallardo, M., Eckhard, U., Delgado, L.M., de Roo Puente, Y.J., Hoyos-Nogués, M., Gil, F.J., Perez, R.A. 2021. Antibacterial approaches in tissue engineering using metal ions and nanoparticles: from mechanisms to applications. *Bioact. Mater.* 6: 4470–4490.
- Grey, B.E., Steck, T.R. 2001. The viable but nonculturable state of *Ralstonia solanacearum* may be involved in long-term survival and plant infection. *Appl. Environ. Microbiol.* 67: 3866–3872.
- Guilger-Casagrande, M., Bilesky-José, N., Sousa, B.T., Oliveira, H.C., Fraceto, L.F., Lima, R. 2022. Effects of biogenic silver and iron nanoparticles on soybean seedlings (*Glycine max*). *BMC Plant Biol.* 22: 255.
- Gulati, K., Adachi, T. 2023. Profiling to probing: atomic force microscopy to characterize nano-engineered implants. *Acta Biomater.* 170: 15–38.
- Guo, Y., Mao, Z., Ran, F., Sun, J., Zhang, J., Chai, G., Wang, J. 2023. Nanotechnology-based drug delivery systems to control bacterial-biofilm-associated lung infections. *Pharmaceutics* 15: 2582.
- Gupta, A., Saharan, B.S. 2026. Nanotech innovations in Agriculture: Enhancing plant growth and Navigating microbial interactions. *Next Bioeng* 2: 100012.
- Haider, F.U., Zulfiqar, U., ul Ain, N., Hussain, S., Maqsood, M.F., Ejaz, M., Yong, J.W.H., Li, Y. 2024. Harnessing plant extracts for eco-friendly synthesis of iron nanoparticles (Fe-NPs): characterization and their potential applications for ameliorating environmental pollutants. *Ecotoxicol. Environ. Saf.* 281: 116620.
- Hanna, D.H., Nady, D.S., Wasef, M.W., Fakhry, M.H., Mohamed, F.S., Isaac, D.M., Kirolos, M.M., Azmy, M.S., Hakeem, G.E., Fathy, C.A. 2025. Plant-derived nanoparticles: green synthesis, factors and bioactivities. *Next Mater.* 9: 101275.
- Hao, R., Li, D., Zhang, J., Jiao, T. 2021. Green synthesis of iron nanoparticles using green tea and its removal of hexavalent chromium. *Nanomaterials* 11: 650.
- Hasan, E., Manzar, Z., Haroon, N., Raza, A., Ali, S.N., Lateef, M., Begum, S. 2024. A facile synthesis of iron oxide nanoparticles as a nano-sensor to detect levofloxacin and ciprofloxacin in human blood and evaluation of their biological activities. *RSC Adv.* 14: 36093–36100.
- Heredia-Ponce, Z., de Vicente, A., Cazorla, F.M., Gutiérrez-Barranquero, J.A. 2021. Beyond the wall: exopolysaccharides in the biofilm lifestyle of pathogenic and beneficial plant-associated *Pseudomonas*. *Microorganisms* 9: 445.
- Hossain, N., Mobarak, M.H., Mimona, M.A., Islam, M.A., Hossain, A., Zohura, F.T., Chowdhury, M.A. 2023. Advances and significances of nanoparticles in semiconductor applications—a review. *Results Eng.* 19: 101347.
- Hosseinzadeh, E., Foroumadi, A., Firoozpour, L. 2023. What is the role of phytochemical compounds as capping agents for the inhibition of aggregation in the green synthesis of metal oxide nanoparticles? a DFT molecular level response. *Inorg. Chem. Commun.* 147: 110243.
- Hu, X., Cai, W., Zhang, L., Zhu, Z., Okita, T.W., Tian, L. 2025. Molecular dialog of *Ralstonia solanacearum* and plant hosts with highlights on type III effectors. *Int. J. Mol. Sci.* 26: 3686.
- Iravani, S. 2014. Bacteria in nanoparticles synthesis: current status and future prospects. *Int. Sch. Res. Notices* 2014: 1–18.
- Izuafa, A., Chimbekujwo, K.I., Raji, R.O., Oyewole, O.A., Oyewale, R.O., Abioye, O.P. 2025. Application of nanoparticles for targeted management of pests, pathogens and disease of plants. *Plant Nano Biol.* 100177.
- Jamzad, M., Kamari Bidkorpeh, M. 2020. Green synthesis of iron oxide nanoparticles by the aqueous extract of *Laurus nobilis* L. leaves and evaluation of the antimicrobial activity. *J. Nanostruct. Chem.* 10: 193–201.
- Jia, Z., Li, J., Gao, L., Yang, D., Kanaev, A. 2023. Dynamic light scattering: a powerful tool for in situ nanoparticles sizing. *Colloids Interfaces* 7: 15.

- Juszczuk-Kubiak, E. 2024. Molecular aspects of the functioning of pathogenic bacteria biofilm based on quorum sensing (QS) signal-response system and innovative non-antibiotic strategies for their elimination. *Int. J. Mol. Sci.* 25: 2655.
- Kamal, A., Saba, M., Kamal, A., Batool, M., Asif, M., Al-Mohaimeed, A.M., Al Farraj, D.A., Habib, D., Ahmad, S. 2023. Bioinspired green synthesis of bimetallic iron and zinc oxide nanoparticles using mushroom extract and use against *Aspergillus niger*; the most devastating fungi of the green world. *Catalysts* 13: 400.
- Kamran, M., Ali, H., Saeed, M.F., Bakhat, H.F., Hassan, Z., Tahir, M., Abbas, G., Naeem, M.A., Rashid, M.I., Shah, G.M. 2020. Unraveling the toxic effects of iron oxide nanoparticles on nitrogen cycling through manure-soil-plant continuum. *Ecotoxicol. Environ. Saf.* 205: 111099.
- Kathiresan, K., Durairaj, S. 2025. Green synthesis of iron oxide nanoparticles using date seed extracts for biomedical applications. *Int. J. Environ. Sci. Technol.* 1–22.
- Khairy, A.M., Tohamy, M.R., Zayed, M.A., Ali, M.A. 2021. Detecting pathogenic bacterial wilt disease of potato using biochemical markers and evaluate resistant in some cultivars. *Saudi J. Biol. Sci.* 28: 5193–5203.
- Khan, S., Nawaz, A., Shah, M.K.A., Latif, M.S., Haroon, M., Khan, A., Elsayed, T.M.A. 2025a. Formulation and characterization of isavuconazole-loaded chitosan/sodium alginate nanoparticles for dermal delivery: in vitro and in vivo evaluation for enhanced antifungal therapy. *J. Pharm. Innov.* 20: 156.
- Khan, H., Piccolella, S., Pacifico, S. 2025b. Harnessing plant extracts for green NP synthesis: toward a sustainable future. *Mater. Today Sustain.* 101195.
- Khan, S., Akhtar, N., Rehman, S.U., Shujah, S., Rha, E.S., Jamil, M. 2022. *Bacillus subtilis* synthesized iron oxide nanoparticles (Fe₃O₄ NPs) induced metabolic and anti-oxidative response in rice (*Oryza sativa* L.) under arsenic stress. *Toxics* 10: 618.
- Khoirotin, Faaizatunnisa, N., Munasir. 2023. Green synthesis of Fe₃O₄ nanoparticles using green betel leaf extract for methylene blue adsorption. *Nat. Life Sci. Commun.* 22: e2023042.
- Kiwumulo, H.F., Muwonge, H., Ibingira, C., Lubwama, M., Kirabira, J.B., Ssekitoileko, R.T. 2022. Green synthesis and characterization of iron-oxide nanoparticles using *Moringa oleifera*: a potential protocol for use in low and middle income countries. *BMC Res. Notes* 15: 149.
- Koleva, L., Umar, A., Yasin, N.A., Shah, A.A., Siddiqui, M.H., Alamri, S., Riaz, L., Raza, A., Javed, T., Shabbir, Z. 2022. Iron oxide and silicon nanoparticles modulate mineral nutrient homeostasis and metabolism in cadmium-stressed *Phaseolus vulgaris*. *Front. Plant Sci.* 13: 806781.
- Kouhbanani, M.A.J., Beheshtkhoo, N., Taghizadeh, S., Amani, A.M., Alimardani, V. 2019. One-step green synthesis and characterization of iron oxide nanoparticles using aqueous leaf extract of *Teucrium polium* and their catalytic application in dye degradation. *Adv. Nat. Sci. Nanosci. Nanotechnol.* 10: 015007.
- Kumar, M., Ranjan, R., Dandapat, S., Srivastava, R., Sinha, M.P. 2022. XRD analysis for characterization of green nanoparticles: a mini review. *Glob. J. Pharm. Pharm. Sci.* 10: 555779.
- Kun, S.O.N.G., Wei, D.I.N.G. 2022. Nonphytotoxic copper oxide nanoparticles are powerful “nanoweapons” that trigger resistance in tobacco against the soil-borne fungal pathogen *Phytophthora nicotianae*. *J. Integr. Agric.* 21: 3245–3262.
- Li, G., Zhang, H., Han, Y. 2023. Applications of transmission electron microscopy in phase engineering of nanomaterials. *Chem. Rev.* 123: 10728–10749.
- Lu, Z., Yu, D., Nie, F., Wang, Y., Chong, Y. 2023. Iron nanoparticles open up new directions for promoting healing in chronic wounds in the context of bacterial infection. *Pharmaceutics* 15: 2327.
- Madusanka, H.K.S., Aruggoda, A.G.B., Chathurika, J.A.S., Weerakoon, S.R. 2023. Effect of seed priming of green synthesized iron oxide magnetic nanoparticles using *Salvinia molesta* plant extract on seed germination and seedlings growth of tomato (*Solanum lycopersicum*). *Int. J. Adv. Res. Innov. Ideas Educ.* 9: 2669–2686.
- Mahmoud, R.K. 2024. Effect of ZnO-NPs on *Rhizoctonia solani* causing root and stem rot on broad bean (*Vicia faba* L.). *SABRAO J. Breed. Genet.* 56: 6.
- Malak, A., Moftha, N.S.H., El-Zaya, E.M., Mohamed, A.S., Saad, D.Y. 2022. Therapeutic effect of silver/chitosan/ascorbic acid nanocomposites on ethanol-induced gastric ulcers in rats. *Pharmacology* 26.
- Mammari, N., Lamouroux, E., Boudier, A., Duval, R.E. 2022. Current knowledge on the oxidative-stress-mediated antimicrobial properties of metal-based nanoparticles. *Microorganisms* 10: 437.
- Mim, J.J., Rahman, S.M., Khan, F., Paul, D., Sikder, S., Das, H.P., Khan, S., Omy, N.T., Shuvo, M.R.H., Hossain, N. 2025. Towards smart agriculture through nano-fertilizer-A review. *Mater. Today Sustain.* 30: 101100.
- Mogazy, A.M., Mohamed, H.I., El-Mahdy, O.M. 2022. Calcium and iron nanoparticles: A positive modulator of innate immune responses in strawberry against *Botrytis cinerea*. *Process Biochem.* 115: 128–145.
- Mohammed, A.M., Saud, W.M., Ali, M.M. 2020. Green synthesis of Fe₂O₃ NPs using *Olea europaea* leaf extract and their antibacterial activity. *Dig. J. Nanomater. Biostruct.* 15: 175–183.
- Mohammed, J.N., Swalaha, F.M. 2025. Emerging trends in synergetic use of biofloculants and nanoparticles for pollutant removal from water: A review. *Water Conserv. Sci. Eng.* 10: 1–17.
- Mukhtiar, A., Alawadi, H.F., Naqve, M., Seleiman, M.F., Mahmood, A., Majeed, M.I., Hafeez, M.B., Khan, N. 2024. Role of iron oxide nanoparticles in maize (*Zea mays* L.) to enhance salinity stress tolerance. *Not. Bot. Horti Agrobot. Cluj-Napoca* 52: 13695.
- Müller, T., Behrendt, U. 2021. Exploiting the biocontrol potential of plant-associated pseudomonads—A step towards pesticide-free agriculture? *Biol. Control* 155: 104538.
- Mushtaq, H., Batool, I., Taj, M.B., Ali, O.M., Alnajeebi, A.M., Yahya, R., Alelwani, W., Alsabbab, M.M., Alghamdi, S. 2025. Phyto-mechanochemical synthesis of iron oxide nanoparticles using *Polygonum paronychioides* and their pesticidal and antimicrobial applications. *New J. Chem.* 49: 11705–11728.
- Mutar, H.M., Jabber Al Kaabi, H.K., Alhasanawi, B.N., Neama, A.J. 2025. Green synthesis of iron nanoparticles using propolis and their in vitro antifungal efficacy against *Trichophyton* fungi. *J. Nanostruct.* 15: 1860–1869.
- Nahari, M.H., Al Ali, A., Asiri, A., Mahnashi, M.H., Shaikh, I.A., Shettar, A.K., Hoskeri, J. 2022. Green synthesis and characterization of iron nanoparticles synthesized from aqueous leaf extract of *Vitex leucoxylon* and its biomedical applications. *Nanomaterials* 12: 2404.
- Naik, H., Manoharadas, S., Bommayasamy, N., Gobi, M., Thomas, J., Dewala, S.R., Amareesan, N. 2025. Mycogenic synthesis of iron oxide NPs (IONPs) for managing bacterial wilt in tomato (*Solanum lycopersicum*) caused by *Ralstonia solanacearum*: Disease suppression, growth promotion and soil health improvement. *New J. Chem.* 49: 5854–5864.

- Narware, J., Chakma, J., Singh, S.P., Prasad, D.R., Meher, J., Singh, P., Bhargava, P., Sawant, S.B., Pitambar, Singh, J.P., Manzar, N. 2025. Nanomaterial-based biosensors: A new frontier in plant pathogen detection and plant disease management. *Front. Bioeng. Biotechnol.* 13: 1570318.
- Ndou, N., Rakgotho, T., Nkuna, M., Doumbia, I.Z., Mulaudzi, T., Ajayi, R.F. 2023. Green synthesis of iron oxide (hematite) nanoparticles and their influence on Sorghum bicolor growth under drought stress. *Plants* 12: 1425.
- Németh, Z., Csóka, I., Semnani Jazani, R., Sipos, B., Haspel, H., Kozma, G., Kónya, Z., Dobó, D.G. 2022. Quality by design-driven zeta potential optimisation study of liposomes with charge imparting membrane additives. *Pharmaceutics* 14: 1798.
- Newberry, D. 2022. The history of nanoscience and nanotechnology. *Nanotechnol. Past Present* 13: 13–22.
- Nizamov, S., Sazdovska, S.D., Mirsky, V.M. 2022. A review of optical methods for ultrasensitive detection and characterization of nanoparticles in liquid media with a focus on the wide field surface plasmon microscopy. *Anal. Chim. Acta* 1204: 339633.
- Noman, M., Ahmed, T., Shahid, M., Nazir, M.M., Li, D., Song, F. 2024. Salicylic acid-doped iron nano-biostimulants potentiate defense responses and suppress Fusarium wilt in watermelon. *J. Adv. Res.* 59: 19–33.
- Nughwal, A., Bharti, R., Thakur, A., Verma, M., Sharma, R., Pandey, A. 2025. Green synthesis of iron oxide nanoparticles from Mexican prickly poppy (*Argemone mexicana*): Assessing antioxidant activity for potential therapeutic use. *RSC Adv.* 15: 10287–10297.
- Nyabadza, A., McCarthy, É., Makhesana, M., Heidarinassab, S., Plouze, A., Vazquez, M., Brabazon, D. 2023. A review of physical, chemical and biological synthesis methods of bimetallic nanoparticles and applications in sensing, water treatment, biomedicine, catalysis and hydrogen storage. *Adv. Colloid Interface Sci.* 321: 103010.
- Osman, A.I., Zhang, Y., Farghali, M., Rashwan, A.K., Eltaweil, A.S., Abd El-Monaem, E.M., Mohamed, I.M., Badr, M.M., Ihara, I., Rooney, D.W., Yap, P.S. 2024. Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural and food applications: A review. *Environ. Chem. Lett.* 22: 841–887.
- Panwar, H., Chaudhary, H., Kumar, P., Phukon, H., Kalita, D., Dubey, R.C. 2025. nanoparticles for control of soil-borne fungal pathogens. *Nanofertilizers Sustain. Agric.* 79–99.
- Pasieczna-Patkowska, S., Cichy, M., Flieger, J. 2025. Application of Fourier transform infrared (FTIR) spectroscopy in characterization of green synthesized nanoparticles. *Molecules* 30: 684.
- Pavlicevic, M., Vaidya, S., Arsenaault, T., Bharadwaj, A., Musante, C., Yu, Y., Shabtai, I., Liquori, J., Hernandez-Viezcas, J.A., Oyanedel-Craver, V., Gardea-Torresdey, J.L. 2025. Upcycling plant waste: Iron nanoparticles synthesized from Cannabis sativa enhance biomass and antioxidative properties in soybean (*Glycine max*). *Environ. Sci. Nano* 12: 1669–1684.
- Pechyen, C., Tangnorawich, B., Toommee, S., Marks, R., Parcharoen, Y. 2024. Green synthesis of metal nanoparticles, characterization and biosensing applications. *Sens. Int.* 5: 100287.
- Pereira, M.C., Oliveira, L.C.A., Murad, E. 2012. Iron oxide catalysts: Fenton and Fentonlike reactions—A review. *Clay Miner.* 47: 285–302.
- Phiri, T.M., Bhattarai, G., Chiwina, K.E., Fan, Q., Xiong, H., Alatawi, I., Dickson, R., Joshi, N.K., Rojas, A., Ling, K.S., Shi, A. 2024. An evaluation of bacterial wilt (*Ralstonia solanacearum*) resistance in a set of tomato germplasm from the United States Department of Agriculture. *Agronomy* 14: 350.
- Pirttilä, A.M., Mohammad Parast Tabas, H., Baruah, N., Koskimäki, J.J. 2021. Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. *Microorganisms* 9: 817.
- Rai, M., Ingle, A.P., Pandit, R., Paralikar, P., Shende, S., Gupta, I., Biswas, J.K., da Silva, S.S. 2018. Copper and copper nanoparticles: Role in management of insect-pests and pathogenic microbes. *Nanotechnol. Rev.* 7: 303–315.
- Rami, M.R., Meskini, M., Sharafabad, B.E. 2024. Fungal-mediated nanoparticles for industrial applications: Synthesis and mechanism of action. *J. Infect. Public Health* 17: 102536.
- Rauf, A., Ahmad, Z., Ajaj, R., Zhang, H., Ibrahim, M., Muhammad, N., Al-Awthan, Y.S., Bahattab, O.S., Ullah, I. 2025. Green synthesis an eco-friendly route for the synthesis of iron oxide nanoparticles using aqueous extract of *Thevetia peruviana* and their biological activities. *Sci. Rep.* 15: 18316.
- Raval, N., Maheshwari, R., Kalyane, D., Youngren-Ortiz, S.R., Chougule, M.B., Tekade, R.K. 2019. Importance of physicochemical characterization of nanoparticles in pharmaceutical product development. *Basic Fundam. Drug Deliv.* 369–400.
- Ravi, L., Jain, P., Maddali, V., Madhushree, S.H., Kajagar, S., Doodi, S., Jeevan Kumar, M., Rentala, S. 2025. Green synthesized heteromorphic iron oxide nanoparticles ($\text{Fe}_3\text{O}_4\text{NPs}$) as potential food additive, food supplement & food colorant sensor and their physiological impact on *Drosophila melanogaster*. *Discov. Appl. Sci.* 7: 225.
- Rojo, C., Carmona, E.R., Hernández-Saravia, L.P., Villacorta, A., Marcos, R., Carevic, F.S., Apablaza, V.H., Nelson, R. 2024. Utilization of orange peel waste for the green synthesis of iron nanoparticles and its application to stimulate growth and biofortification on *Solanum lycopersicum*. *Waste Biomass Valorization* 15: 6343–6356.
- Roy, A., Singh, V., Sharma, S., Ali, D., Azad, A.K., Kumar, G., Emran, T.B. 2022. Antibacterial and dye degradation activity of green synthesized iron nanoparticles. *J. Nanomater.* 2022: 3636481.
- Ruffatto, K., Minello, L.V.P., Furtado, B.G., Johann, L., Sperotto, R.A. 2025. nanoparticles as tools for enhancing plant resistance to biotic stress in the context of climate change. *Physiol. Plant.* 177: e70227.
- Şahin, A., Altunsoy, Ş., Kızılbey, K. 2024. An approach for cationic dyes removal from wastewater: Green synthesis of iron nanoparticles using *Prunus avium* stems extracts. *Kuwait J. Sci.* 51: 100226.
- Sahli, C., Moya, S.E., Lomas, J.S., Gravier-Pelletier, C., Briandet, R., Hémadi, M. 2022. Recent advances in nanotechnology for eradicating bacterial biofilm. *Theranostics* 12: 2383.
- Saleem, S., Khan, M.S. 2023. Phyto-interactive impact of green synthesized iron oxide nanoparticles and *Rhizobium pusense* on morpho-physiological and yield components of greengram. *Plant Physiol. Biochem.* 194: 146–160.
- Seleim, M.A., Abo-Elyousr, K.A., Abd-El-Moneem, K.M., Saeed, F.A. 2014. First report of bacterial wilt caused by *Ralstonia solanacearum* biovar 2 race 1 on tomato in Egypt. *The plant pathol. j.* 30: 299.
- Salim, S., Hari, N., Sudhi, S., Nair, A.J. 2025. Green synthesis, characterisation and bioactivity of iron oxide nanoparticles using *Myristica fragrans* leaf extract. *Microbe* 100481.

- Sengupta, I., Bhattacharya, P., Talukdar, M., Neogi, S., Pal, S.K., Chakraborty, S. 2019. Bactericidal effect of graphene oxide and reduced graphene oxide: Influence of shape of bacteria. *Colloid Interface Sci. Commun.* 28: 60–68.
- Shabbir, M.A., Naveed, M., Rehman, S.U., Ain, N.U., Aziz, T., Alharbi, M., Alsahammari, A., Alasmari, A.F. 2023. Synthesis of iron oxide nanoparticles from *Madhuca indica* plant extract and assessment of their cytotoxic, antioxidant, anti-inflammatory and anti-diabetic properties via different nanoinformatics approaches. *ACS Omega* 8: 33358–33366.
- Shah, A., Khalil, A.T., Ahmad, K., Iqbal, J., Shah, H., Shinwari, Z.K., Maaza, M. 2021. Biogenic nanoparticles: Synthesis, mechanism, characterization and applications. *Biogenic Nanopart. Cancer Theranostics* 27–42.
- Shah, S.T., Sari, I.P., Yanto, D.H.Y., Chowdhury, Z.Z., Bashir, M.N., Badruddin, I.A., Hussien, M., Lee, J.S. 2025. Nature's nanofactories: Biogenic synthesis of metal nanoparticles for sustainable technologies. *Green Chem. Lett. Rev.* 18: 2448171.
- Sharma, D., Kanchi, S., Bisetty, K. 2019. Biogenic synthesis of nanoparticles: A review. *Arab. J. Chem.* 12: 3576–3600.
- Sharma, D., Sharma, A., Gautam, H.R. 2025. Green synthesized nanoparticles for disease management in vegetable crops: A review. *Plant Nano Biol.* 100179.
- Sharma, V., Thakur, S. 2025. Green nano-pesticides from plant sources: Synthesis, mechanisms, environmental impacts and prospects for sustainable agriculture. *Pestic. Biochem. Physiol.* 106543.
- Shirsat, S., K., S. 2024. Iron oxide nanoparticles as iron micronutrient fertilizer—Opportunities and limitations. *J. Plant Nutr. Soil Sci.* 187: 565–588.
- Shoudho, K.N., Uddin, S., Rumon, M.M.H., Shakil, M.S. 2024. Influence of physicochemical properties of iron oxide nanoparticles on their antibacterial activity. *ACS Omega* 9: 33303–33334.
- Shukla, G., Gaurav, S.S., Singh, A., Rani, P. 2022. Synthesis of mycogenic silver nanoparticles by *Fusarium pallidoreum* and evaluation of its larvicidal effect against white grubs (*Holotrichia* sp.). *Mater. Today Proc.* 49: 3517–3527.
- Siddique, M.I., Silverman, E., Louws, F., Panthee, D.R. 2024. Quantitative trait loci mapping for bacterial wilt resistance and plant height in tomatoes. *Plants* 13: 876.
- Sidkey, N. 2020. Biosynthesis, characterization and antimicrobial activity of iron oxide nanoparticles synthesized by fungi. *Al-Azhar J. Pharm. Sci.* 62: 164–179.
- Singaravelu, S., Motsoene, F., Abrahamse, H., Dhillip Kumar, S.S. 2025. Green-synthesized metal nanoparticles: A promising approach for accelerated wound healing. *Front. Bioeng. Biotechnol.* 13: 1637589.
- Singh, A., Gaurav, S.S., Shukla, G., Rani, P. 2022a. Assessment of mycogenic zinc nano-fungicides against pathogenic early blight (*Alternaria solani*) of potato (*Solanum tuberosum* L.). *Mater. Today Proc.* 49: 3528–3537.
- Singh, A., Basnal, N., Shukla, G., Chaudhary, N., Singh, S., Gaurav, S.S. 2022b. Evaluation of efficacy of phyto-synthesized iron oxide nanoparticles in contributing drought resilience in wheat (*Triticum aestivum* L.). *Nanotechnology* 33: 485101.
- Singh, K., Sethi Chopra, D., Singh, D., Singh, N. 2022c. Green synthesis and characterization of iron oxide nanoparticles using *Coriandrum sativum* L. leaf extract. *Indian J. Biochem. Biophys.* 59: 450–454.
- Singh, A., Gaurav, S.S., Shukla, G., Rani, P. 2021. Evaluation of mycosilver nanofungicides as potential control agent against *Phytophthora infestans*. *Plant Cell Biotechnol. Mol. Biol.* 22: 157–168.
- Singh, A., Rawat, T., Govardhan, O., Sharma, C., Lohani, P., Kumar, A., Hassan, S., Singh, H., Kumar, S., Yadav, R.R., Gautam, S. 2025a. Impact of green synthesized iron oxide nanoparticles from *Rhododendron arboreum* on wheat (*Triticum aestivum*): Evaluation of morpho-physiological, biochemical and yield attributes. *Biocatal. Agric. Biotechnol.* 103640.
- Singh, K.M., Jha, A.B., Dubey, R.S., Sharma, P. 2025b. Nanoparticle-mediated mitigation of salt stress-induced oxidative damage in plants: Insights into signaling, gene expression and antioxidant mechanisms. *Environ. Sci. Nano* 12: 2983–3017.
- Singh, J., Kumar, A., Nayal, A.S., Vikal, S., Shukla, G., Singh, A., Singh, A., Goswami, S., Kumar, A., Gautam, Y.K., Verma, Y. 2024a. Comprehensive antifungal investigation of green synthesized silver nanoformulation against four agriculturally significant fungi and its cytotoxic applications. *Sci. Rep.* 14: 5934.
- Singh, N., Singh, M.K., Raghuvansi, J., Yadav, R.K., Azim, Z. 2024b. Green synthesis of nano iron oxide using *Embllica officinalis* L. fruit extract and its impact on growth, chlorophyll content and metabolic activity of *Solanum lycopersicum* L. *J. Appl. Biol. Biotechnol.* 12: 173–181.
- Sivalingam, A.M. 2025. Biological characterization of synthesized iron nanoparticles (FeNPs) from *Avicennia marina* for phenol red removal. *Sens. Bio-Sens. Res.* 100843.
- Sun, H.Q., Lu, X.M., Gao, P.J. 2011. The exploration of the antibacterial mechanism of Fe³⁺ against bacteria. *Braz. J. Microbiol.* 42: 410–414.
- Suppiah, D.D., Julkapli, N.M., Sagadevan, S., Johan, M.R. 2023. Eco-friendly green synthesis approach and evaluation of environmental and biological applications of iron oxide nanoparticles. *Inorg. Chem. Commun.* 152: 110700.
- Talebi, S. 2024. Green synthesis of Fe₃O₄@Cu nanocomposite using aqueous extract of *Aloe vera* plant and evaluation of its antifungal activity. *Biol. Mol. Chem.* 2: 253–264.
- Tariq, M., Mohammad, K.N., Ahmed, B., Siddiqui, M.A., Lee, J. 2022. Biological synthesis of silver nanoparticles and prospects in plant disease management. *Molecules* 27: 4754.
- Tran, T.K., Nguyen, M.K., Lin, C., Hoang, T.D., Nguyen, T.C., Lone, A.M., Khedulkar, A.P., Gaballah, M.S., Singh, J., Chung, W.J., Nguyen, D.D. 2024. Review on fate, transport, toxicity and health risk of nanoparticles in natural ecosystems: Emerging challenges in the modern age and solutions toward a sustainable environment. *Sci. Total Environ.* 912: 169331.
- Tyagi, P.K., Gupta, S., Tyagi, S., Kumar, M., Pandiselvam, R., Daştan, S.D., Sharifi-Rad, J., Gola, D., Arya, A. 2021. Green synthesis of iron nanoparticles from spinach leaf and banana peel aqueous extracts and evaluation of antibacterial potential. *J. Nanomater.* 2021: 4871453.
- Ullah, J., Gul, A., Khan, I., Shehzad, J., Kausar, R., Ahmed, M.S., Batool, S., Hasan, M., Ghorbanpour, M., Mustafa, G. 2024. Green synthesized iron oxide nanoparticles as a potential regulator of callus growth, plant physiology, antioxidative and microbial contamination in *Oryza sativa* L. *BMC Plant Biol.* 24: 939.

- Vinukonda, A., Bolledla, N., Jadi, R.K., Chinthala, R., Devadasu, V.R. 2025. Synthesis of nanoparticles using advanced techniques. *Next Nanotechnol.* 8: 100169.
- Wang, Z., Luo, W., Cheng, S., Zhang, H., Zong, J., Zhang, Z. 2023. *Ralstonia solanacearum*—a soil borne hidden enemy of plants: research development in management strategies, their action mechanism and challenges. *Front. plant scie.* 14: 1141902.
- Yadav, A., Yadav, K., Ahmad, R., Abd-Elsalam, K.A. 2023. Emerging frontiers in nanotechnology for precision agriculture: Advancements, hurdles and prospects. *Agrochemicals* 2: 220–256.
- Yekeen, M.O., Ibrahim, M., Wachira, J., Pramanik, S. 2025. Green synthesis and characterization of iron oxide nanoparticles using *Egeria densa* plant extract. *Appl. Biosci.* 4: 27.
- Yosri, N., Khalifa, S.A., Guo, Z., Xu, B., Zou, X., El-Seedi, H.R. 2021. Marine organisms: Pioneer natural sources of polysaccharides/proteins for green synthesis of nanoparticles and their potential applications. *Int. J. Biol. Macromol.* 193: 1767–1798.
- Zain, M., Ma, H., Nuruzzaman, M., Chaudhary, S., Nadeem, M., Shakoor, N., Azeem, I., Duan, A., Sun, C., Ahamad, T. 2023. Nanotechnology based precision agriculture for alleviating biotic and abiotic stress in plants. *Plant Stress* 10: 100239.
- Zanbili, F., Poursattar Marjani, A. 2025. Innovative green and bio-based approaches for photosensitive nanoparticles synthesis: A review on methodologies, characterization and applications. *Micro Nano Syst. Lett.* 13: 3.
- Zhang, Y.J., Ze, H., Fang, P.P., Huang, Y.F., Kudelski, A., Fernández-Vidal, J., Hardwick, L.J., Lipkowski, J., Tian, Z.Q., Li, J.F. 2023. Shell-isolated nanoparticle-enhanced Raman spectroscopy. *Nat. Rev. Methods Primers* 3: 36.
- Zúñiga-Miranda, J., Guerra, J., Mueller, A., Mayorga-Ramos, A., Carrera-Pacheco, S.E., Barba-Ostria, C., Heredia-Moya, J., Guamán, L.P. 2023. Iron oxide nanoparticles: Green synthesis and their antimicrobial activity. *Nanomaterials* 13: 2919. doi.org/10.3390/nano13222919