

Review Article

***Cymbopogon nardus*–mediated Silver Nanoparticles: Synthesis, Mechanisms, and Prospects for Plant Disease Management**

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DOI: 10.14416/j.asep.2026.07.015

Received: 18 March 2026; Revised: 10 May 2026; Accepted: 25 June 2026; Published online: 27 July 2026

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Abstract

Silver Nanoparticle (AgNP) synthesis through traditional chemical methods has raised environmental concerns, leading to the development of alternate plant-mediated methods. One such plant source for the synthesis of AgNPs is *Cymbopogon nardus* L. (citronella) because of its abundance of phytochemicals. Oxygenated monoterpenoids, including citronellal and geraniol, assist in reducing the Ag⁺ form of silver to the Ag⁰ form and help stabilize nanoparticles during their formation. The size, polydispersity, and stability of AgNPs are influenced by the AgNO₃ concentration and the pH, temperature, and reaction time applied during their synthesis. AgNPs range in size from 10-50 nm and possess pronounced antibacterial and antifungal activities through mechanisms such as membrane disruption, the release of silver ions, and the generation of reactive oxygen species, which vary depending on the type of bacteria or fungus targeted by the AgNPs. Potential applications of AgNPs include seed treatments, foliar sprays, and soil pathogen control. Other factors that may influence the efficacy and safety of AgNPs include the potential for phytotoxicity and environmental transformations. This review provides an overview of the current body of research in this area and highlights the need for standardised protocols for AgNP synthesis, validation of the underlying mechanisms supporting the biologically active properties of AgNPs, and field level evaluation of the potential use of *C. nardus*-mediated AgNPs in plant disease management.

Keywords: Antimicrobial Activity, *Cymbopogon nardus*, Green Synthesis, Plant Disease Management, Phytochemicals, Silver Nanoparticles

1 Introduction

Nanotechnology is a cutting-edge scientific methodology that is becoming more accepted as an effective agricultural practice, with various applications to crop protection (e.g., pesticides), nutrient delivery systems to improve crop yield, suppression of plant diseases, and the development of sustainable agricultural production techniques. Of the various nanomaterials that are being tested and studied for use within the agriculture sector,

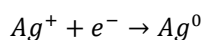
silver nanoparticles (AgNPs) are considered to have the most potential for providing agricultural benefits due to their broad-spectrum antimicrobial properties, high surface areas, highly reactive physicochemical nature, and the ability of AgNPs to interact with the target microbe via multiple mechanisms. Thus, AgNPs could provide viable alternatives or supplementary pesticides to the traditional chemical pesticides typically used to control crop pests and pathogens, especially in situations where pests or pathogens have developed a resistance to



traditional chemical pesticides or where repeated pesticide applications raise environmental and health concerns [1], [2].

There are two routes available for synthesizing silver nanoparticles; they can be produced through chemical and physical methods. The use of chemical reducing agents (e.g., sodium borohydride, hydrazine, sodium citrate, etc.) and stabilizers/surfactants is frequently utilized in the production of silver nanoparticles because these agents facilitate the controlled formation of nanoparticles by controlling their size, shape, and morphology. Chemical synthesis methods can produce silver nanoparticles with a good degree of control; however, there are many concerns associated with chemical synthesis, such as the use of toxic reagents, high energy input, hazardous residues, and higher production costs. These concerns limit the acceptance of chemical synthesis methods for the production of silver nanoparticles within sustainable agriculture, as they have long-term impacts on farmers' safety, consumers' safety, soil health, water resources, and non-target organisms.

The process of green synthesis is a more environmentally friendly method of producing nanoparticles through the use of biological materials, such as plant extracts, microorganisms, algae, fungi, and agricultural waste [3]. Among these different biological materials, the use of plant-based materials is appealing because they are easily obtainable, inexpensive, scalable (capable of large-scale production), and contain bioactive compounds that are naturally found in plants and can help to reduce metal ions for the production of metallic (metal) nanoparticles [4]. The use of phytochemicals (chemicals found in plants), such as phenolics, flavonoids, alkaloids, proteins, sugars, terpenoids, aldehydes, and alcohols, results in the reduction of silver ions to form silver nanostructures through the following fundamental reaction :



The reduced silver atoms (i.e., metallic silver) then undergo nucleation, growth, and stabilization to ultimately produce nanoscale particles [5].

Cymbopogon nardus L. (*C. nardus*) is a perennial herb that grows in many tropical and subtropical regions around the world and is commonly called citronella. It produces essential oils that include various terpenoids, such as citronellal, citronellol, geraniol, citral, and limonene, which possess antibacterial, antifungal, antioxidant, and insect repellent activities [6], [7].

Some of these terpenoids are capable of donating electrons and may also bind to metal surfaces; thus, *C. nardus* is theoretically capable of serving as a valuable bioresource for producing AgNPs via plant-mediated synthesis.

However, while the theoretical potential is high, the literature on the synthesis of AgNPs using *C. nardus* as a biosource is far less extensive than that available for other plant systems (e.g., *C. citratus*) and for other species within the same genus. This limitation is significant because much of the evidence for the synthesis of AgNPs using *C. nardus* as a biosource depends on indirect evidence obtained from related plant species.

The aim of this review is to synthesise current information regarding the synthesis of silver nanoparticles using *C. nardus*, including the impact of phytochemicals on the synthesis process and the mechanisms involved. Due to limited direct research on applications of *C. nardus* to plant disease management, this review uses a structured comparative evidence approach to examine evidence from other *Cymbopogon* species with similar applications to identify existing patterns while distinguishing species-specific data. The review also discusses the antimicrobial mechanisms and the physicochemical characterisation of nanoparticles, as well as challenges related to reproducibility, colloidal stability, environmental fate, and scale-up feasibility. This review highlights the current understanding of the mechanism of silver nanoparticle synthesis from *C. nardus* and the evidence gaps that remain, thereby supporting future research and potential agricultural applications.

2 Phytochemical profile of *C. nardus* and its functional role in AgNP formation

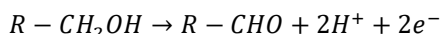
The phytochemical profile of *C. nardus* consists of a wide variety of compounds, primarily identified as oxygenated monoterpenoids (Table 1). Citronella oil contains the predominant phytochemical components citronellal, citronellol, geraniol, and citral, while the other constituents are limonene, β -caryophyllene, elemol, geranyl acetate, and eugenol. The phytochemical constituents of citronella provide its characteristic fragrance and biological properties and may also contribute to its potential use in synthesizing nanoparticles [6].

The possible roles of the phytochemicals from citronella in producing AgNPs are two fold: 1) as reducing agents, phytochemicals that can donate electrons to reduce Ag^{+} to Ag metal (i.e., nanoparticles); and 2) as stabilizing or capping agents, which can attach to the surface of nanoparticles

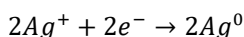
Table 1. Major phytochemicals of *C. nardus*, functional groups, expected role in AgNP synthesis, and antimicrobial relevance.

Compound	Chemical Group	Role in AgNP Synthesis and Their Biological Attributes	References
Citronellal	Monoterpenoid aldehyde	Reducing agent; antifungal, antibacterial	[12], [13]
Geraniol	Monoterpenoid aldehyde	Reducing and capping agent; antioxidant, antimicrobial	[14], [15]
Citronellol	Monoterpenoid aldehyde	Capping and stabilizing agent; antimicrobial, anti-inflammatory	[16]
Citral	Aldehyde	Reducing agent; antifungal, antiviral	[17]
Limonene	Monoterpenoid hydrocarbon	Secondary stabilizer; antimicrobial	[18], [19]
B-Caryophyllene	Sesquiterpene	Surface modifier; antimicrobial	[20]

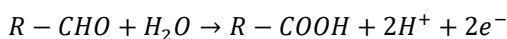
and prevent their aggregation [8]. The presence of hydroxyl, carbonyl, aldehyde, and several other oxygen-containing functional groups is important due to their ability to participate in redox reactions and surface coordination. For example, geraniol contains a hydroxyl group that may be oxidized during Ag^+ reduction. A simplified representation is:



The released electrons can then reduce silver ions:



Similarly, aldehydic compounds such as citronellal may undergo oxidation to carboxylic acid derivatives:



A reaction that supplies electrons for the reduction of Ag^+ is also electron donating. Despite being simplified representations of the complex phytochemical system, these equations provide a useful framework for understanding how compounds derived from citronella, along with other organic compounds, may act as substrates during AgNP synthesis [9], [10].

The process by which phytochemicals provide stability to AgNPs occurs after silver nuclei have been formed and begin to grow into small particles. Once the silver nuclei are created, phytochemicals adsorb onto the surface of the silver particles, where they attach via oxygen or nitrogen bonds, forming a "capping" layer of organic material. The capping layer prevents the accumulation and conglomeration of the silver particles through sterics and, in some cases, electrostatic repulsion. Furthermore, when the phytochemical capping agent contains hydroxyl or carboxyl groups,

alkaline conditions can promote their deprotonation, leading to an increase in the negative surface charge of the AgNPs and improved colloidal stability [11].

3 Green synthesis of *C. nardus*-mediated silver nanoparticles

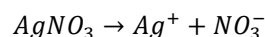
3.1 Preparation of plant extract

Harvesting and processing *C. nardus* are often the first steps in the synthesis process (Figure 1). Fresh leaves or aerial parts of the plant are generally rinsed with both tap and distilled water to eliminate soil, dust, and any surface contaminants. After cleaning, the plant material can be air-dried, shade-dried, dried in an oven at low temperature, or used fresh, depending on the design of the extract preparation process. The dried or fresh plant material is chopped, ground, or powdered to maximise surface area and enhance the efficiency of extraction [21].

Extraction can be carried out using distilled water, ethanol, methanol, or hydroalcoholic solvents. Aqueous extraction is often preferred during green synthesis due to safety, cost, and environmental considerations. However, the use of organic solvents such as ethanol or methanol may lead to the extraction of different phytochemicals, particularly less polar terpenoids and phenolic compounds. Because of this, it is important to note that the polarity of the extraction solvent can influence the yield and composition of the phytochemicals, which can ultimately affect the size, shape, and stability of the resulting nanoparticles [22].

3.2 Silver precursor and reduction process

Silver nitrate is a frequently employed precursor because of its propensity to dissociate easily in solutions containing water. The reaction involved in this process is as follows:



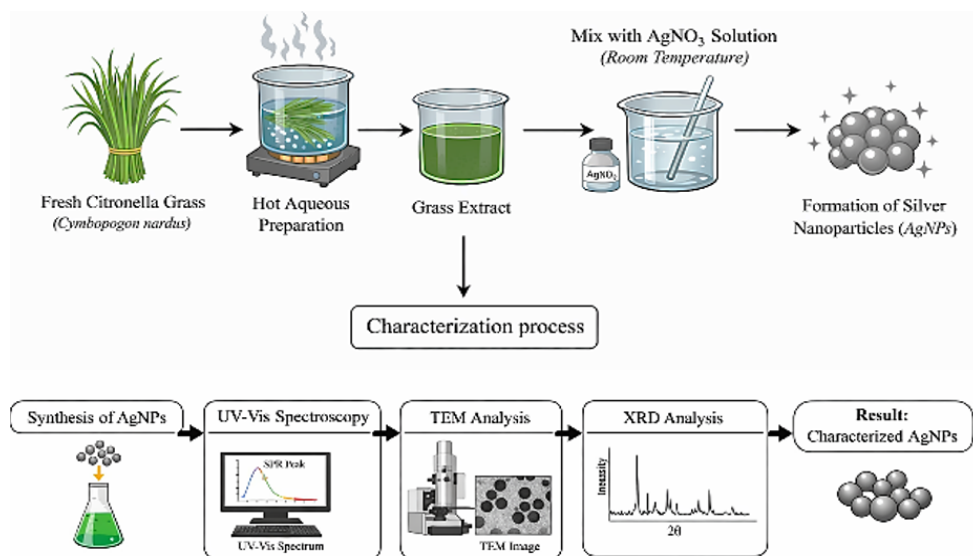
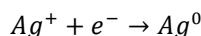


Figure 1. Green synthesis process of AgNPs using *C. nardus*.

Once silver ions are generated, they will interact with the phytochemicals present in the plant extract. The general reaction for the reduction of silver ions to metallic silver can be represented as follows:



Once sufficient metallic silver atoms have formed in the suspension, they aggregate into clusters called nuclei, which subsequently grow into metal nanoparticles (AgNPs) through the deposition of additional silver atoms onto existing particles and the fusion (coalescing) of individual particles to form larger metal particles. The presence of nanoparticles can often be observed through a change in the color of the liquid from light yellow or light brown to dark brown due to surface plasmon resonance (SPR). While the color change alone may not provide definitive confirmation of nanoparticle formation, it can serve as an initial indication that the reduction process has occurred [11].

3.3 Surface plasmon resonance and optical confirmation

AgNPs (Silver Nanoparticles) exhibit surface plasmon resonance properties due to the collective oscillation of their conduction electrons when illuminated with light, generating a strong absorption peak at approximately 400–450 nm in the UV-Vis spectrum for spherical AgNPs. Reported SPR peaks are typically found within this range

for *Cymbopogon*-based synthesis of AgNPs, indicating that the nanoparticles were most likely produced as near-spherical or spherical particles [23].

Generally, narrow and sharp SPR peaks indicate a relatively uniform particle size and minimal aggregation of AgNPs, whereas broadening or shifting of the SPR peak (e.g., toward longer wavelengths) usually indicates larger particle sizes, greater polydispersity, and/or aggregation of AgNPs. It should be stressed, however, that UV-Vis spectroscopic data cannot be used in isolation to determine the size and shape of AgNPs. Complementary techniques such as electron microscopy, dynamic light scattering (DLS), powder X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and zeta potential measurements are required for comprehensive nanoparticle characterization [24].

4 Influence of Synthesis Parameters on Particle Size, PDI, Morphology, and Stability

4.1 $AgNO_3$ precursor concentration

The concentration of $AgNO_3$ governs the relative abundance of Ag^+ ions available for reaction in solution. Lower precursor (0.5–1 mM) promotes nucleation and controlled growth, which often yields smaller and more uniform nanoparticles. Higher precursor concentrations (3 mM and above) not only accelerate the rate of reduction and increase absorbance but also often lead to excessive particle collisions, secondary nucleation, organic aggregation, and larger particles [25], [26].

4.2 Extract-to-precursor volume ratio

The ratio of plant extract to AgNO_3 influences the amount of reducing and capping molecules present in the reaction system. An increase in the amount of extract can increase the rate of reduction because more phytochemicals are available to donate electrons. It may also increase capping density, thereby limiting nanoparticle size and preventing their agglomeration. However, if too much extract is used, excessive organic material may be introduced into the system, which could lead to particle coalescence, significant broadening of absorbance peaks, and/or reduced colloidal stability [27], [28].

The relationship may be expressed conceptually as:

High extract ratio
→ *higher reducing power*
+ *higher capping density*
→ *smaller and more stable AgNPs*

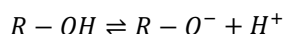
In contrast:

Low extract ratio → *insufficient capping*
→ *aggregation*
+ *larger particles*

4.3 pH

One of the most significant parameters associated with plant mediated silver nanoparticle (AgNP) synthesis is pH. The use of *Cymbopogon*-derived extracts for AgNP synthesis has been shown to be most effective under mildly alkaline to strongly alkaline conditions (approximately 8 - 11); indeed, many researchers have also reported that AgNPs are produced more rapidly, with smaller particle sizes and greater stability, within the pH range of 9-11 [29], [30].

Under alkaline pH conditions, deprotonation of hydroxyl and carboxyl groups occurs, increasing their electron-donating capability and improving the surface charge on these molecules:



The deprotonated molecules possess a greater reducing capability and can bind more effectively to nanoparticle surfaces, thereby promoting faster reduction of Ag^+ and enhancing the stability of the resulting AgNPs [4].

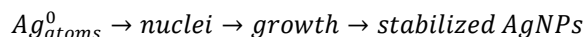
4.4 Temperature

Temperatures can alter synthesis kinetics by affecting molecular motion and the frequency of collisions between reactants, particularly Ag^+ ions and phytochemical reducing agents. Moderate heating, typically achieved within the range of 60-80 °C, can accelerate nucleation and reduce the time required for nanoparticle synthesis. However, excessive heating may result in the degradation of sensitive phytochemicals or the loss of volatile compounds present in essential oils through evaporation. Thus, determining the optimum temperature involves balancing an increased reaction rate with the preservation of phytochemicals responsible for the reduction and stabilization of the nanoparticles [31], [32].

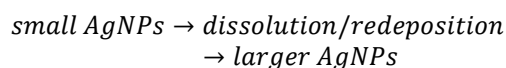
4.5 Reaction time

The time frame in which a reaction occurs can influence the extent of $\text{Ag}^+(\text{aq})$ reduction and, consequently, the growth behavior of AgNPs. Short-time reactions may not allow complete reduction of silver ions, resulting in lower nanoparticle yields. With longer reaction times, complete reduction may be achieved; however, the growth of AgNPs may be influenced by one of two phenomena: aggregation or Ostwald Ripening [33].

A simplified conceptual growth pathway:



Thus, if there is too much time between the start and end of the reaction:



4.6 Polydispersity index

The polydispersity index indicates how uniform the size distribution of nanoparticles is within a suspension. Generally, a PDI of less than 0.30 indicates that the nanoparticle suspension has a fairly uniform or monodisperse population of nanoparticles, whereas a PDI greater than 0.50 suggests a broad distribution of nanoparticle sizes and poor uniformity [34]. Low PDIs are especially important for agricultural applications because large variations in nanoparticle size can lead to variable antimicrobial activity and varying phytotoxicity effects.

While many studies have demonstrated trends regarding how variables affect one another (Table 2),

historically, little has been published regarding the quantitative relationships between different synthesis parameters and the resulting nanoparticle properties. For example, there are very few studies that provide either a kinetic model or a regression-based correlation that directly connects pH, precursor concentration, and extract volume to final particle size, polydispersity index (PDI), or morphology. The absence of a predictive model limits the ability to precisely replicate synthesis conditions and achieve consistent control over nanoparticle properties

5 Comparative Evidence Synthesis and Structure–Property–Activity Relationships

Given the limited number of studies assessing the impact of *Cymbopogon nardus* on the synthesis of silver nanoparticles, a comparative framework has been developed to provide a structured overview of evidence derived directly from *C. nardus*, as well as supporting evidence from other *Cymbopogon* species. This approach makes it possible to identify and evaluate consistent trends in synthesis behavior, nanoparticle characteristics, and antimicrobial activity while maintaining clarity regarding the limitations of species-specific data.

Table 3 outlines previously reported studies that have focused directly on the synthesis of silver

nanoparticles (AgNPs) using *C. nardus*. The table provides information on the synthesis conditions, the size of the resultant nanoparticles, and the antimicrobial activity of the AgNPs produced. The findings from these studies demonstrate that *C. nardus* extracts can produce AgNPs within the nanometer range generally associated with biological activity (approximately 10–30 nm in size). Furthermore, the surface plasmon resonance (SPR) peaks of the AgNPs synthesized using *C. nardus* extracts are consistently observed within the typical wavelength range of 400–450 nm. These findings indicate that the phytochemicals present in citronella possess sufficient reducing and stabilizing capabilities to facilitate the production of nanoparticles with characteristics comparable to those synthesized using other plant-based systems.

Due to the limited number of direct studies, broad conclusions cannot yet be drawn regarding *C. nardus*-mediated synthesis. However, by incorporating comparative data from other *Cymbopogon* species, including *C. citratus* and other related taxa that have been studied more extensively, several trends can be identified (Table 4). Across these studies, alkaline pH conditions (pH range 9–11) are consistently associated with smaller and more stable nanoparticles due to faster reduction rates and a greater number of available functional groups resulting from deprotonation. Similarly, lower

Table 2. Factors influencing the characteristics and efficiency of the synthesis of AgNP from *C. nardus*.

Parameter	Effects on Synthesis	Range	References
Concentration of AgNO ₃	The higher the concentration, the higher the absorbance, the faster the reaction, as well as the NPs tend to be larger and vice versa	0.01 - 5 mM	[35], [30], [36]
Volume Ratio	The higher the extract, the faster the rate of reduction, leading to more stable and smaller NPs. This is due to the high concentration of the capping agents present in the extract	1:5 - 1:27	[37], [35], [38]
Temperature	Typically, at higher temperatures (>60°C), it accelerates the rate of reaction and favors a smaller size of NPs due to faster nucleation	27 - 80°C	[37], [39], [23]
pH Level	AgNP synthesis favors alkaline conditions (9, 10, 11), which leads to more uniform and stable NPs	9 -11	[30], [38]
Reaction Time	A shorter time may result in incomplete reduction and a much lower yield. On the other hand, long or extended time allows complete reduction but may result in larger particles. Optimization of reaction time is necessary. It can also vary depending on if its machine-mediated or at room temperature	30 min -24 hr	[30], [38]

Table 3. Studies on *C. nardus*-derived AgNP showing direct synthesis and anti-microbial performance.

Study	Plant Source	Size	SPR	Target	Activity	Mechanism	Application
[40]	<i>C. nardus</i> EO	—	400–450	—	8.67% yield	Geraniol reduction	Scalable production
[21]	<i>C. nardus</i> leaf	~15.7 nm	435	<i>E. coli</i> , <i>S. aureus</i>	Strong inhibition	ROS + Ag ⁺	Antimicrobial stability

Table 4. Comparative data from other *Cymbopogon* spp. to identify consistent trends.

Study	Plant Source	Size (nm)	SPR (nm)	Target	Activity	Mechanism	Application Relevance
[36]	<i>C. citratus</i>	19	440	<i>P. theae</i>	100% inhibition	ROS + membrane damage	Fungicide alternative
[41]	<i>C. citratus</i>	10–100	—	<i>Candida albicans</i> and <i>Streptococcus mutans</i>	99.66% of <i>S. mutans</i> and 99.56% of <i>C. albicans</i>	Cell membrane and mitochondria disruption	Antimicrobial
[42]	<i>C. citratus</i>	~40	432	Multiple bacteria	ZOI up to 34.67 mm	DNA interaction	Broad-spectrum control
[43]	<i>C. proximus</i>	15–45	448	Bacterial pathogens	27 mm ZOI	Cell wall disruption	Sustainable antimicrobial
[29]	<i>C. citratus</i>	~39	411	MDR bacteria	Strong ZOI	Enzyme inhibition	Reduce chemical inputs
[44]	<i>C. citratus</i>	13–30	420	<i>E. coli</i> , <i>S. aureus</i>	MIC 15–35 µg/mL	ROS + DNA damage	Eco-friendly pathogen control

precursor concentrations and higher extract-to-metal ratios promote controlled nucleation and reduced aggregation, thereby producing nanoparticles with narrower size distributions. Surface plasmon resonance peaks are consistently reported within the 400–450 nm range across all studies, suggesting similar optical properties and generally comparable nanoparticle morphologies regardless of the *Cymbopogon* species used for synthesis. The range of 400–450 nm for all conducted studies indicates that there is no difference in the morphology of the size of the nanoparticles, nor their optical properties, regardless of the species of the plant that was used in the synthesis. The findings from these studies are summarized in Table 5, which provides an overview of how different synthesis conditions influence nanoparticle properties and antimicrobial activity.

These observations indicate a clear relationship between nanoparticle structure, physicochemical properties, and biological activity. Synthesis conditions (e.g., pH, precursor concentration, and extract-to-metal ratio) influence nucleation rates and growth dynamics, which in turn determine nanoparticle characteristics such as size, morphology, and polydispersity. These characteristics ultimately govern the biological activity of the synthesized nanoparticles. In general, nanoparticles smaller than

50 nm exhibit greater antimicrobial activity due to their higher surface area, enhanced interaction with microbial cell membranes, and increased capacity to release silver ions and generate reactive oxygen species.

Although these trends are relatively consistent across studies, several limitations must be considered. Variations in experimental design, including extraction methods, microbial test organisms, assay conditions, and reporting approaches, limit the ability to make direct quantitative comparisons among studies. Additionally, many studies do not report sufficient statistical information, such as standard deviations or polydispersity index (PDI) values, reducing the reliability of cross-study comparison. Finally, while comparative evidence from related species provides valuable insights into general trends within the *Cymbopogon* genus, caution should be exercised when extrapolating these findings to *C. nardus* because of the limited number of direct investigations available. Based on the synthesis of findings presented in Table 1-3, it can be concluded that a consistent structure-property-activity relationship exists in the *Cymbopogon*-mediated AgNP system. However, more systematic studies focusing specifically on *C. nardus* are needed to validate these relationships, particularly in the context of plant disease management.

**Table 5.** Consolidated trends linking nanoparticle properties to antimicrobial activity and application.

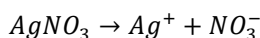
Parameter	<i>C. nardus</i>	Supporting Species	Key Insight
Size	~15 nm	13–95 nm	Most effective range <50 nm
SPR	400–450 nm	411–448 nm	Consistent nanoparticle formation
Activity	Moderate–high	High	Strong antimicrobial performance
Mechanism	ROS + Ag ⁺	Same	Conserved across species
Application	Early-stage	Field + lab validated	Strong translational potential

*Due to the limited number of studies specifically investigating *C. nardus*-mediated AgNPs, selected studies from closely related *Cymbopogon* species were included to support comparative quantitative analysis.

6 Mechanistic Pathway of Phytochemical-Mediated AgNP Formation

6.1 Dissociation of silver nitrate

The process begins with the dissociation of silver nitrate:

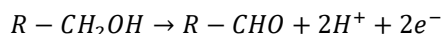


The Ag⁺ ions act as a metal precursor for the formation of silver nanoparticles [11].

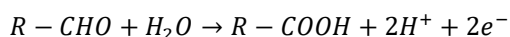
6.2 Oxidation of phytochemicals and electron release

Citronellal, geraniol, and similar substances are capable of donating electrons through the oxidation of their aldehyde or alcohol functional groups [45]–[47]. The electrons produced from the oxidation of geraniol-type alcohols and citronellal-type aldehydes facilitate the reduction of Ag⁺ to Ag⁰ (Figure 2). The reactions involved in these electron-transfer processes are as follows:

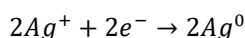
Oxidation of Geraniol-like Alcohols:



Oxidation of Citronellal-like Aldehydes:



The electrons released from the oxidation of geraniol- or citronellal-type compounds drive the following silver reduction reaction:



Thermodynamically, the standard reduction potential (E°) of Ag⁺/Ag⁰ (+0.80 V) indicates that these oxidation-reduction reactions are thermodynamically favorable. However, quantitative redox potentials for specific phytochemicals such as citronellal and geraniol have not been extensively reported under nanoparticle synthesis conditions. Consequently, many mechanistic explanations remain theoretical and have yet to be validated through electrochemical or computational studies.

6.3 Nucleation and growth based on the LaMer model

Ag⁰ (silver atoms) build up in solution once reduced from silver ions, when their concentrations reach the point of supersaturation or the threshold level required for particle nucleation. According to the LaMer model of nanoparticle formation, there are three main stages to nanoparticle formation, three major stages occur during nanoparticle synthesis: monomer generation, burst nucleation, and controlled growth [48]. Rapid reduction creates a large number of nuclei within a very short time frame, resulting in smaller particle sizes. Conversely, slow reduction produces fewer nuclei but allows much greater growth of each particle, thus producing larger particle sizes.

This can be summarised as:

Fast reduction → many nuclei

→ smaller AgNPs

Slow reduction → fewer nuclei → larger AgNPs

6.4 Capping and stabilization

Following nucleation and growth, phytochemicals become adsorbed onto the nanoparticle surface, forming a protective organic layer through interactions with silver atoms.

Reduction of Ag^+ to Ag^0

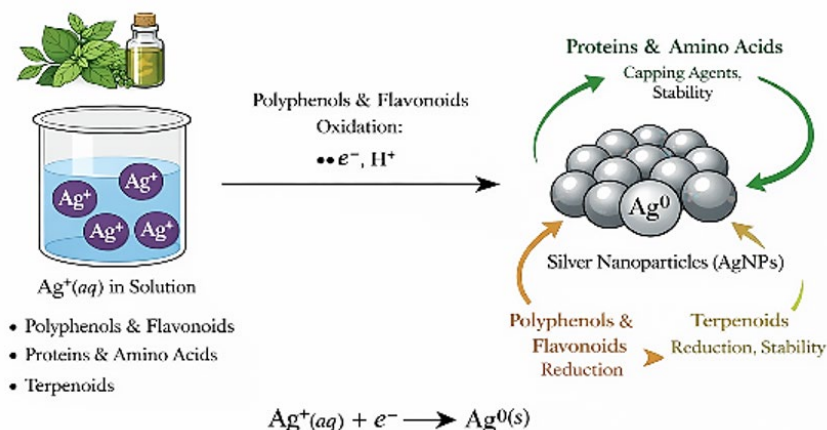
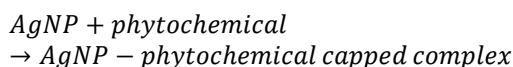


Figure 2. Schematic representation of the simplified view of the reduction of Ag^+ to Ag^0 .

Oxygen-containing functional groups, particularly hydroxyl ($-\text{OH}$) and carbonyl ($-\text{C}=\text{O}$) groups, interact with the silver surface and form an organic corona around the nanoparticles. This organic corona provides steric stabilization through its physical presence and contributes to electrostatic stabilization through the deprotonation of surface-bound functional groups [27], [49], [50].

The process of stabilizing the silver nanoparticles via either protonation or deprotonation can be represented conceptually as follows:



Therefore, this capping layer is critical for stabilizing the nanoparticles, as uncoated nanoparticles possess an elevated surface energy and have a tendency to aggregate. However, to directly validate the binding affinity and stabilization energy of functional groups on the surface of silver nanoparticles, it requires advanced analytical techniques such as XPS (X-ray photoelectron spectroscopy) or molecular docking studies, as very limited validation of functional group binding and stabilization on *C. nardus*-mediated AgNPs has been accomplished, and most interpretations rely on indirect FTIR data.

6.5 Spectroscopic validation

Several characterization techniques can substantiate the proposed mechanism. AgNP formation and subsequent development into more complex metallic nanoparticles

can be verified by UV-Vis spectroscopic data showing the existence of the distinctive surface plasmon resonance (SPR) peak between 400 and 450 nm. FT-IR can be employed to demonstrate changes in the functional groups involved in nanoparticle reduction and capping. The crystalline structures of metallic silver can be compared by applying X-ray diffraction (XRD), whereas the oxidation states of silver can be calculated through the use of X-ray photoelectron spectroscopy (XPS) by assessing Ag 3d peaks, typically observed near 368 eV (metallic silver) to 374 eV (oxidized silver). The morphology and core size of the AgNPs can be supported by scanning and transmission electron microscopy (SEM and TEM, respectively) with colloidal behavior in a suspension and evaluated by means of dynamic light scattering (DLS) and Zeta potential measurements [51].

7 Characterization of *C. nardus* -mediated Silver Nanoparticles

7.1 UV-visible spectroscopy

Surface plasmon resonance allows the monitoring of AgNPs formation using UV-Vis spectrometry. The SPR peak of AgNPs typically falls in the range of 400–450 nm. A narrow SPR peak indicates that the nanoparticles (NPs) are consistent in shape and stable. In contrast, a broader peak suggests that the NPs have aggregated or exhibit a wider size distribution. The SPR peaks (420–435 nm) measured for *C. nardus*-mediated AgNPs suggest that the NPs are predominantly spherical or nearly so [21], [40].



Table 6. FTIR band assignments of *Cymbopogon*-mediated AgNPs showing functional group involvement in reduction and stabilization.

Wavenumber (cm ⁻¹)	Plant Extract Spectrum	AgNP Spectrum	Assignment	Interpretation	Reference
~3200–3500	Broad O–H peak	Shifted/broadened	O–H stretching (phenols/alcohols)	Involved in reduction and capping	[21]
~2850–2950	Present	Reduced intensity	C–H stretching (alkanes)	Organic backbone of phytochemicals	[21], [35]
~1650–1750	Strong peak	Shifted	C=O stretching (aldehydes/ketones)	Oxidation during Ag ⁺ reduction	[40]
~1500–1600	Present	Slight shift	C=C stretching (aromatic)	Structural stabilization	[53]
~1350–1450	Present	Slight shift	C–N stretching / bending	Binding to nanoparticle surface	[30], [35]
~1000–1200	Present	Shifted	C–O stretching	Interaction with AgNP surface	[54], [35]

7.2 Fourier transform infrared spectroscopy

FTIR spectroscopy is used to identify functional groups involved in the reduction and stabilization of *C. nardus*-mediated AgNPs (Table 6). Bands in the range of 3200 to 3500 cm⁻¹ are generally attributed to O–H stretching from alcohols and/or phenolic compounds. Those in the range of 2850–2950 cm⁻¹ are most commonly associated with C–H stretching (aliphatic). Carbonyl C=O from carbonyls (aldehydes, ketones, esters, or carboxylic acids) typically occurs around 1650–1750 cm⁻¹. C–O stretching vibrations are generally found between 1000–1200 cm⁻¹, whereas bands near 1350–1450 cm⁻¹ may result from C–H bending, C–N stretching, or a combination of both, depending on the composition of the extract [21], [52].

7.3 SEM and TEM

Nanoparticle size and morphology can be determined using a scanning electron microscope (SEM) and a transmission electron microscope (TEM). SEM provides information regarding the surface morphology of nanoparticles, whereas TEM provides more detailed information on particle shape and core size at the nanoscale level. In most studies involving plant-mediated synthesis of AgNPs, the nanoparticles are reported to be spherical or quasi-spherical in shape. For agricultural antimicrobial applications, AgNPs with particle sizes ranging from 10–50 nm are generally considered optimal due to their high surface area and enhanced ability to interact with microbial cells [55]–[57].

7.4 XRD

X-ray diffraction (XRD) analysis is used to confirm the crystalline nature of AgNPs. Metallic silver nanoparticles typically exhibit characteristic face centered cubic (FCC) diffraction peaks corresponding to the (111), (200), (220), and (311) crystallographic planes. Sharp diffraction peaks indicate formation of crystalline AgNPs, whereas broader peaks may indicate smaller crystallite sizes. Crystallite size can be calculated via the Debye-Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos \theta}$$

Where D is the crystallite size, K is the shape factor (typically 0.9), λ is the X-ray wavelength, β is the full width at half maximum and θ is the Bragg angle [58], [59].

7.5 DLS and Zeta potential

Dynamic light scattering (DLS) provides information on the hydrodynamic diameter of nanoparticles, which is typically larger than the particle size measured by TEM because it includes the metallic core, phytochemical capping layer, and surrounding hydration shell. Polydispersity index (PDI) values below 0.3 generally indicate a relatively uniform nanoparticle population [60].

The measurement of the molecular stability and molecular charge of the particles in suspension can be assessed through the Zeta Potential or Zeta Value (ζ).

The general meaning of the Zeta Value is as follows:

- | ζ | > 30 mV = strong electrostatic stability
- | ζ | = 20 – 30 mV = moderate stability
- | ζ | < 20 mV = weak stability or aggregation risk

For AgNPs produced from *C. nardus*, available physicochemical and bioactivity data suggest that nanoparticle stability and surface charge are strongly influenced by synthesis conditions and the dispersion medium. Although detailed Zeta Potential data are not routinely presented in published research studies, similar *Cymbopogon*-based AgNPs typically exhibit negative Zeta Values ranging from -37 mV to between -3mV, and the range of Zeta Values is chemically reasonable, with -37 mV exhibiting a high level of stabilisation (e.g., capping and use of alkaline medium) and -3mV exhibiting a low level of electrostatic repulsion which allows for an increase in the propensity to form aggregates. [21], [23], [40].

8 Antimicrobial mechanisms of *C. nardus*-mediated AgNPs

8.1 General multi-target mechanism

AgNPs exhibit broad-spectrum antimicrobial activity because they act through multiple biocidal mechanisms (Figure 3). These mechanisms include membrane disruption, the release of dissolved silver ions (Ag^+), the generation of reactive oxygen species (ROS), inhibition of intracellular enzymes, damage to DNA, and the contribution of bioactive phytochemicals associated with nanoparticle capping layers. In the case of *C. nardus* -mediated AgNPs, phytochemicals such as citronellal, geraniol, and citronellol may further enhance antimicrobial activity because these compounds themselves possess antibacterial and antifungal properties [61], [62].

A simplified sequence of antimicrobial action can be represented as follows:

AgNPs → microbial attachment
→ membrane disruption
→ Ag^+ release
→ ROS generation
→ metabolic failure
→ cell death

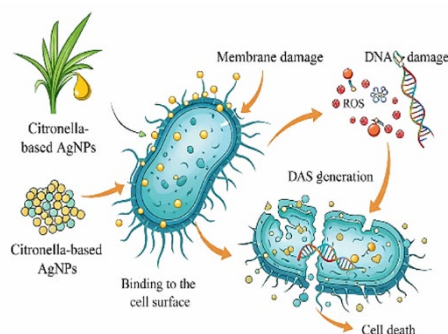
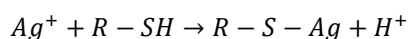


Figure 3. Antimicrobial mechanism of citronella-based AgNP against phytopathogen.

8.2 Mechanism of antibacterial action

The antibacterial activity of AgNPs begins with their attachment to the bacterial cell surface. Interaction with the cell envelope compromises membrane integrity, resulting in increased membrane permeability and leakage of proteins, ions, and other intracellular components. In addition, Ag^+ ions released from AgNPs can bind to thiol ($-\text{SH}$) groups present in proteins and enzymes through the following reaction:



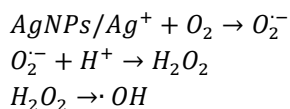
This interaction modifies enzyme structure and inhibits key metabolic pathways, including electron transport and ATP synthesis. Silver ions (Ag^+) can also interact with DNA and ribosome components during protein synthesis, leading to inhibition of these processes. Gram-negative bacteria are generally more susceptible to AgNPs than Gram-positive bacteria because their thinner peptidoglycan layer provides a less effective barrier between AgNPs and the cell membrane. However, susceptibility remains dependent on factors such as nanoparticle size, surface charge, coating composition, and the microbial species being evaluated [63].

8.3 Mechanism of antifungal action

The thicker and more complex structure of the fungal cell wall, which is composed primarily of chitin, glucans, and mannoproteins, suggests that AgNPs may exert antifungal effects through surface interactions, reactive oxygen species (ROS) generation, lipid peroxidation of cell membranes, and inhibition of spore germination and hyphal growth. ROS generated in the presence of AgNPs include superoxide radicals, hydroxyl radicals, and hydrogen peroxide, all of which can damage lipids, proteins, and nucleic acids.



The ROS-mediated antifungal mechanism can be represented as follows:



These reactive oxygen species initiate oxidative damage to fungal membranes and intracellular organelles, ultimately leading to reduced mycelial growth, decreased spore germination, and cell death. In addition, phytochemicals such as citronellal and geraniol may contribute to antifungal activity by disrupting membrane integrity and interfering with ergosterol-related pathways [64], [65].

8.4 Phytochemical–silver synergy

The potential synergistic interaction between AgNPs and citronella-derived phytochemicals is promising; however, its implications should be interpreted with caution. The phytochemical corona surrounding AgNPs may facilitate interactions between nanoparticles and microbial cell surfaces by enhancing nanoparticle stability, reducing aggregation, and delivering bioactive compounds closer to the site of infection. Despite these observations, the current understanding of phytochemical–AgNP interactions remains largely descriptive. Consequently, claims of synergy should be regarded as plausible mechanisms rather than fully validated conclusions supported by direct experimental evidence. There is still limited experimental evidence differentiating between the major antimicrobial mechanisms of action of AgNPs from *C. nardus*, such as reactive oxygen species (ROS) generation versus bacterial membrane disruption. Most conclusions regarding the antimicrobial effects of AgNPs derived from *C. nardus* are based on inferences made from studies of other AgNPs and not from species-specific experimental evidence.

9 Application in plant disease management

9.1 Relevance to phytopathogenic fungi and bacteria

AgNPs show potential for plant disease management due to the sensitivity of many pathogens (fungi/bacteria) to oxidative stress, membrane damage, and ion-related toxicity. Examples of fungal pathogens include *Fusarium*, *Magnaporthe*, *Alternaria*, *Aspergillus*, and *Penicillium*. The use of AgNPs on many of these pathogens

has been shown to prevent spore germination, mycelial growth, and/or toxin production [66], [67]. Similarly, AgNPs inhibit bacterial plant pathogens through membrane damage, disruption of cellular metabolism, and interference with essential biochemical processes [68]–[70].

9.2 Seed treatment

Seed treatment represents one of the most practical applications of AgNPs because of the small amount required to treat each seed (due to seed size). Evidence suggests that AgNP application can reduce the incidence of seed-borne pathogens and improve seedling vigor. However, concentration is a critical factor. Low concentrations of AgNPs may promote seed germination and early seedling growth, whereas excessive concentrations can inhibit root development and induce oxidative stress [71].

9.3 Foliar spray

Foliar application of AgNPs may be an effective method for controlling several plant diseases, such as leaf spot, blight, and rust, by providing contact between these pathogens and AgNPs on the surface of leaves. Proper formulation of AgNPs for foliar application will be necessary to prevent aggregation, create adhesion to the leaf surface, and prevent phytotoxicity [72].

9.4 Soil drench

Soil application of AgNPs provides a potential means of controlling soil-borne pathogens; however, it also presents greater environmental risks due to interactions between AgNPs and soil organic matter, clay minerals, salts, and microbial communities. In addition, aggregation and transformation of AgNPs in soil may reduce their efficacy, while excessive accumulation could adversely affect beneficial soil microorganisms [73].

9.5 Dose–response and percent inhibition

Radial growth inhibition for antifungal tests is commonly calculated by means of using the following equation:

$$\text{Percent Inhibition} = \frac{C - T}{C} \times 100$$

where C = the colony diameter of the control and T = the colony diameter of the treatment.

Future studies should report on agriculturally relevant parameters such as EC50, MIC, MFC, phytotoxic concentrations, reduction of disease severity, and yield response for agricultural relevance. Any dose of active ingredient or product that effectively inhibits the growth of pathogens, while causing damage to the plant, is not considered to be agriculturally relevant [74].

10 Phytotoxicity, dose thresholds, and plant response

Plants often respond to AgNPs in a biphasic manner. At low concentrations, AgNPs may stimulate germination, root growth, or enzymatic activity, whereas higher concentrations may induce phytotoxicity by increasing ROS production and damaging chloroplasts, resulting in reduced chlorophyll content and inhibited root and shoot growth [75], [2].

Generally, the hormone response curve for plants can be described as follows:

Low dose → stimulation

High dose → inhibition/toxicity

In many systems, low AgNP concentrations are either beneficial or well tolerated, whereas higher concentrations increase the risk of phytotoxicity. These responses depend on crop species, nanoparticle size, surface coating, and duration of exposure. For example, a recent tomato study [68] reported improved germination and growth at concentrations of 5–20 ppm, whereas chlorophyll and carotenoid contents declined at 25–50 ppm. This finding suggests the existence of a crop-specific "window of effectiveness" rather than a universally applicable concentration range. Similarly, studies involving other crops have reported beneficial responses within the 20–40 ppm range, further demonstrating that optimal AgNP concentrations vary among plant species. Important indicators of plant response include germination percentage, root length, shoot length, chlorophyll content, malondialdehyde (MDA) concentration, antioxidant enzyme activity, reactive oxygen species (ROS) levels, total biomass, disease severity, and crop yield [76]–[78].

11 Colloidal stability and Zeta potential in agricultural systems

Colloidal stability is critical for maintaining nanoparticle surface area and preserving their

physicochemical properties. In the case of colloidal silver (AgNPs), their stability in aqueous solution is governed primarily by the amount of surface charge and the existence of protective agents (capping agents). While they may remain stable in pure water, the complexity of agricultural systems might affect the stability of AgNPs. For example, irrigation water, fertilizers, salts, soil particles, organic matter, and pH changes can all influence the stability of AgNPs [79], [80]. Zeta potential is commonly used to assess the electrostatic stability of nanoparticles. The thresholds are generally considered as:

$|\zeta| > 30 \text{ mV} = \text{high stability}$

$|\zeta| = 20 - 30 \text{ mV} = \text{moderate stability}$

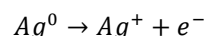
$|\zeta| < 20 \text{ mV} = \text{low stability}$

Though plant-mediated AgNPs may depend not only upon electrostatic stabilization but could also exhibit a degree of steric stabilization due to phytochemical capping. Electrosteric stabilization can occur when the capping layer retards aggregation even in the presence of a low zeta potential [49].

12 Environmental fate and transformation pathways

Nanoparticles do not remain unchanged once they are placed into use, as they undergo dissolution, sulfidation, aggregation, oxidation, and formation of an eco-corona (sulfidation converts elemental silver into, among other compounds, silver sulfide, which is less soluble than elemental silver; thus, the dissolution process produces the soluble silver ions that have some antimicrobial activity but may also affect non-target organisms). These transformations affect AgNP antimicrobial effectiveness, mobility, bioavailability, toxicity, and persistence of AgNPs (Table 7).

Dissolution releases Ag^+ ions:



The formation of the ionic silver creates silver ions that provide antimicrobial activity, but they may pose a risk to non-target organisms. Sulfidation of silver can also provide some toxicity reduction due to the oxidative reduction of silver to silver sulfide (Ag_2S):

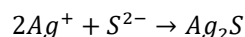


Table 7. Summary of environmental transformation.

Process	Environmental Driver	Impact on Bioavailability	Impact on Toxicity
Dissolution	High O_2 , Low pH	Increase (Active Ag^+)	Increase
Sulfidation	Anaerobic soil, S-residues	Major decrease	Major decrease
Aggregation	High salinity (Ca^{2+} , Mg^{2+})	Decrease (Lower mobility)	Decrease
Eco-Corona	Humic/fulvic Acids	Variable	Variable (Often masks toxicity)

Because silver sulfide is less soluble than ionic silver, sulfidation generally reduces both toxicity and antimicrobial activity. Aggregation may occur when salts, dissolved ions, or organic matter reduce the repulsive forces between nanoparticles, causing them to cluster together. Eco-corona formation occurs when natural organic matter, proteins, or soil-derived compounds adsorb onto AgNP surfaces, altering their physicochemical behavior, environmental fate, and biological interactions [81], [82].

13 Reproducibility, batch variability, and statistical validation

Reproducibility is a significant limitation of the field of plant-mediated AgNP research. The chemical composition of plant extracts can be chemically complex and can vary based on several parameters, including plant age, harvest time, geographical origin, extraction method, drying procedure, solvent type, and storage conditions. These factors can influence both the reducing power and capping efficiency of the extract.

To improve reproducibility, future studies should report the results of independent synthesis batches separately. Nanoparticle characterization should include the number of independent synthesis batches, mean particle diameter, particle size standard deviation, polydispersity index (PDI), zeta potential, UV-Vis peak position, and appropriate statistical comparisons among batches.

For nanoparticle size analysis using TEM images, at least 100 particles should ideally be measured to obtain representative size distributions. Similarly, antimicrobial assays should report inhibition zone diameters for all experimental replicates, together with the mean and standard deviation. Statistical analyses should include appropriate procedures such as analysis of variance (ANOVA) followed by suitable post hoc tests.

For antifungal assays:

$$\text{Percent Inhibition} = \frac{C - T}{C} \times 100$$

For the coefficient of variation:

$$CV(\%) = \frac{SD}{Mean} \times 100$$

A low CV indicates better reproducibility.

14 Scale-up and practical deployment

The technical, economic, or regulatory challenges associated with scaling up *C. nardus*-mediated AgNP synthesis must be addressed before commercial deployment becomes feasible. While laboratory-scale synthesis allows precise control of small reaction volumes, larger-scale production introduces challenges related to mixing efficiency, reaction kinetics, temperature gradients, and variability in extract composition. These factors can lead to differences in nanoparticle size, morphology, and stability.

The extraction process also leads to difficulties in the standardization of plant extracts. Variations in the levels of citronellal, geraniol and other phytochemicals may arise due to differences in plant source material and extraction methods. Consequently, industrial-scale production may require routine characterization of extracts before AgNP synthesis. Analytical techniques that may assist in the standardization of the reducing potential of the extract include GC-MS, HPLC, FTIR and total phenolic content.

Continuous-flow synthesis may provide superior results in comparison with batch synthesis because of better control of miscibility, temperature and reaction duration. Waste valorization could utilize spent citronella biomass remaining after essential oil extraction, allowing recovery of water-soluble phytochemicals for nanoparticle production.

However, the regulatory classification that products produced using AgNPs within the agricultural sector will ultimately be classified into has yet to be determined. Depending on the jurisdiction, such products may be regulated as pesticides, nanomaterials, or plant protection products, each of which is subject to different legal and safety

requirements. Consequently, comprehensive data regarding product residues, crop safety, environmental persistence, food-chain transfer, and effects on non-target organisms will be required before commercial approval can be obtained.

15 Future research directions

Future research should emphasize acquiring direct evidence of AgNP production mediated by *C. nardus* over indirect evidence based on inherent properties associated with each of the AgNP-producing species studied to date. The principal objective of this research will be mechanistic resolution, including the identification of the specific compound(s) that are oxidized (i.e. the reducing agent) when oxidizing Ag^+ to Ag^0 via reduction. Mechanistic resolution can be accomplished via the use of gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), Fourier transform infrared spectroscopy (FTIR) and reflection X-ray photoelectron spectroscopy (XPS) and time-resolved UV-visible spectroscopy. For the standardization of synthetic optimization, studies should utilize both factorial experimental designs and response surface methodology to determine the effect of the following factors on AgNP characteristics: pH, concentration of AgNO_3 , ratio of extract to AgNO_3 , temperature, and time of synthesis. Response variables of interest will be particle size, polydispersity index (PDI), zeta potential, surface plasmon resonance peak (SPR peak), yield, and antimicrobial activity. In addition, mechanism-resolved antimicrobial studies will require the assessment of ROS production, Ag^+ release, membrane disruption, germination of fungal spores, morphological variation of fungal hyphae and viability of pathogens tested. Assays designed for plant disease management should go beyond assaying “generalist” consumables (i.e. random samples) that potentially may be aggressively subject to strict levels of scrutiny) and focus only on commercially significant pathogens within agriculture to ensure accuracy and accountability. Additionally, with respect to crop safety, experiments utilizing a dose–response methodology should be employed to establish the effective dosing of AgNPs for antimicrobial activity and for the determination of the margin of safety for phytotoxicity. Field trials for studying disease control will need to assess both: i) disease severity; ii) yield/production; and iii) residue/non-target effects. Finally, it will be essential for researchers to study the environmental fate of AgNPs

produced by *C. nardus*, including dissolution, sulfidation, adsorption to environmental material (e.g. soils), and formation of “eco-corona” in naturally occurring soils and irrigation or rainwater.

16 Conclusion

Cymbopogon nardus-mediated (citronella) silver nanoparticles represent a viable novel form of green nanotechnology for managing plant diseases via environmentally benign methods. The high levels of phytochemical compounds found in citronella (e.g., oxygenated monoterpenoids such as citronellal, citronellol, geraniol, and citral) enable the reduction of silver ions and stabilization of silver nanoparticles.

The mechanisms involved in the synthesis of silver nanoparticles through *C. nardus* extracts can be explained by five stages: AgNO_3 dissociation, phytochemical oxidation (of the monoterpenes), Ag^+ reduction to Ag^0 , nucleation, and growth and capping of the nanoparticles. The suitable conditions for the synthesis of nanoparticles include controlling the AgNO_3 concentration, the ratio of extract to precursor, the pH (approximately 9–11), temperature, and the duration of the reaction, which favorably influence the production of small, stable, and biologically active nanoparticles.

Characterization should utilize multiple techniques, including UV-Vis, FTIR, SEM/TEM, XRD, DLS, and zeta potential to verify nanoparticle formation, surface chemistry, morphology, crystallinity, size distribution, and stability. *C. nardus*-mediated silver nanoparticles possess multiple modes of antimicrobial action that may positively contribute to their anticancer activity, including membrane disruption, silver ion release, generation of reactive oxygen species, inhibition of metabolic processes and possible silver-phytochemical synergism.

However, caution should be taken when interpreting these findings, as few studies have focused specifically on *C. nardus* and its silver nanoparticles. When utilized as seed treatments, foliar sprays, or soil-applied agents in plant disease management, optimal dosing and evaluation of phytotoxicity are critical.

Before agricultural application of *C. nardus*-mediated silver nanoparticles can occur, critical gaps remain, including reproducibility, statistical validation, environmental fate, feasibility of large-scale production, regulatory classification, and field performance. *C. nardus*-mediated silver nanoparticles show great promise as a new and sustainable means of controlling plant diseases. However, to ensure these

nanoparticles can be effectively used, substantial direct evidence, development of standardized protocols for use, and continued testing for effectiveness and safety are necessary. Before large-scale field application, major unanswered questions that will need to be addressed regarding the current use of *C. nardus*-mediated AgNPs include: reproducibility, statistical validation, environmental fate, scale-up feasibility, regulatory classification, and field-level performance. Overall, *C. nardus*-mediated AgNPs represent a sustainable, emerging method of protecting crops and future development depends upon obtaining stronger direct scientific evidence, developing industry-standard protocols, and critical evaluation of both efficacy and safety.

Acknowledgments

The authors express their gratitude to the Department of Science and Technology - Science Education Institute (DOST-SEI) for the graduate scholarship and financial support under the Accelerated Science and Technology Human Resource Development Program (ASTHRDP), which facilitated the publication of this review article.

Author Contributions

A.G.A.B.: conceptualization, writing, creation of original draft, formal analysis, resources, writing, reviewing and editing the manuscript; R.T.A.: reviewing and editing the manuscript, and supervision of the project; D.S.P.: reviewing and editing the manuscript; S.P.K.: reviewing and editing the manuscript. All authors have read and agreed upon all the contents of the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors utilized the ChatGPT tool to enhance the language and readability of the manuscript.

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