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PLANT-MEDIATED SILVER NANOPARTICLES: ANTIBACTERIAL ACTIVITY AND THEIR INFLUENCE ON PLANT GROWTH CHARACTERISTICS

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Abstract

Metal nanoparticles (MNPs) have a range of appealing physical and chemical properties to produce antimicrobial agents. The growing demand for green MNPs has received significant research attention to developing efficient, quick, affordable, and environmentally sustainable methods of MNPs synthesis. Silver nanoparticles (AgNPs) are among the most frequently investigated noble MNPs known for their potential in antimicrobial applications. The plant extracts as reducing and capping agents to obtain AgNPs and are favoured due to their efficient and non-hazardous activity. A wide range of AgNPs bioactivities has shown their capability to combat different microbial resistance issues. Plant-mediated AgNPs are an easy and sustainable method to produce noble antimicrobial NPs. On the other hand, these NPs have positively or negatively impacted the plant growth parameters. In this review, we highlight the plant-mediated synthesis of AgNPs, their potential in antimicrobial activity for various fields and their influence on plant growth parameters.

INTRODUCTION

Nanotechnology fosters a great sense of excitement in several life sciences, especially in biotechnology and biomedical tools [1-3]. Feynman [4] first articulated the notion of nanotechnology, which outlined the likelihood of synthesis by direct atom manipulation. Modern nanotechnology is constructing atomic-scale functional systems using materials with a size range between 1 to 100 nm [5]. Recently, green nanomaterials have gotten great interest as their characteristics and structure differ significantly from bulk materials [6]. The interaction between nanoparticles (NPs) and biological resources results in novel nanomaterials with regulated size, shape, texture, and surface chemistry [7]. Due to their small size, NPs are widely embracing all disciplines of scientific research such as surface science, functional biology, electronics, semiconductor physics, microfabrication and organic chemistry [8].

Synthesis of desired nanoscale materials is one of the most exciting elements of contemporary nanoscience and nanotechnology owing to their diverse use in material sciences, biomedical engineering, molecular biology, medical tools and electronics [9]. Many scientific experimental techniques used physical and chemical methods to synthesise metal nanoparticles (MNPs) [10]. However, these techniques employ expensive chemicals and may release toxic chemical elements accumulated on the surface of NPs, adversely affecting many biological and medical uses [11]. This amplifies the growing demands for environmentally friendly NPs synthesis via "green synthesis" and other innovative techniques.

Green nanotechnology seems to be of great relevance in manufacturing NPs products that are safe for all beings and have profitable long-term viability [12]. Due to their exceptional optical, chemical, and electrical characteristics, the green production of MNPs attracts special attention [13]. Green NPs synthesis techniques are safer, non-hazardous, affordable, and more sustainable than other methods that employ biological entities such as fungi, bacteria, yeast, and plant extracts [14]. Furthermore, the employment of microorganisms or plant-based techniques requires low energy and makes very little or almost no pollution in addition to operating under room temperature [13]. Among all the other biological techniques of NPs synthesis, microbe aided NPs synthesis is not economically feasible since they entail maintaining sanitary conditions and very complex processes for sustaining microbial cultivations [15]. Besides, microorganisms take a much more extended incubation period to reduce metallic ions than plants due to the availability of water-soluble phytochemicals that serve as reducing agents [16].

Plant-mediated synthesis of NPs is the most widely applicable technique of generating NPs and has a significant benefit, as plants are widely dispersed, readily available, and safe to use [17], some examples of plants species as shown in **Figure 1**. Plant extracts also outperform microorganisms in scale-up, bio-safety, and no complexity of maintaining cell cultures [18]. AgNPs are one of the most studied MNPs. They seem to be a convenient, attractive choice of those mentioned above 'green' approaches due to their unique optical, sensing, catalytic and antimicrobial properties [19]. Owing to its large surface-to-volume area, AgNPs are broadly used in various industrial areas such as water treatment, optics, catalysis, electronics, dentistry, food packaging, and clothing [20]. Other types of NPs, including metallic and non-metallic NPs, are reported by only a few previous investigations since their safety is less than AgNPs [21]. In addition, several experiments have been carried out to produce AgNPs using plants extracts in biological and pharmaceutical industries [22]. These NPs present potent antimicrobial efficiency at low concentrations [7]. Moreover, AgNPs have been widely studied for their exceptional optical, electrical and thermal properties [7].

With the development of various infectious illnesses and resistance of pathogenic microorganisms to antibiotics, novel antimicrobial agents and AgNPs are the most prospective agents [23]. Furthermore, the wide-spectrum bioactivity of green AgNPs exhibited strong antimicrobial efficacy against gram-positive and gram-negative bacteria [24]. Therefore, this review discusses the potential antimicrobial activity of plant-derived AgNPs and its implications on different plant growth parameters. Thus, more attention has been paid to investigating the advancement of plant-mediated AgNPs in various medical and practical life applications.

AgNPs IN PLANT BIOTECHNOLOGY

Plant biotechnology encompasses genetic transformation as one of its key divisions. Agrobacterium-mediated transformation was notably efficient with the lowest level of gene copy number. However, the agrobacterium-mediated transformation was found to be remarkably active with a low number of gene copies [25]. Plant-mediated Agrobacterium has more persistent transgenic expression and less transgene silencing [26]. Several antibiotic substances such as thimentin, cefotaxime and carbenicillin have been used in bacterial eradication in plant tissue culture medium [27]. However, a higher concentration of these antibiotics may impair the efficiency of plant regeneration and genetic stability [25]. For instance, cefotaxime is a beta-lactam antibiotic agent used to treat and manage bacterial infections [28]. β-lactam reacts with peptidoglycan transpeptidases, causing cell lysis [29]. Significant inhibitory action of β-lactam and their derivatives, such as cefotaxime and carbenicillin on organogenesis, embryogenesis and plant cell development, has been documented [30], particularly in herbaceous and certain woody plant species, including *Citrus sinensis* [31], *Araucaria excelsa* [32], *Tectona grandis* [33], and *Populus euphratica* [34]. It has been demonstrated that long-term exposure of explants to antibiotics could be hazardous and increase the possibility of bacterial resistance [25]. While the agrobacterium-mediated genetic transformation approach entails the

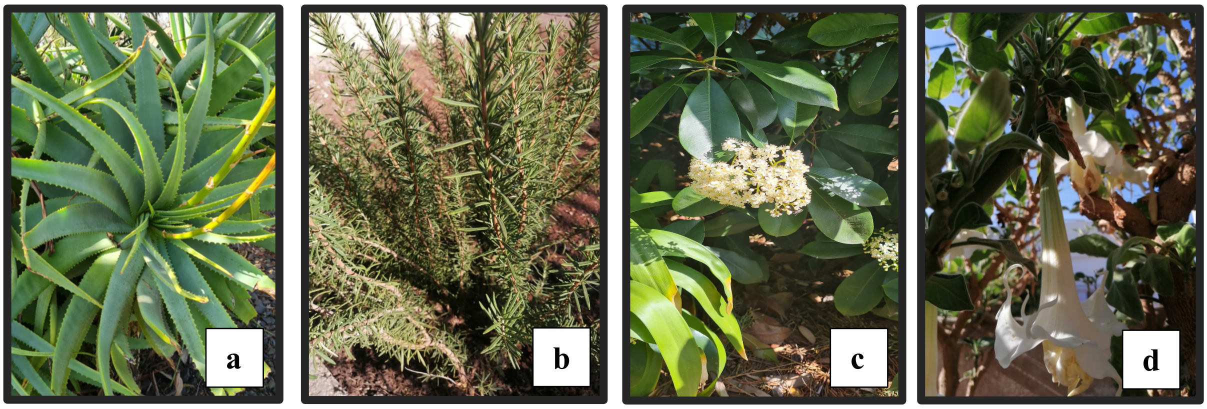

Figure 1. Some plants species are used in the process of synthesis of AgNPs. a) *Aloe vera,* b) *Rosmarinus officinalis,* c) *Skimmia laureola* and d) *Datura metel.*

introduction of external genes into the genetic makeup of the plant, this can result in the manifestation of novel characteristics or the alteration of preexisting ones [35]. Thus, a new approach that does not modify the genetic composition of the plant is required. The use of plant-mediated AgNPs represents an new approach with a novel mechanism to enhance plant growth [36]. Furthermore, agrobacterium-mediated genetic transformation leads to the occurrence of heritable genomic alterations in the plant [37]. The inserted genes integrate into the plant's genome and are heritable, being transmitted to future generations by enabling meticulous regulation of the introduced characteristics, and providing specific enhancements such as resistance to pests, tolerance to herbicides, or improved nutritional composition [35]. This approach may trigger the release of the introduction of genetically modified organisms into the ecosystem and raise a prospect of ecological impacts [38]. In addition, agrobacterium-mediated genetic transformation requires skilled strategies and proficiency, making it a considerably complex technique [39].

By contrast, plant-mediated AgNPs does not cause any alterations in the plant's genetic material [40]. Moreover, they do not cause any alterations in the plant's genetic makeup [41]. The effects are often transient and linked to the existence of NPs within or near plant tissues [42]. Thus, plant-mediated synthesis may represent a more environmentally friendly and sustainable method [43].

Due to their crystalline surface structure and large surface area to volume, MNPs have improved electrochemical activity [44]. AgNPs can inhibit microbial contamination through several actions. Ag^+ ions bind with protein sulfhydryl groups and substantially prevent bacterial development by repressing respiration enzymes, transporting components of an electron, and interrupting DNA functions [25]. Moreover, the presence of Ag^+ ions condenses DNA molecules and prevents their replication ability [45]. Ag^+ ions may also bind with thiol groups which inactivate the bacterial protein [45].

AgNPs are synthesised through chemical, physical and biological methods. The application of physical and chemical techniques is cumbersome and not affordable [46]. Physical and chemical production of NPs leads to the absorption of some hazardous substances on the NPs' surface, which can have detrimental impacts on their application. Thus, there is a growing demand for low-cost and eco-friendly NPs. Researchers have attempted bio-extracts of plants and microbes to produce cost-effective and eco-friendly NPs [47]. Metal ions are reduced intracellularly or extracellularly in the biosynthesis of metal NPs due to bio-extracts. Ag has been widely used to treat several illnesses [47]. Before introducing antibiotic therapies, silver was used as a disinfectant agent to treat skin burns and wounds [48].

PLANT MEDIATED AgNPs

Manufacture of AgNPs can be done through different techniques, for example, microwave-assisted, radiation-assisted [49], thermal decomposition [50], solution reduction, physical routs, chemical and electrochemical reactions and recently via biological means [51]. With the evolution of chemical and physical synthesis methods, the risk of environmental pollution is increased due to hazardous generated byproducts released from these methods [51]. Biosynthesis of AgNPs has been developed owing to their environmentally friendly and cost-effective properties [16]. Furthermore, no hazardous chemicals are used in this method [52]. Different fungal species, such as *penicillium* sp. [53], *Botryosphaeria rhodina* [54], *Phanerochaete chrysosporium* [55], and *Aspergillus sydowii* [56] owing to their strong binding and metal-accumulation capabilities. The fabrication of AgNPs using fungi is simple compared to other microorganisms due to the ease of monitoring mycelial growth in the laboratory versus to bacteria [57]. AgNPs have been manufactured using different bacterial species such as, *Bacillus brevis* [58], *Enterobacter hormaechei* [59], and *Pantoea ananatis* [60] were employed for the production of AgNPs with biological activities. However, bacteria used for the synthesis of NPs can be easily genetically modified to produce the desired NPs, and they grow rapidly [61]. Nevertheless, to get a clear filtrate from colloidal broth, expensive and complex equipment are required [62]. On the other side, algae exhibited the ability to rapidly multiply and bind inorganic metal ions [61]. Various algae species have been demonstrated their ability to synthesis AgNPs including, *Chlorella vulgaris* [63], *Gelidiella acerosa* [64], and *Spirulina platensis* [65].

In plant-mediated AgNPs, roots, leaves, bark, fruits, flowers and shoots are extracted to generate AgNPs [66]. The standard steps (**Figure 2**) for the synthesis of AgNPs from plants are: i) preparation of the plant part extract, ii) the addition of the Ag ion ($AgNO_3$) to the extract, iii) keeping under room temperature for several hours and waitin g for the dark brown colour of the solution which is the indication of the AgNPs production, iv) centrifugation to form pellet and v) characterize the NPs via different microscopy devices [5]. Plant-derived AgNPs may be stimulated by various reducing agent compounds such as alkaloids, carbonyl groups, amines, phenolics, terpenoids and pigments [46]. There have been many plants species were used in the biosynthesis of AgNPs, such as *Saraca indica* [67], *Viola serpens* [68], *Rosmarinus officinalis* [69], *Ocimum tenuiflorum* [70], *Ammannia baccifera* [71], *Hibiscus cannabinus* [72], *Vitex negundo* [73], *Podophyllum hexandrum* [74], *Piper pedicellatum* [75], *Moringa oleifera* [76], *Solanum torvum* [77], *Artocarpus heterophyllus* [78], *Andrographis paniculate* [79] and *Brucea javanica* [80]. Plant-derived methods are advantageous because they are simple, affordable environmentally benign [81]. Plant bioactive compounds reduce metal ions to a fine shape, different sizes, and solid antimicrobial MNPs [82].

Biosynthesis of NPs, combining nanotechnology and biotechnology, attracted popularity as the green chemistry principles advocated for developing environmentally friendly technologies for manufacturing nanomaterials [83]. Ubiquitous chemicals and starting components used in chemical synthesis reactions are

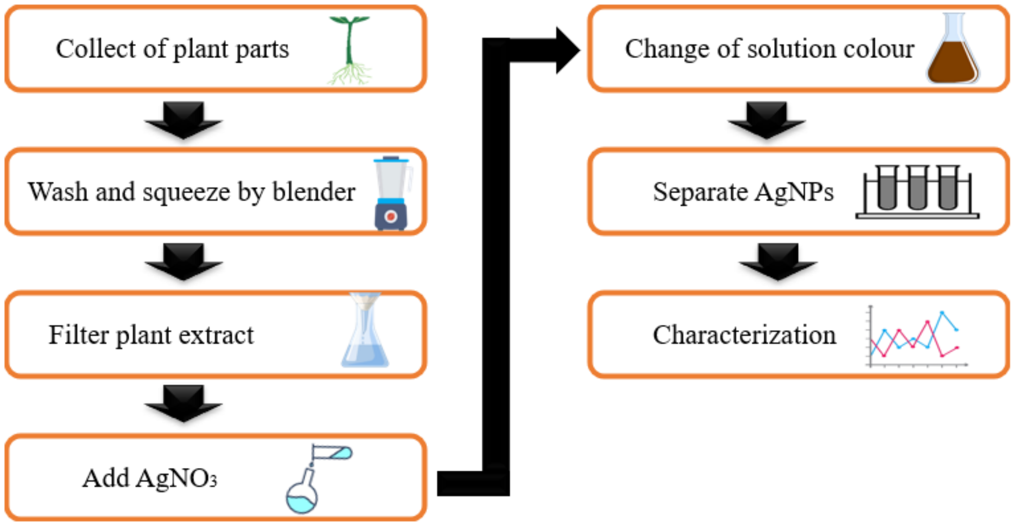

Figure 2. Standard protocol used in the manufacturing of plant-based AgNPs.

potentially toxic and harmful [52]. Concerns about the environment have led to the development of biomimetic technologies as an alternative to hugely hazardous chemical procedures. Thus, researchers were inspired to create MNPs using biological processes due to the need to comply with green chemistry principles where plant extracts [84], enzymes [85], and microorganisms [86] play a key role in NPs synthesis. Biologically created NPs possess great promise applications (**Figure 3**). Among MNPs, particularly AgNPs is highly crucial in the world today as most diseases have evolved into antibiotic resistant species [36].

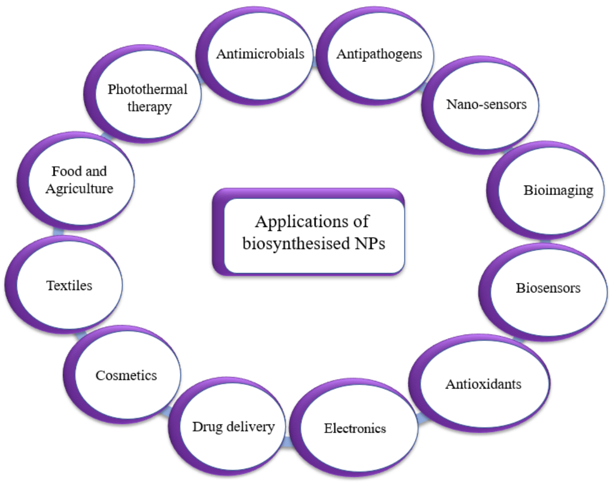

Figure 3. The potential applications of biosynthesised NPs including environmental and biomedical applications.

Using plants in a one-step process makes large-scale manufacturing of nanoparticles more spontaneous, cost-effective, and environmentally benign [36]. Several microbes, including bacteria, fungi, yeasts and algae, are also used for the same purpose [87]. The presence of phytochemicals in plants is mainly responsible for the synthesis of NPs. The main phytochemicals essential for reducing ions are flavonoids, quinines, carboxylic acids, amides, terpenoids, aldehydes, and ketones [52]. In most cases, dried or powdered plant materials are used, while using fresh plant parts has been reported occasionally [88]. Since plants contain a wide variety of chemicals, a single extract can serve as a green chemical agent with several applications [89].

Using plant extracts is a great idea since it keeps trees out of risk and makes it simple to store plant parts, including leaves, stems, and bark [90]. Furthermore, using plants and their derivatives is favourable because using other biological entities like bacteria or fungi is not cost-effective as they need special isolation equipment and aseptic conditions [91]. Plant-based synthesis of NPs is a simple process that can be easily amplified for large-scale fabrication of NPs [92]. Therefore, the biosynthesis of NPs using plants are environmentally friendly, cost-effective, and almost using harmless products.

It is well evidenced that plants can hyper-accumulate and actively reduce metallic ions [93]. These distinctive features make plants a green way to synthesise MNPs [93]. They are recognized as natural chemical factories that require little maintenance and are thus a low-cost method [94]. The main advantage of this technique is that its kinetics are significantly greater or equivalent to chemical NPs synthesis approaches [95]. Optimisation of synthesis parameters like temperature, reaction time, pH, metal ion concentration, and extract concentration controls the size and shape of the produced NPs [96]. Therefore, this plant-based synthesis route can be referred to as the synthetic process that complies with all of the principles of green chemistry and can be used for the environmental sustainability [97].

On the other hand, the presence of contaminants derived from plant extracts can compromise the stability and purity of the synthesised AgNPs, hence influencing their potential utility across many domains [98]. In addition, the process of plant-mediated synthesis can lead to the production of NPs that exhibit diverse sizes and shapes, which in turn influence their physicochemical characteristics and overall performance [99]. The presence of heterogeneity can impede the exact manipulation necessary for specific applications [100]. To fully exploit the benefits of plant-mediated AgNPs and mitigate their drawbacks, it is crucial to tackle these obstacles.

ANTIBACTERIAL EFFICIENCY OF PLANT-BASED AgNPs

NPs have an active response and influence on microorganisms. An antimicrobial property of NPs suggests that possible reverse effects pose harm to microorganisms [101]. Ag has been widely used since ancient times to treat illnesses [47]. Before introducing antibiotic therapies, Ag was used as a disinfectant agent to treat skin burns and wounds [48]. The antibacterial effect of AgNPs is not similar to Gram-negative and Gram-positive bacteria [102]. The antibacterial activity of AgNPs on Gram-negative and Gram-positive bacteria has been widely investigated [103-106]. The antibacterial activity of AgNPs can be assessed by the agar well diffusion method [107]. This method involves placing antibiotic disks on the bacterial patches grown in an agar medium, then measuring the inhibition zone around the infected area [108]. Some reports have shown that Gram-negative bacteria are more susceptible to AgNPs than Gram-positive bacteria [109-111]. At the same time, other researchers have found reverse results [112, 113]. **Table 1** shows some list of plant species mediated AgNPs and their antibacterial effect on bacterial pathogens.

The bacterial cell membrane is negatively charged, while AgNPs are positively charged [118]. When the AgNPs accumulate on the bacterial cell membrane, it brings structural changes in the membrane, thus increasing the cell permeability [119]. As a result, uncontrolled movement among cytoplasmic membrane contributes to cell death [48, 119]. The idea is that AgNPs can trigger genetic material damage by attaching with the bacterial cell, therefore hindering the transcription and translation mode [82].

The antibacterial mechanism of AgNPs is partially understood [114]. To identify the antibacterial effect of AgNPs the biocidal action and inhibitory action approaches can be used. In the first approach, AgNPs biocidal activity kills bacterial cells, whereas, in the second approach, bacterial cell division is interrupted [120]. AgNPs antibacterial action is greatly influenced by pH, the concentration of $AgNO_3$, temperature, size of AgNPs and the type of bacteria [121]. This is because the smaller size of AgNPs has a larger surface, which offers a robust bactericidal activity than the larger particles [119]. AgNPs may interact with the bacterial cell membrane by attaching to the sulfur-containing proteins [122], affecting cell permeability and respiration system and triggering cell death [114]. Moreover, AgNPs may react with the cell membrane and reach inside the bacteria [123]. The antibacterial activity of AgNPs is also due to the binding with thiol group molecules present in respiratory enzymes of bacteria [124]. Likewise, AgNPs penetrate bacterial cells blocking DNA replication and cell reproduction [125] (**Figure 4**). Furthermore, it has been indicated the cell membrane damage is caused by the bacterial external membrane [126]. AgNPs can disturb the structure of the lipid bilayer of the bacterial cell membrane which causes a high permeability. This alteration enables ions and other cellular constituents to escape from the cell. Thus, alterations in the integrity of the membrane may harm the functional and structural stability of the bacterial cell [127]. Some researchers imply that AgNPs can stimulate the bacterial membrane pits and gaps and, subsequently, cell fragmentation [128]. It has also been recognized that Ag ions bind with enzymatic sulfhydryl groups, creating metabolic instability and cell death [129]. The smaller AgNPs have a wider binding surface and more antibacterial activity than the larger AgNPs [12]. Membrane thickness and molecular composition of Gram-negative and Gram-positive bacteria are responsible for the variation in their susceptibility to AgNPs [130]. Ag ions can penetrate cells via the bacterial cell wall, accumulating damaged DNA and affecting protein synthesis [12]. In some research, NPs have been shown to cause reactive oxygen species in plants [131]. The mechanism of nanoparticle-induced reactive oxygen species generation, differs amongst NPs kinds, and the biological process remains unknown [132].

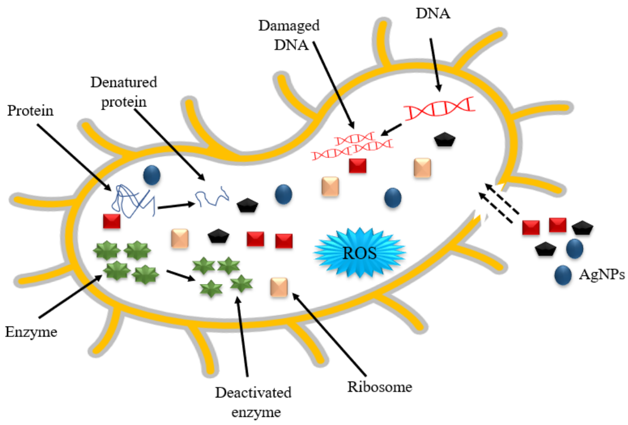

Figure 4. Schematic illustration of potential antimicrobial activity of AgNPs attacking bacterial cell. AgNPs may target bacterial cells using various methods. These include generating reactive oxygen species (ROS) that cause damage to the cell's membrane, proteins, and DNA. Additionally, AgNPs can directly interact with the cell membrane by dissolving and releasing metal ions, which can inhibit the electron transport chain. AgNPs can also regulate bacterial metabolic processes.

EFFECT OF GREEN AgNPs ON PLANT GROWTH

With great awareness of nanotechnology and its applications, authors have studied the interactions and behaviour between NPs and the environment [133-135]. NPs reach the environment naturally or by engineering approach [136]. Such particles have the potential to invade the plant and disrupt its physiological functions [132]. Recently, attention has been raised towards the ultimate fate of released AgNPs into the plants [137]. AgNPs can affect plant physiological, biochemical, and structural

Table 1. List of plant species mediated AgNPs and their antibacterial effect on bacterial pathogens.

Plant species	Microbial pathogen	Susceptibility test	Factors influenced antimicrobial activity	Main findings	References
Carambola	*Escherichia coli, Pseudomonas aeruginosa*	Agar well diffusion	AgNPs concentration and size	Synthesized AgNPs successfully inhibit both bacterial pathogens.	[114]
Boswellia ovalifoliolata Shorea tumbuggaia Svensonia hyderobadensis	*Proteus, Pseudomona, Klebsiella, Bacillus* and *E Escherichia coli, Aspergillus, Fusarium, Curvularia* and *Rhizopus*	Disc diffusion	Plant extract	AgNPs may have a signnificant advantage outperform conventional antibiotics.	[1]
Dioscorea batatas	*Bacillus substilis* and *Staphylococcus aureus, Escherichia coli, Saccharomyces cerivisae and Candida albicans*	Disc diffusion	Temperature	The gram positive bacteria (*Bacillus substilis* and *Staphylococcus aureus*) were more responsive to AgNPs at room temperature than at 80°C.	[115]
Phyllanthus pinnatus	*Vibrio cholera, Shigella flexneri*	Disc diffusion	NPs concentration	The higher concentration of AgNPs showed the greater bactericidal inhibition.	[107]
Emblica officinalis	*Escherichia coli, K. pneumonia, Staphylococcus aureus, Bacillus subtilis*	Disc diffusion	Plant extract	AgNPs proved to have a greater antibacterial effect on gram-negative unlike to the gram-positive bacteria.	[51]
Artemisia nilagirica	*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus subtilis*	Agar well diffusion	Type of the microorganism	microbial sensitivity to AgNPs varies for each bacterial pathogen.	[116]
Cleome viscosa	*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*	Disc diffusion	NPs concentration	Highest concentration of AgNPs displayed higher inhibitory activity against bacterial growth.	[117]
Crataegus douglasii	*Staphylococcus aureus, Escherichia coli*	Disc diffusion	Plant extract concentrations, reaction temperature and PH	AgNPs have shown a high antibacterial activity against both bacterial pathogens.	[16]
Peganum harmala	*Escherichia coli, Staphylococcus aurous, Bacillus cereus, Enterococcus faecalis*	Disc diffusion	AgNPs concentration	AgNPs exhibited a greater antibacterial activity against *Escherichia coli* (Gram negative) than against *Staphylococcus aurous* and *Bacillus cereus* (Gram positive), while no inhibitory zone was detected for *Enterococcus faecalis*.	[22]
Moringa oleifera	*Klebsiella pneumoniae, Staphylococcus aureus*	disc diffusion	Copper ions concentrations in the water.	The obtained AgNPs were suggested to be used in water purification.	[7]

functions. They can increase the oxygen radicals, modify gene expression, disrupt DNA or destroy cells [132]. In another work, plant-based AgNPs enhanced and improved the germination of wheat grain [138]. Contrary findings were demonstrated that elevated concentrations of AgNPs cause oxidative stress, resulting in lower growth and biomass in rice plants [139]. It has been also found that AgNPs at low and high concentrations inhibited plant growth by limiting the absorption of both macro and micro elements, thereby disturbing the ionic balance in the plants [140].

Das et al. [141] observed significant developments in leaf number and leaf area index, chlorophyll content, and pod yield of *Phaseolus vulgaris* after using of low concentrations of plant mediated AgNPs. Sehnal et al. [132] observed adverse effects on germinated Zea mays seeds, including reduction in fresh biomass and root development. They also found alterations in stem, root and leaves morphology. Green AgNPs can change the expression of proteins involved in primary metabolic and cell defence system. Increasing of hydrogen peroxide level can significantly affects plant growth and causes cell death. Vishwakarma et al. [142] studied the effect of biosynthesized AgNPs using *Aloe vera* extract on hydroponically gown Brassica seedlings. Their results revealed that different concentrations (1 and 3 mM) of AgNPs reduced the seedlings growth which attributed to the aggregation of AgNPs leading to the inhibition in photosynthesis.

Almutairi and Alharbi [143] noticed a beneficial effect on maize germination percentage and rate after being exposed to plant-based AgNPs. This may be because NPs are small enough to get through the seed coat promote gemination process. The potential mechanism is that NPs help seeds absorb more water [144]. Likewise, Krishnaraj et al. [145] didn't find toxicity impacts or morphological alterations when plants exposed to AgNPs.

CONCLUSION

Plants-based AgNPs comply with all green chemistry strategies exhibiting improved characteristics that might be implemented in almost any field of science and real life. Green chemistry concepts pursue a technique that makes use of sustainable raw materials and avoids dangerous byproducts. Plant-mediated AgNPs synthesis has become such a technique for constructing unique and essential nanoscale particles. It can also help prevent potentially hazardous laboratory incidents that can occur from dangerous chemicals and sophisticated equipment. The impact of AgNPs was reported to be positive and negative on several plant growth parameters such as the length of roots and shoots, fresh and dry weight, and seed germination. Moreover, it has been shown that AgNPs are indispensable to modern-day life, from killing microorganisms to battling against cancer cells. AgNPs with good antibacterial activity against various microorganisms successfully provides excellent potency and promise in medicines used against bacterial infections. While the development of AgNPs has such a dominant impact, their risk concerns should be tackled. Hence, it is imperative that the cytotoxicity of AgNPs in living beings must be subjected to further investigation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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