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Management of Plant Diseases with Green Synthesized Nanoparticles Using Plant Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Nanotechnology is a rapidly advancing field that has effectively tackled various issues across multiple industries, including agriculture. Nanoparticles (NPs) can typically be synthesized using two different techniques: top-down approach and bottom-up approach. In top-down approach, NPs are generated by reducing the size of a larger material, resulting in agglomerates with nano sized particles. Bottom-up approach involves building nanoscale structures from atomic and molecular components. In agriculture, nanotechnology plays a role in the production of nanoscale fertilizers, pesticides, and herbicides. Nanoparticles are used in plant disease management as well. The green synthesis of nanoparticles is often referred to as biosynthesis. It uses a range of biological sources like microorganisms or their derivatives as well as plant extracts, instead of synthetic chemicals, with minimal impact on human health and the environment. Green-synthesized

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nanoparticles (GSNPs) are known for their ease of production and cost-effectiveness. They offer several advantages, including their ability to induce systemic resistance to diseases and exhibit fungicidal and bactericidal properties.

Keywords: Nanoparticles; green synthesis; plant extract; plant disease management.

1. INTRODUCTION

Agriculture faces numerous hurdles, including inefficient use of resources, expensive capital equipment, threats from pests and environmental factors, and a declining interest among the younger generation. In this context, the adoption of cutting-edge technologies plays a crucial role in overcoming these agricultural challenges and boosting crop yields. Nanotechnology is a rapidly advancing field that has effectively tackled various issues across multiple industries, including agriculture [1]. Nanotechnology involves working with nanomaterials, the particles typically having sizes ranging from 1 to 100 nanometers [2]. Prof. Norio Taniguchi coined the term nanotechnology, and Richard Feynman is known as the father of nanotechnology. The distinctive features of nanoparticles, such as large surface area to volume ratio, surface plasmon resonance (SPR), and their unique biological, optical, and electrical properties, have significantly increased their demand. Furthermore, the production of nanoparticles has garnered substantial popularity among scientists and researchers worldwide in recent years [3]. Nanotechnology has found widespread applications in everyday life and is progressively becoming more significant in society. It is being utilized in various fields, including medicine, pharmaceuticals, electronics, energy, environmental sciences, chemistry, food industries, and more recently, agriculture [4]. In agriculture, nanotechnology plays a role in producing nanoscale fertilizers, pesticides, and herbicides [5]. Nanoparticles are used in plant disease management as well.

2. SYNTHESIS OF NANOPARTICLES

Nanoparticles (NPs) can typically be synthesized using two different techniques. The first method, the top-down approach, involves physical processes like sonication, laser ablation, radiation, and thermal decomposition. In this approach, NPs are generated by reducing the size of a larger material, resulting in agglomerates with nano sized particles. Drawbacks of this method are, producing a variety of particle sizes (polydispersity), introducing imperfections (including contamination from the initial material), requiring significant energy and specialized laboratory equipment, and being costly [6]. The second approach, the bottom-up method, involves building nanoscale structures from atomic and molecular components. NPs are formed through chemical and biological synthesis. Chemical synthesis techniques include electrochemistry, vapor flux condensation, the sol-gel method, and chemical reduction. Among these, chemical reduction, which utilizes chemicals like sodium borohydride and sodium citrate [7], is one of the most commonly used methods for NP generation. Nonetheless, chemical methods often involve multiple chemical species or molecules, which can increase particle reactivity and toxicity. They may also adversely affect human health [8] and the environment due to the decomposition of chemical groups, the generation of by-products, and high energy demand [9].

Different methods of nanoparticle synthesis: Nanoparticles can be synthesized using various methods, generally categorized into physical, chemical, and biological methods. These methods employ different sources for the synthesis process, such as reducing agents or electron donors. Some of the methods are listed.

Even though all these methods have been used for nanoparticle synthesis, the widespread application of physical and chemical approaches in agriculture and healthcare has been relatively limited [10]. The challenges linked to the synthesis of nanoparticles by physical and chemical methods stem from several factors. These include the use of potentially hazardous chemicals, the need for costly equipment and machinery, the requirement for larger laboratory spaces, demanding processing conditions like high temperature and pressure, and significant energy consumption [11,12,13]. Moreover, the time required for synthesis, the generation of harmful by-products, the overall high costs involved, and the adverse environmental effects further compound the difficulties associated with nanoparticle production [14].

Table 1. Methods of nanoparticle synthesis

Methods of nanoparticle synthesis		
Physical methods	**Chemical methods**	**Biological methods**
• Gas phase deposition • Electron beam lithography • Powder ball milling • Pulsed laser ablation • Aerosol	• Coprecipitation • Sonochemical • Thermal decomposition • Microemulsion • Hydrothermal • Electrochemical deposition	• Plants • Fungi • Algae • Bacteria • Biopolymers

[3]

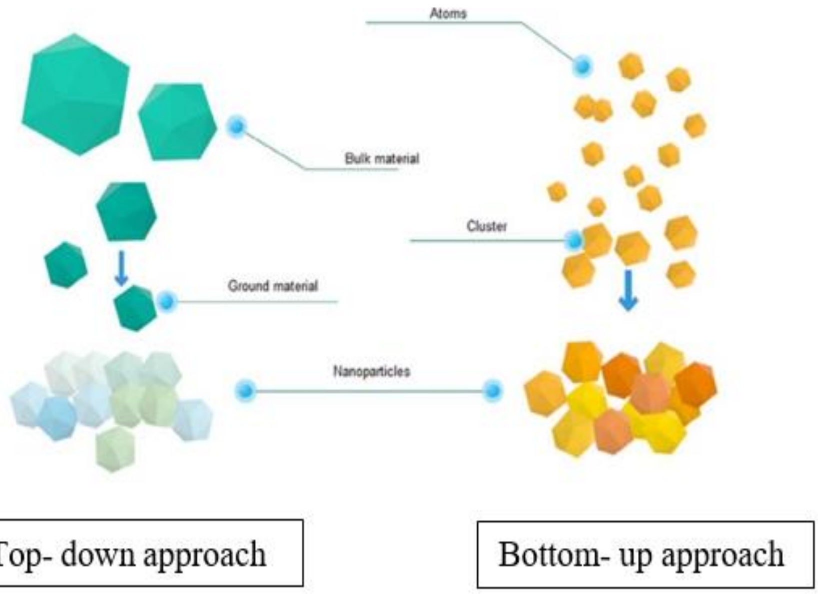

Fig. 1. Techniques of nanoparticle synthesis [4]

3. GREEN SYNTHESIS OF NANOPARTICLES

The green synthesis of nanoparticles is often referred to as biosynthesis, involving a range of biological sources. In green nanoparticle synthesis, microorganisms or their derivatives, as well as plant extracts, are used instead of synthetic chemicals, with minimal impact on human health and the environment. Utilizing green nanotechnologies can be a potent approach to address the intricate scientific and technological hurdles in enhancing the safety of the entire agricultural and food production chain [15]. Dr. Kattesh V. Katti is known as the father of green nanotechnology. Green nanomaterials offer significant potential in developing nano-based pesticide formulations due to their small size, large surface area, and properties that can be tailored for specific targets. This has the potential to enhance the effectiveness, safety, and economic impact of conventional pesticides by prolonging their duration of action, reducing the necessary dosage, enabling the controlled release of active ingredients, ensuring stability, and minimizing runoff and environmental residues [15].

The green synthesis of nanoparticles involves three key components: reducing agents, solvents, and capping agents. Biomolecules, which can be naturally occurring or produced by plants and microbes, such as polyphenols, flavonoids, terpenoids, tannins, alkaloids, polysaccharides, proteins, amino acids, and vitamins, serve as reducing agents during nanoparticle synthesis [2]. These biomolecules reduce metal ions to a zero-valence state, and the functional groups within these primary biopolymers and phytochemicals play a role in stabilizing the resulting nanoparticles. Among various biological methods, utilizing plants for nanoparticle synthesis is preferred due to their widespread availability, safety, lack of toxicity, and the presence of a wide range of phytoconstituents that can act as reducing agents [16].

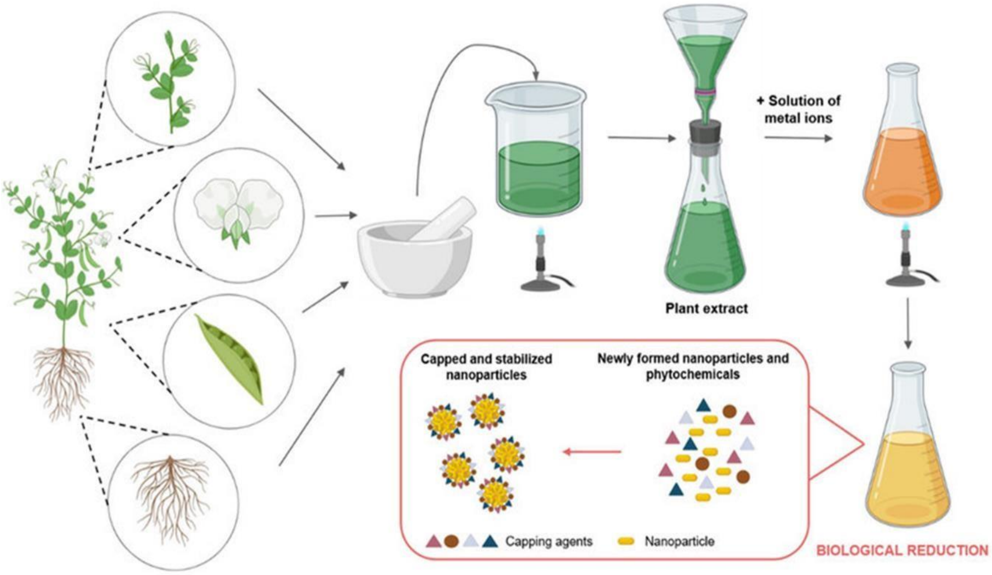

Fig. 2. General steps of green synthesis of nanoparticles [69]

The green synthesis of nanoparticles is regarded as a safe approach to nanoparticle production and has numerous advantages compared to physical and chemical methods. This method is environmentally friendly, cost-efficient, provides high yields, steady, involves a simple and easy synthesis process, relies on renewable and biocompatible materials, avoids the use of dangerous toxic chemicals, allows for easy accessibility and easy handling, and is an energy- saving process, among other benefits [16,17]. However, a few drawbacks are associated with it, such as the potential ecological imbalance caused by the overexploitation of natural biological sources and the seasonal variation in the availability of phytochemicals [18].

Different parts of plants, including fruits, leaves, stems, and roots, have been widely employed for the green synthesis of nanoparticles because of the valuable phytochemicals they contain [19]. In nanoparticle synthesis using plant materials, the specific part of the plant to be used is cleaned and boiled with distilled water. Afterward, the resulting mixture is squeezed, filtered, and combined with the desired solutions for nanoparticle synthesis. As the solution colour changes, indicating the formation of nanoparticles, they can be separated and collected. This natural plant extract-based synthesis is environmentally friendly and cost-effective, eliminating the need for intermediate chemical reagents. Nanoparticle synthesis occurs in athree-step sequence: (i) the reduction of metal ions, often signaled by observable colour changes, (ii) the clustering of nanoparticles; and (iii) the stabilization of these nanoparticles [13].

4. FACTORS AFFECTING GREEN SYNTHESIS OF NANOPARTICLES

Solution pH: The pH level of a solution plays a critical role in synthesizing nanoparticles from plant sources [20]. It has been emphasized that the pH of the solution can significantly affect the time it takes for synthesis, as well as the size and shape of the resulting nanoparticles [21]. The formation of nucleation centers during nanoparticle synthesis is highly dependent on the pH level. An increase in pH can lead to the formation of more nucleation centers, which in turn accelerates the reduction of metallic ions into metal nanoparticles. The time it takes to reduce metal salts is closely associated with the pH of the reaction medium because pH affects the interaction between the functional groups present in the plant extract and the metal ions [22]. Scientific research has demonstrated that the production of smaller nanoparticles is more likely to occur in a basic solution compared to an acidic one [23].

Reaction time: The incubation and reaction period length plays a crucial role in determining the characteristics, morphology, and yield of nanoparticles [24]. Changes in the incubation time and storage conditions also impact the properties of the nanoparticles produced [25].

Extending the incubation period can result in aggregation and decrease the potential for reducing nanoparticles during the synthesis process [26]. Certain research findings have indicated that effective nanoparticle synthesis tends to occur with longer reaction times [27].

Temperature: Temperature is another influential factor in the synthesis of nanoparticles, and it has a similar impact on their morphological properties as pH does. Temperature also affects the formation of nucleation centers, with lower temperatures leading to a reduced formation of these centers, subsequently slowing down the synthesis rate [28]. Because of the specific secondary metabolites present in plant extracts, it has been recommended to carry out nanoparticle synthesis at room temperature to prevent the degradation and alteration of functional groups [29]. Nevertheless, research has demonstrated that triangular-shaped nanoparticles are formed at lower temperatures, whereas spherical-shaped nanoparticles are generated at higher temperatures [30]. Studies have reported that smaller volumes of plant extract are needed for stable nanoparticle synthesis at higher temperatures, and larger-sized nanoparticles tend to be produced at higher temperatures [31].

Effect of plant extract concentration: The concentration of plant extract plays a crucial role in synthesizing metal nanoparticles as it provides the electrons needed for reducing metal ions. A reduction in the amount of plant extract results in a reduced formation of nanoparticles [32]. On the other hand, employing a larger volume of plant extract in metal nanoparticle synthesis leads to a higher quantity of phytochemicals, accelerating the reduction of metal salt. Nevertheless, the faster this reduction occurs, the smaller the size of the resulting metal nanoparticles [33].

Concentration and nature of metal salt: The choice of metal salt employed in the synthesis significantly impacts the characteristics, structure, and size of the nanoparticles produced. For instance, copper salts such as copper chloride, copper sulphate, copper acetate, and copper nitrate are commonly used for nanoparticle synthesis. Research findings have indicated that when copper chloride salt is utilized, triangular and tetrahedral-shaped copper nanoparticles (Cu-NPs) are formed, whereas rod-shaped Cu-NPs are obtained with copper acetate salt [34]. When copper sulphate salt is used, spherical Cu-NPs are synthesized [35]. It has also been reported that an increase in the concentration of metal salt leads to an increase in the size of the nanoparticles [36].

Pressure: Numerous research investigations have demonstrated that pressure plays a role in influencing the morphological characteristics of nanoparticles synthesized from plant sources [37].

5. CHARACTERIZATION OF NANOPARTICLES

Metal nanoparticles are characterized for several purposes, including tracking the completeness of reduction, identifying the functional groups involved in the bio-reduction process, assessing purity levels, and analyzing their morphological attributes. The commonly employed techniques for these purposes are highlighted below;

UV–visible spectrophotometry: The interaction between plant extract and metal salt results in observable colour changes in the solution due to the excitation of surface plasmon vibrations in the NPs. The absorption band corresponding to each metal can be confirmed by analyzing the solution with a UV spectrophotometer. UV-visible spectrophotometry tracks the characteristic peaks generated by metal salt-derived nanoparticles (NPs) at various absorption wavelengths during the synthesis process. For instance, in the case of Cu-NPs, a distinctive absorption occurs in the range of 520-600 nm within the visible region due to surface plasmon resonance (SPR). UV-visible spectroscopy is also employed to estimate the aggregation state, size, and size distribution of NPs [38].

Fourier transform infrared (FTIR) spectroscopy: FTIR spectroscopy can be used to detect functional groups capable of donating electrons for the reduction of metal salts [39]. FTIR spectrophotometer measures the wavelength of light against infrared intensity. Researchers typically compare the FTIR spectra of the plant extract and the synthesized nanoparticles to determine which functional groups are responsible for reducing the metal ions [40].

X-ray diffraction (XRD): The X-ray diffraction (XRD) technique is utilized to gather structural data regarding the crystalline nature of nanoparticles [41]. During XRD analysis, the high-energy X-ray rays emitted by the machine penetrate deeply into the nanoparticles, yielding valuable insights into their structure [42]. The formation of nanoparticles in the nanoscale range is typically characterized by broadening the peaks observed in XRD analysis.

Scanning electron microscopy (SEM): The morphological characteristics of nanoparticles are assessed through the use of scanning electron microscopy (SEM). Additionally, SEM analysis can be employed to estimate the average size of nanoparticles with the assistance of certain statistical software tools [37].

Transmission electron microscopy (TEM): TEM provides higher magnification, superior resolution, and more precise information regarding shape, crystallinity, and size compared to SEM [37]. Additionally, TEM is particularly advantageous due to its ability to distinguish between crystalline and amorphous structures using selected area electron diffraction techniques, which makes it even more beneficial for nanoparticle characterization [43].

Atomic force microscopy (AFM): Atomic force microscopy (AFM) is used for morphological assessment of nanoparticles [44].

Energy dispersive X-ray spectroscopy (EDX): EDX spectroscopy is widely accepted as a suitable method for determining the elemental composition of nanoparticles. This is achieved by examining the distinct groups of peaks in the X-ray spectrum produced by the unique atomic structure of each element, facilitating the identification of these elements [45].

6. MECHANISM OF ACTION OF NANOPARTICLES AGAINST PHYTOPATHOGENS

Green-synthesized nanoparticles (GSNPs) have primarily been studied for their effectiveness in combatting phytopathogens. However, our understanding of how these nanoparticles inhibit or kill microorganisms remains limited. The mechanism of action of nanoparticles can be broadly categorized as disruption of the peptidoglycan layer in bacterial cell walls, toxicity resulting from the release of toxic metal ions into the cytoplasm leading to imbalances in nutrient uptake, impairment of membrane function including membrane damage and the loss of membrane potential, generation of reactive oxygen species (ROS) and the production of antioxidants, damage to genetic material such as double-helix strand breaks, dysfunction of proteins, etc.

Apart from the impact of metal ions, different metabolites found in plant extracts have been observed to induce cell death in pathogens and stimulate systemic resistance in plants [46]. Alkaloids, phenolics, and natural compounds present in plant extracts have been shown to possess bactericidal and fungicidal properties against plant pathogens, thereby enhancing the efficacy of green-synthesized nanoparticles [15].

Antimicrobial and disease-managing properties of nanoparticles green synthesized from plant extracts: Green synthesized nanoparticles are reported to be effective against several plant pathogenic fungi, bacteria, and viruses.

Table 2. Mechanism of action of different metal nanoparticles against phytopathogens

Nanoparticle type	Ionic form	Mechanism of action	Phytopathogen type
AgNPs	Ag^+	• Release of ions that are toxic to pathogens • Increase the permeability of the bacterial membrane • Disruption of the bacterial membrane • Damage to the cell components (lipids, DNA and proteins) • Inhibition of enzyme activity • Inhibition of DNA replication	Bacteria and fungi
Al_2O_3NPs	Al^{3+}	• Release of ions that are toxic towards pathogens • Increase ROS production • Depolarization of cell membranes	Bacteria
AuNPs	Au^{3+}	• Disruption of bacterial membrane and alteration of metabolism • Damage to cell organelles (cell wall and mitochondria) • Inhibition of DNA uncoiling and transcription mediated by binding of AuNPs to bacterial DNA	Bacteria
CeO_2NPs	Ce^{3+}, Ce^{4+}	• Inhibition of ion transport through pumps • Induction of oxidative degradation of lipids and/or proteins of the pathogen's plasma	Gram-positive bacteria and fungi

Nanoparticle type	Ionic form	Mechanism of action	Phytopathogen type
		membrane • Impairment of electron flux and bacterial respiration • Inhibition of fungal enzyme activity	
CdONPs	Cd^{2+}	• Release of ions that are toxic against pathogens • Induction of oxidative stress on bacterial cells • Increase ROS production • Interrupted transmembrane electron transport and mitochondria damage	Bacteria
CuNPs/CuONPs	Cu^{2+}	• Inhibition of enzyme activity essential to the microorganisms • Increase ROS production • Damage to essential molecules such as DNA	Bacteria and fungi
SeNPs	Se^{6+}, Se^{4+}	• Intracellular ATP depletion • Induction of oxidative stress through ROS production • Alteration of bacterial membrane potential • Disruption of bacterial membrane • Inhibition of fungal spore germination	Bacteria and fungi
SiNPs/SiO_2NPs	Si^{4+}	• Induction of mechanical damage to bacterial membrane • Increased ROS production • Induction of oxidative stress	Bacteria and fungi
TiO_2NPs	Ti^{4+}	• Increase in ROS production • Induction of photocatalytic damage	Bacteria and fungi
ZnONPs	Zn^{2+}	• Release of ions that are toxic towards pathogens • Increased ROS production • Disruption of mitochondrial function • Induction of changes in cell morphology and release of cell components	Bacteria
Fe_2O_3NPs	Fe^{3+}, Fe^{2+}	• Induction of oxidative stress through ROS production • Destruction of cell membranes, inducing changes in cell morphology and release of cell components • Damage to essential molecules such as proteins and DNA • Release of iron ions leading to oxidative damage by Fenton reaction	Bacteria and fungi

[4]

Table 3. Green synthesized nanoparticles against phytopathogenic fungi

Plant	Plant part used	NP	Pathogen	Effective concentration	Experimental approach	Reference
Oryza sativa	Leaf	Ag	*Rhizoctonia solani*	-	-	[47]
Solanum tuberosum	Leaf	Ag	*Alternaria alternata, R. solani, Botrytis cinerea, Fusarium oxysporum*	22.8 µg/mL	*In vitro*	[48]
Abelmoschus esculentus	Seed	Au	*Puccinia graminis tritici,* *Aspergillus flavus, Aspergillus niger, Candida albicans*	-	-	[49]
Citrus sinensis	Fruit	Cu	*Colletotrichum capsici*	-	*In vitro*	[50]
Aloe vera	Leaf	Se	*Colletotrichum coccodes,* *Penicillium digitatum*	-	*In vitro*	[51]
Curcuma longa	Roots	TiO_2	*Fusarium graminearum*	0.2–20 mg/mL	*In vitro*	[52]
Eclipta alba	Leaf	ZnO	*Sclerospora graminicola*	-	Field	[53]
Punica granatum	Peels	ZnO	*Aspergillus niger*	50µg/mL	*In vitro*	[54]

Table 4. Green synthesized nanoparticles against phytopathogenic bacteria

Plant	Plant part used	NP	Pathogen	Effective concentration	Experimental approach	Reference
Piper nigrum	Stem	Ag	*Citrobacter freundii* *Erwinia cacticida*	-	*In vitro*	[55]
Azadirachta indica	Leaf	Ag	*Xanthomonas oryzae* pv. *oryzae*	-	*In vitro*	[56]
Phyllanthus emblica	Fruit	Ag	*Acidovorax oryzae*	30 µg/mL	*In vitro*	[57]
Cymbopoga citratus	Leaf	Al_2O_3	*Pseudomonas aeruginosa*	2000 µg/mL	*In vitro*	[58]
Gloriosa superba	Leaf	CeO_2	*Pseudomonas aeruginosa*	100 µg/mL	*In vitro*	[59]
Carica papaya	Leaf	CuO	*Ralstonia solanacearum*	250 µg/mL	*In vitro* & greenhouse	[60]
Rosmarinus officinalis	Flower	MgO	*X. oryzae* pv. *oryzae*	-	*In vitro*	[61]
Withania somnifera	Leaf	Se	*Bacillus subtilis*	25 µg/mL	*In vitro*	[62]
Cynodon dactylon	Leaf	Si	*Pseudomonas aeruginosa*	60 µg/mL	*In vitro*	[63]
Trigonella foenum- graecum	Leaf	TiO_2	*Bacillus subtilis*	10 mg/mL	*In vitro*	[64]
Solanum lycopersicum	Fruit	ZnO	*X.oryzae* pv. *oryzae*	-	*In vitro*	[65]

Table 5. Green synthesized nanoparticles having both antifungal and antibacterial properties

Plant	Plant part used	NP	Pathogen	Effective concentration	Experimental approach	Reference
Azadirachta indica	Leaf	Ag	*R. solanacearum,* *Aspergillus* sp. *Fusarium* sp.	-	*In vitro*	[66]
Leucaena leucocephala	Leaf	CdO	*P. aeruginosa,* *Aspergillus niger*	500 µg/mL	*In vitro*	[67]
Ocimum sanctum	Leaf	Cu	*Alternaria carthami, A. niger,* *Colletotrichum gloeosporioides,* *Colletotrichum lindemuthianum, Drechslera sorghicola,* *F. oxysporum,* *Macrophomina phaseolina,* *Rhizoctonia bataticola, R. solani,* *Xanthomonas axonopodis* pv. *citri,* *X. axonopodis* pv. *punicae*	10–60 µg/mL	*In vitro*	[68]

7. CHALLENGES

The use of biological sources for nanoparticle synthesis may impose constraints on the possibility of large-scale commercial production of nanoparticles. This limitation arises because the availability and prevalence of the specific plants or biological materials used can fluctuate depending on the season and geographic location. Furthermore, it is crucial to consider the optimal growth stage of these plants when utilizing them for nanoparticle synthesis, as this factor significantly influences the quality and yield of the nanoparticles [69].

Green-synthesized nanoparticles (GSNPs) can exhibit a wide range of sizes, which can pose challenges in achieving uniformity in their properties for various applications. Additionally, GSNPs are often less stable and prone to oxidation, which can impact their long-term effectiveness and shelf life [1].

One significant drawback is that many experiments investigating nanoparticles' effects have primarily focused on short-term crops or immediate impacts. This limited scope means that we may not have a comprehensive understanding of the cumulative and long-term effects of nanoparticles on plants, the environment, and even human health. As nanoparticles become more widely used in agriculture and other fields, it becomes increasingly important to conduct research that addresses their potential long-term impacts and cumulative toxicity. This will help to ensure the safe and sustainable application of nanoparticles in various sectors [15].

8. FUTURE PROSPECTS

It is essential to conduct field trials with various crops and diseases to evaluate the efficacy of all synthesized nanoparticles compared to commercial pesticides and biocontrol agents. Research should explore the use of less phytotoxic metals like Cu, Zn, Mn, Fe, and Mg as potential alternatives to costly silver nanoparticles (AgNPs). A thorough understanding of the structural properties of green nanoparticles, including morphology, size, functional groups, loading capacity, and their impact on plants, should be established. A more efficient, rapid, and scalable protocol for formulating green nanoparticles is necessary for successful large-scale production. The effects of nanoparticles on soil, wildlife, plant biodiversity, crop yields, and farmer income should be investigated. The toxicity and effectiveness of green-synthesized nanoparticles should be compared to chemically synthesized nanoparticles to assess their suitability for various applications.

9. CONCLUSION

Green-synthesized nanoparticles (GSNPs) are known for their ease of production and cost-effectiveness. They offer several advantages, including their ability to induce systemic resistance to diseases and exhibit fungicidal and bactericidal properties. However, despite their promising attributes, questions remain about their efficacy and long-term sustainability when used in field conditions. There is a lack of comprehensive knowledge regarding the extended or prolonged effects of GSNPs on plants and the environment. This knowledge gap hinders our understanding of how GSNPs may impact crops and ecosystems over extended periods. Research efforts should address these questions to ensure the safe and effective utilization of GSNPs in agriculture and environmental applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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