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Green synthesis of silver nanoparticles and their impact on plant microbial symbioses

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Abstract
Nanotechnology offers a plethora of opportunities in every sector of the economy, which can influence our everyday lives. Several nanoparticles are being developed and employed daily across diverse fields such as agriculture, food, biomaterials, composites and polymers industries and health sector owing to their small size and large surface to volume ratio. Among these, silver nanoparticles (AgNPs) are of leading interest in the present scenario, due to their distinctive properties such as chemical stability, good conductivity, catalytic activities etc. Generally, these nanoparticles are prepared by a variety of chemical and physical methods that are expensive, difficult to scale-up and may lead to generation of toxic by-products. Biological synthesis of AgNPs offers a green alternative. Green synthesis of AgNPs using microorganisms in particular for the ecofriendly has additional advantages due to their high growth rate, ease of cultivation and ability to grow in ambient conditions of temperature, pH and pressure. Therefore, microorganisms can serve as potential biofactories for clean, non-toxic and environmentally friendly synthesis of AgNPs. Soil microorganisms can be exposed to and affected by nanoparticles (NPs) that are either purposely released into the environment (e.g., nanoagrochemicals and NP-containing amendments) or reach soil as nanomaterial contaminants. It is crucial to evaluate the potential impact of NPs on key plant microbe symbioses such as mycorrhiza and rhizobia, which are vital for health, functioning and sustainability of agricultural ecosystems. NPs may have neutral, negative or positive effects on development of mycorrhizal and rhizobial symbioses. The net effect of NPs on mycorrhizal development is driven by various factors. Rhizobia show differential responses to NPs depending on the NPs concentration and the properties of NPs, rhizobia, and growth substrate. This is so contradicting like we can use microorganisms for synthesis of NPs and the same NPs may be toxic to these beneficial microorganisms. So to safeguard these ecologically paramount associations, studies with respect to appropriate application rate and comprehensive (preferably case-specific) assessment of the context parameters i.e., the properties of NPs, microbial symbionts, and soil are needed to combat nano particles' toxicity to beneficial micro organisms and effective usage of micro organisms for synthesis of nanoparticles.

Keywords: nanoparticles, Green synthesis, mycorrhiza, rhizobia, tripartite symbiosis, toxicity

Introduction
Nanotechnology explores a variety of promising approaches in the area of material sciences on a molecular level, and silver nanoparticles (AgNPs) are of leading interest in the present scenario. The Greek word 'Nano' meaning 'dwarf' or something very small and depicts one thousand millionth of a meter (10^{-9} m).The Nanoscience is the study of structures and molecules on scales of nanometers (1 and 100 nm).The term nanotechnology was coined by Norio Taniguichi in 1974 while the concept of nanotechnology was articulated by Richard Feynman in his seminal 1959 lecture entitled "There's plenty of room at the bottom" (Feynman, 1960) [5]. The nanoparticles can be broadly categorised as Inorganic nanoparticles and organic nanoparticles. Inorganic nanoparticles incorporate Semi-conductor Nps (ZnO, ZnS, CdS), Metallic Nps (Au, Ag, Cu, Al) and Magnetic NPs (Co, Fe, Ni) while the Organic nanoparticles incorporate carbon based materials (fullerenes, quantum dots, carbon nanotubes). Among these, silver nanoparticles (AgNPs) are of leading interest in the present scenario, due to their distinctive properties such as chemical stability, good conductivity, catalytic activities etc. Silver has medicinal properties. They exhibit broad-spectrum antimicrobial activity with lesser tendency to induce antimicrobial resistance. Their size and shape can easily be adjusted. They offer high surface-area-to-volume ratio.

Green synthesis of nanomaterials refers to the synthesis of different metal nanoparticles using bioactive agents such as plant materials, microorganisms and various biowastes including vegetable waste, fruit peel waste, egg shell, agricultural waste and so on.

Green synthesis of metallic nanoparticles has become a new and promising field of research in recent years. Green synthesis of nanoparticles has gained significant importance in recent years as it has several merits such as it is simple, cost-effective, nanoparticles formed have good stability, less time consumption, non-toxic by-products, environment-friendly and can be easily scaled up for large-scale synthesis. Chemical synthesis methods lead to the presence of toxic chemical species adsorbed on the surface of nanoparticles therefore, green synthesis has attracted attention for the synthesis of various metal and metal oxide nanoparticles. Green synthesis approaches are found to be more reliable and economic route to synthesize these metal nanoparticles.

Wang *et al*. (2021) [9] synthesized AgNPs extracellularly using the culture supernatants of *Aspergillus sydowii* and observed that its synthesis is affected by temperature, pH and substrate concentration. The biosynthesized AgNPs showed effective antifungal activity against many clinical pathogenic fungi and antiproliferative activity cancer cells *in vitro* (Saravanan et al., 2017) [7]. The green synthesis of AgNPs was performed using an exopolysaccharide (EPS) producing strain *Leuconostoc lactis*. Spherical-shaped AgNPs stabilized by EPS thin biofilm, bacterial EPS was used as both, reducing and stabilizing agent.

By all this we can conclude as silver nanoparticle has wide applications and now recent days nano urea also gaining lots of importance because in less quantity we can cover large area. Meanwhile Large-scale and versatile applications of NPs have inevitably led to their increasing presence in the environment and consequent risks, which are of utmost significance from health and environmental perspectives. Nanoparticles entry into soil can occur through application of nanoagrochemicals, NP-containing amendments (e.g., biosolids, sludge and manure) and contamination by industrial wastes, plant litter, animal feces, carcasses, exuviae, and atmospheric deposition.

It is crucial to evaluate the potential impact of NPs on key plant microbe symbioses such as mycorrhizas and rhizobia, which are vital for functioning and sustainability of both natural and agricultural ecosystems. It is important to know the potential impact of NPs on key plant microbe symbioses such as mycorrhizas and rhizobia, which are vital for functioning and sus tainability of natural and agricultural ecosystems.

Mycorrhizal `symbioses

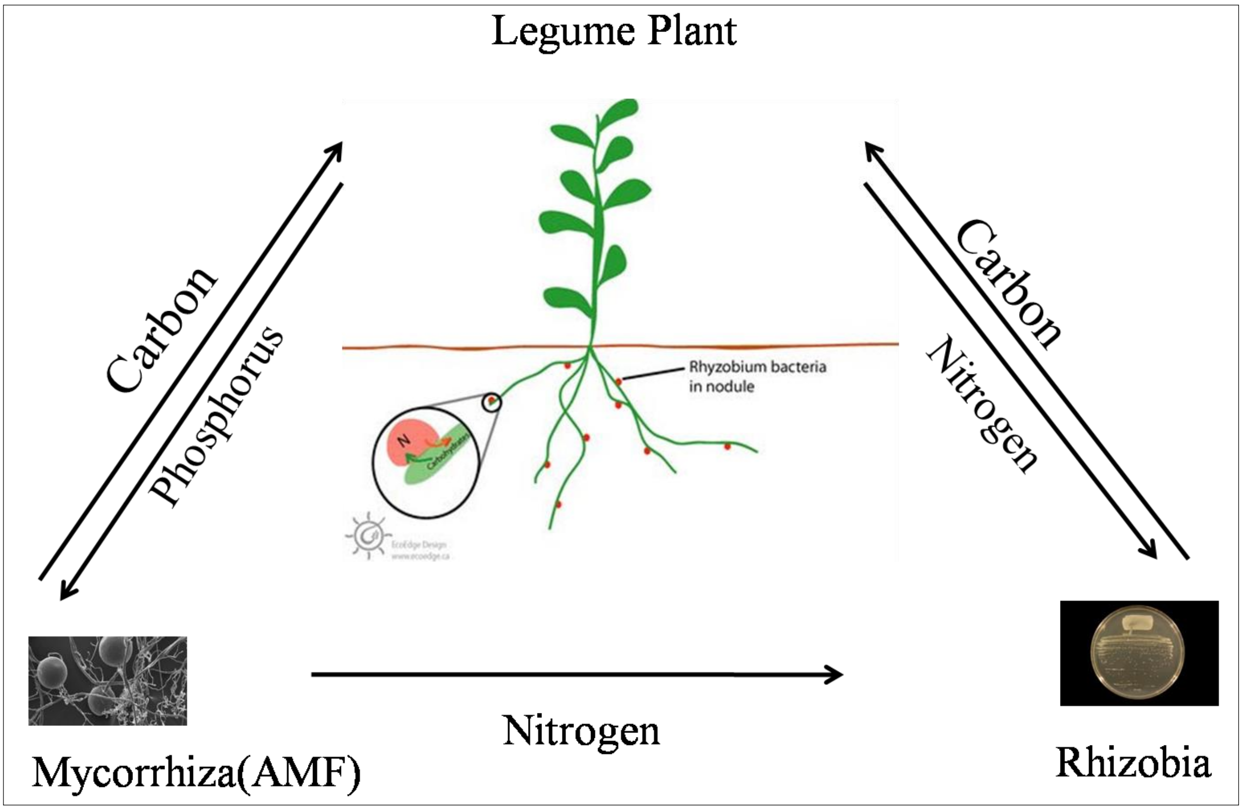
Mycorrhiza is a association between a fungus and a plant. There are 2 types of mycorrhiza those are ectomycorrhizal fungi which grows extracellularly and endomycorrhizal fungi which grows intracellularly.

Rhizobial symbioses

Rhizobia are diazotrophic bacteria that fix nitrogen. Gram negative, motile, non-sporulating rods and they forms nodules. Nitrogen is deficient nutrient and most commonly supplied plant nutrient, Nitrogen which is supplied through fertilizers has severe environmental concerns. Inoculation with rhizobia tends to increase yield. Rhizobia after infection, produce nodules in the legume where they fix nitrogen gas (N_2) from the atmosphere.

Tripartite Symbiosis

Plants colonized by Arbuscular mycorrhizal fungi (AMF) greatly enhance Phosphorus (P) and Nitrogen (N) acquisition, especially by extra radical mycelium. On the other hand, soil bacteria referred to as rhizobia establish a symbiotic relationship with legume plants by making novel root organ known as nodules, which fix atmospheric dinitrogen (N_2) and transfer it to the host plant. The symbiotic relationship of both AMF and rhizobia with the same host leguminous plants is termed a "tripartite symbiosis".



Effects of different nanoparticles on development of mycorrhizal & rhizobial symbioses

Nanoparticles may have neutral, negative, or positive effects on development of mycorrhizal and rhizobial symbioses. The net effect of Nanoparticles on mycorrhizal development is driven by various factors including Nanoparticles type, speciation, size,P concentration, fungal species, and soil physicochemical properties. As expected for potentially toxic substances, Nanoparticles concentration was found to be the most critical factor determining the toxicity of Nanoparticles against mycorrhizas, as even less toxic Nanoparticles such as ZnO Nanoparticles can be inhibitory at high concentrations and highly toxic Nanoparticles such as Ag Nanoparticles can be stimulatory at low concentrations. Likewise, rhizobia show differential responses depending on the Nanoparticles concentration and the properties of Nanoparticles, rhizobia, and growth substrate.

Ways to combat NP toxicity

After assessment the risks could be managed by applying three thumb rules as

- Risk prevention
- Risk mitigation
- Risk communication

Risk prevention and mitigation can be done by proper assessment and identification of risk. Using less toxic, biodegradable or green nano materials as agrochemicals, use of nano materials with proper safety, handling of nano materials without exposure risks can be avoided.

Risk Communication and complete knowledge providing to the associated mankind and farmers in relation to risks associated to nano agrochemicals would help in better management of hazards and threats.

Conclusion

Although, AgNPs have received much attention in recent years and green synthesis technology offers a potentially easy, efficient, clean, nontoxic and environmentally friendly method, it is necessary to carefully evaluate the toxicological effects of the NPs on the ecosystem for better application and controlled application and prevents environmental pollution. Though the natural concentrations of NPs in soils are low the inadvertent release of engineered NPs and the continuous use and abuse increase their concentration in different environments. Out of the total production, 63–91% of NPs end up in landfills worldwide, which paves the way for their release in water, soil, and atmosphere (Rizwan et al., 2017). In soil, the major route of NPs entry includes the use of NPs based agrochemicals and NPs used in soil remediation. Also, NPs are used as additives in pesticides to enhance the solubility of essential ingredients or to protect its ingredients from premature degradation (Chhipa, 2017) [3]. Apart from these, the accidental transport of NPs from other environmental compartments also adds NPs o the soil system. Due to the deposition of NPs in soils, the interaction between metal-based NPs and soil microorganisms is certain which may affect the soil beneficial bacteria and fungi adversely. Therefore, it becomes imperative to assess the overall impact of NPs on beneficial soil bacteria and fungi.

In-depth evaluation of the literature shows that Nanoparticles may have negative, neutral or even positive effects on development of mycorrhizal and rhizobial symbioses. Overall, most studies indicating adverse effects of Nanoparticles on mycorrhizas and rhizobia have been performed using either unrealistically high NP concentrations that are unlikely to occur in soil, or simple soil-less media (e.g., hydroponic cultures) that provide limited information about the processes occurring in the real environment/agrosystems. Now a days nano urea is gaining more attention, because of its wide usages if farmers are dumping more nano materials then we have to face consequence of NP' toxicity to important microbial associations like mycorrhiza and rhizobia. so it is important to know about nano paticles' pros and cons. More studies in this regard is needed.

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