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Assessment of Seed Germination and Growth of *Vigna ra*diata L in the Presence of Green Synthesised and chemically Synthesised Nanoparticles

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Abstract

With the fast developments in nanotechnology, the production of nanomaterials has constantly been expanding, and phytotoxicity by nanoparticles (NPs) is now becoming a major stress factor for plant growth and productivity. Considering the wide applications of NPs in various industries, a feasible way to overcome this stress is to rely on alternative sustainable routes of synthesis. Green nanotechnology is a fast-growing field that offers sustainable options for NP production. In the present study, we targeted the production of silver nanoparticles in a green protocol using the leaf extract of *Aloe vera* and *Rosa indica* as an alternative to chemically synthesised AgNP and examined its impact on the growth of seedling and root of a commonly cultivated plant, *Vigna radiata* L. AgNP formation by green protocol was evident from the colour change of the solution and confirmed by determining the Plasmon resonance peak. The involvement of various phytocomponents in nanoparticle synthesis was identified by Fourier-transform infrared (FTIR) spectroscopy. We identified that chemically synthesised AgNP could create stress in *Vigna radiata* seeds, thereby inhibiting its germination. The presence of green synthesised AgNPsfrom *Aloe vera* and *Rosa indica* showed a germination rate of 88.46 % and 86.96 %, respectively. Further observation of the root growth parameters have shown that the presence of green synthesised AgNP induced root growth as evident from the increased root length with proper adventitious root formation; indicating an adaptive root development for the plant's response to its environment, which can be further utilized in nano-agriculture sector for crop improvement.

Keywords: Silver nanoparticle, *Aloe vera*, *Rosa indica*, germination, root growth

Introduction

Nanoparticles emerged as the material of choice for many industrial applications due to their nano-size (1 to100 nm) and distinctive physicochemical properties. Silver nanoparticles (AgNPs), owing to their antimicrobial properties [1] and other unique electrochemical features [2] become a common nanomaterial in many commercial products, including electronic devices, household and healthcare products [3,4]. The applications of AgNPs are now extended to the field of agriculture where it is used as an antifungal agent, growth stimulator [5], or fruit ripening agent [6]. The extensive use, in turn, will cause the release of AgNPs into soil and water bodies [7-9], resulting in the large-scale accumulation of AgNPs in the ecosystem. Water bodies contaminated with AgNPs can permeate into agricultural fields during irrigation or fertigation [10]. Due to the unique features to

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infuse into different media, AgNPs can be taken up by crop plants and can easily become part of the food chain [11]. This not only has an impact on food but also causes serious health issues to animals and humans [12, 13].

The entry of AgNPs into the plant system can result in its bioaccumulation via the food chain, thereby facilitating various irreversible changes in biological systems. Furthermore, since metallic silver has lethal effects on many organisms [14] and the chances of leaching of silver ions from AgNPs to the environment is high [15], there lies a grave concern regarding the safety of AgNPs when it gets released into the ecosystem. Hence, there is an immediate need to find any sustainable alternative for nanoparticle synthesis with lesser or no environmental issues.

Previous studies showed that AgNP accumulation in plants negatively impacts germination, initiation of root and development[16], manifesting its effects in a species-specific manner [17]. Very few studies detailed the effect of nanoparticle accumulation in *Vigna radiata,* and so far, no other reports are available regarding the impact of green synthesized AgNPs on germination and growth parameters in *Vigna radiata*. The present study envisaged the synthesis of AgNP via green protocol from *Aloe vera* and *Rosa indica* leaf extracts as a sustainable alternative for AgNP synthesis and evaluated the impact of green synthesized AgNPs on germination of seed and growth of *Vigna radiata* L root system.

Materials and Methods

The leaves of *Aloe vera* and *Rosa indica* were collected from Thodupuzha, Kerala, India (9.8959° N latitude). The leaves were cleaned by thorough washing with deionised water before use.

Production of agNPs: The inner gel was collected from *Aloe vera* leaf, homogenized and filtered to get *Aloe vera* gel extract. This was used to reduce silver nitrate to AgNPwith slight modifications in the previously described protocol [18]. Dried leaves of *Rosa indica* were ground, and aqueous extract was obtained by 30 minutes of boiling in deionized water (1:10 ratio) in a water bath at 60^0C followed by filtration and centrifugation. The fresh extract was added to the 1mmol of fresh $AgNO_3$ solution dropwise till colour change. This solution was centrifuged at 5000rpm for 15 minutes, and the supernatant was collected and used for experiments.AgNPs formation using *Aloe vera* and *Rosa indica* leaf extracts were confirmed by visual determination of colour change.

Characterization of agNPs by spectrometry analysis: The characterisation of AgNP formed was carried out by scanning in a spectral range of 300–700 nm in a UV spectrophotometer (Thermo Scientific). Further identification of functional groups were done by collecting infrared spectra using attenuated total internal reflectance infrared spectroscopy (ATR-FTIR) in a spectral range of 4000–500 cm^{-1}(Thermo Scientific Nicolet iS5 FT-IR spectrometer).

Experimental groups: Surface sterilized healthy seeds of *Vigna radiata* L were used to study the impact of AgNPs on germination of seed and growth parameters. The experimental groups include (i) control – seeds grown in the presence of water (ii) seeds grown in the presence of chemically synthesised AgNPs (iii) seeds grown in the presence of AgNPs from *Aloe vera* extract (AVNP) (iv) seeds grown in the presence of AgNPs from *Rosa indica* extract (RNP). All seeds were grown on sterile cotton bedding in Petri dishes. Chemically synthesised AgNP was synthesised using sodium borohydride as a reducing agent [19].

Determination of growth of seedling (seed germination) and root: Seeds were considered germinated if the radicle extension is ≥ 3 mm. Seed germination was assessed by determining the germination percentage (GP), which is calculated as:

GP = total number of germinated seeds /total number of seeds evaluated × 100. Root

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growth was measured by noting the length and observation of the adventitious root. All the experiments were carried out in triplicates (n= 3) with 20 healthy seeds per petridish. Statistical significance was calculated using the Students-Newman-Keuls test in a GraphPad Instat (Ver. 2.04a, San Diego, USA). A statistically significant data has a p-value less than 0.05.

Results and Discussion

Production of AgNPs from aloe vera and Rosa indica leaf extracts and its characterization

AgNPswereproduced by reducing silver ions in silver nitrate to metallic silver and subsequent agglomeration and stabilization of nanostructure by the action of the phyto-components in the leaf extract. The visual observation of colour change was noted, indicating AgNPs formation (Fig.1).

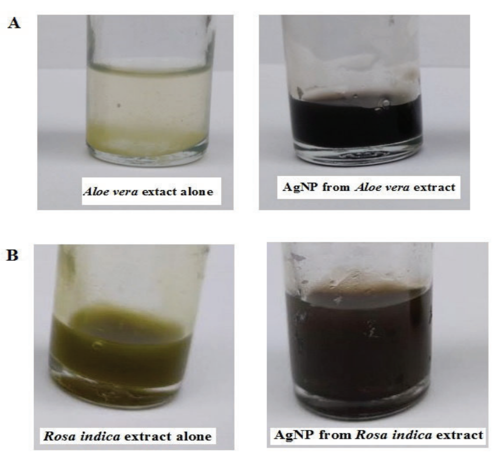

Figure 1: Colour change of *Aloe vera* (A) and *Rosa indica* (B) leaf extract during the synthesis of AgNP.

The formation of AgNPshasbeen confirmed from UV spectrophotometric analysis with the observation of surface Plasmon peak at 400nm (Fig. 2) in green synthesized NP from *Aloe vera* and *Rosa indica.* A noticeable spectral characteristic in accordance with surface plasmon resonance (SPR) as a result of the interactions between free electrons and light waves is observed in all nanometals, including AgNP[20, 21]. The SPR peak width is influenced by the size and conformation of the NPs synthesized, and a spectral range 320–580 nm is characteristic λ max for AgNPs [22, 23]. From the spectrophotometric analysis, we observed this characteristic SPR peak, confirming the formation of AgNPs by the green synthesis protocol.

The data from FT-IR were revealed the possible biomolecules that participate in the bioreduction of silver and stabilization of AgNPs. The FT-IR profile of green synthesised AgNPs from *Aloe vera* extract illustrated seven prominent spectral bands at 3275, 2923, 1743, 1635, 1376, 1245 and 1075 cm^{-1} (Fig 3A). The spectral range of 3000–3600 cm^{-1} represents the stretching vibrations of hydroxyl and amine groups [24]. The typical spectra of carbohydrate monomers like mannose and uronic acid in *Aloe vera* gel at 3275 cm^{-1} is shiftedto3430 cm^{-1}, suggesting interactions with AgNPs. The other peaks observed in Fig 3 A correspond to o-acetyl ester in gel polysaccharide (1743 and 1245 cm^{-1}), carbonyl group stretching vibration (1635 cm^{-1}) and C–H stretching of aliphatic –CH and $–CH_2$ groups (2923 cm^{-1}). From the FT-IR spectra, a possible role of mannose-rich saccharides in the *Aloe vera* gel can be predicted to be the phyto-component involved in AgNP formation.

The FT-IR spectra of AgNPs synthesised from *Rosa indica* extract showed prominent bands at 1162, 1368, 1619, 2767 and 3490 cm-1 (Fig.3B). The absorbance band at 3490 cm^{-1} corresponds to the stretching vibrations of hydroxyl groups. 2767 cm^{-1} and 1619 cm^{-1} represent the stretching of –CH and C=C, respectively. The shifted peak at 1368cm^{-1} might represent the possible interaction between the phenolic compounds and silver to form the AgNPs.

Impact of agNPs on the growth of the seedling

The growth of seedlings was estimated

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by measuring the germination percentage of the experimental groups on both agar and cotton anchorage. It is observed that the presence of chemically synthesised AgNPs completely inhibited the growth of seedlings, whereas control seeds in the presence of water alone showed 100% germination (Fig 4). From the data, it is clear that green synthesised AgNPs from *Aloe vera* (AVNP) and *Rosa indica* (RNP) can effectively overcome the germination inhibition by chemically synthesised AgNPs with a germination percentage of 88.46 % and 86.96 % for AVNP and RNP, respectively (Fig 5).

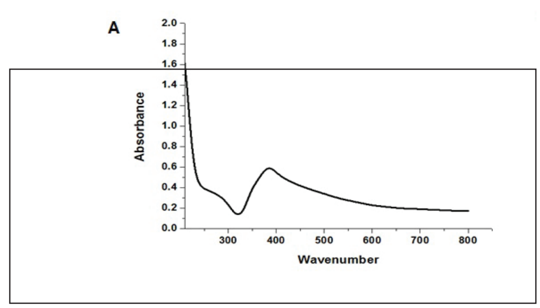
Heavy metal tolerance varies among different plants [25] due to different mechanisms of tolerance like exogenous chelation with extracellular secondary metabolites [26- 28], inactivation by interaction with cell surface ligands [29] or endogenous chelation with biocomponents like phenolic compounds [30–32]. Many studies reported that toxic metals can be complexed by phenolic compounds in a stable manner [32] or can scavenge the heavy metal stress-induced reactive oxygen species [33], thereby inactivating the metal toxicity. Since endogenous mechanisms by the plant species towards metal toxicity remain the same in all the experimental groups, we can conclude that the AgNPs stabilized by phyto-components, which itself are phenolic compounds, can prevent the leaching of metal ions to the biological system, thereby protecting the crop plants from the deleterious effects of metal toxicity.

Impact of agNPs on root growth

Root growth is considered as a prime factor to access the overall performance of crop plants grown in petriplates or containers as root serves as the organ involved in anchorage and uptake of nutrients and water[34]. The central point of entry for AgNPs to the plant system is root cells, where these nanoparticles infiltrate the cell wall and membrane of root cells [35]. It is reported that AgNPs can readily get into the plant body through pores in the root cells [35] and can even induce the formation of bigger pores to permit the entry of larger NPs [36]. Thus root growth might be affected by the presence of AgNPs. In the study, we examined the role of green synthesised AgNPs on root growth and observed that green synthesized AgNPsdidnot affect root growth, and adventitious roots growth was normal. It is also observed that the presence of AgNPs synthesised from *Rosa indica* extract showed increased root length than the control (Table 1). Few previous studies suggested the positive role of silver nanoparticles on plant growth and disease management [37, 38]. Here we can assume that the AgNPs coupled with the bio-components positively impacted germination and growth in *Vigna radiata.*

The extensive use of NPs resulted in their largescale release to the environment from various NP-based products, which could cause probable risks to the ecosystem, especially soil, water and plants. Many studies were carried out to assess the effect of AgNPs on plants and observed that chemically synthesised AgNP could inhibit seed germination and root growth and reduce biomass and leaf area [39, 40]. The present study also observed similar findings indicating the deleterious effects of chemically synthesised silver nanoparticles on seed germination.

Nanoparticles accumulation on plants can result in alterations of gene expression and pathways, thereby affecting the growth and development of that plant [41]. For example, in the presence of chemically synthesised AgNP, we observed a total inhibition of germination of *Vigna radiata* seeds. In contrast, a germination rate of 88.46% and 86.96% was observed in green synthesised AgNPs. These might be due to the gene regulatory mechanisms mediated by the active components present in the green synthesised AgNPs.



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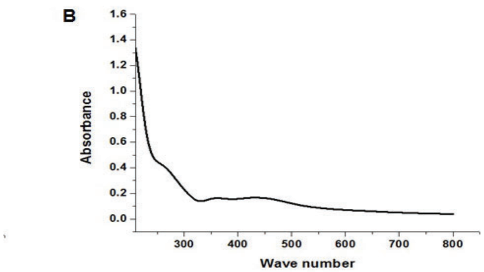

Figure 2: UV-visible spectra of AgNP synthesised from *Aloe vera* (A) and *Rosa indica* (B) leaf extract

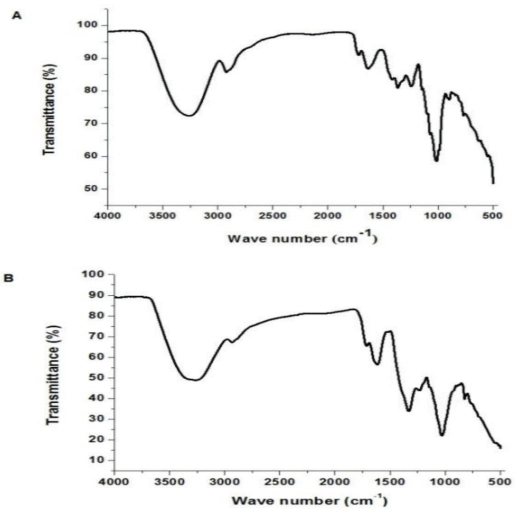

Figure 3: FTIR spectra of AgNP synthesised from *Aloe vera* (A) and *Rosa indica* (B) leaf extract

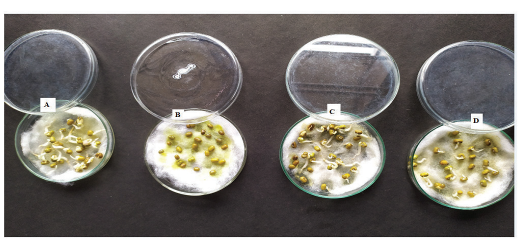

Figure 4: Germination of *Vigna radiata* seeds after four days incubation under controlled conditions. (A) Control in the presence of water alone (B) in the presence of chemically synthesised AgNP (C) in the presence of AgNP synthesised using *Aloe vera* extract and (D) in the presence of AgNP synthesised using *Rosa indica* extract

Table 1: Impact of green synthesised AgNPs on root growth

Experi-mental groups	Root growth	
	Root length (cm)	**No. of ad-ventitious roots**
Control(wa-ter-soaked seeds)	3.3cm	11
AgNP synthesised using *Aloe vera* leaf extract (AVNP)	2.3cm	10
AgNP synthesised using *Rosa indica* ex-tract (RNP)	4.4cm	10

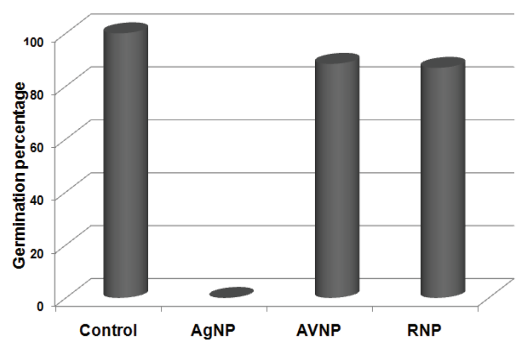

Figure 5: Germination percentage of *Vigna radiata* seeds after four days incubation under controlled conditions. Control: in the presence of water alone; AgNP: in the presence of chemically synthesised AgNP; AVNP: in the presence of AgNP synthesised using *Aloe vera* extract; RNP: in the presence of AgNP synthesised using *Rosa indica* extract

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Later on, we examined the root growth parameters of seeds grown in the presence of green synthesised AgNPs and control. AgNP synthesised from *Rosa indica* induced an increased root growth evident from the higher root length and normal adventitious roots. Thus it can be concluded that the presence of green synthesised nanoparticles in the growth medium has an overall positive impact on the growth of *Vigna radiata* (Fig 6). Earlier reports showed the toxic effect of metal like chromium on the growth of *V. radiata* [42] and our study also observed the deleterious effect of chemically synthesised silver nanoparticles on germination signifying the extent of damage that can be induced by metal toxicity on plant growth. In contrast to this, we observed that green synthesized AgNP can enhance the growth of *V. radiata*. This is in agreement with the study by Thiruvengadam *et al* which reported an enhanced growth of *Zea mays* L in presence of green synthesised silver nanoparticles [43]. Even though there is no doubt regarding the lesser toxicity of green synthesised AgNPs, it is imperative to understand the molecular pathways in detail.

Conclusion

The present study investigated the effects of green synthesised nanoparticles on germination and growth of *Vigna radiata* L. The complete inhibition of seed germination in the presence of chemically synthesised AgNPs points to a serious environmental issue. Even though the amount of nanoparticles leaching to the environment is less, we need to consider the consequences of bioaccumulation of these particles in the ecosystem. To design a sustainable alternative for the chemically synthesised AgNP, we adopted green protocols to synthesise AgNPs using the leaf extracts of *Aloe vera* and *Rosa indica*. The study on the impact of the green synthesised AgNPs on seed germination and growth revealed good germination capability with appreciable growth characteristics like root elongation and lateral root formation in *Vigna radiata* in the presence of green synthesized AgNPs. Thus, our results deliver a sustainable approach for silver nanoparticle synthesis, which can be considered a good alternative in the nano-agriculture sector.

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