Chapter 4
Tripartite Interaction Among Nanoparticles, Symbiotic Microbes, and Plants: Current Scenario and Future Perspectives

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Abbreviations

AM	Arbuscular mycorrhiza
CNT	Carbon nanotubes
DOC	Dissolved organic carbon
GRSPs	Glomalin-related soil proteins
MWCNTs	Multi-walled carbon nanotubes
NP	Nanoparticles
ROS	Reactive oxygen species

4.1 Introduction

Nanoparticles (NPs) are defined as the particles with a size of 1–100 nm in diameter (Auffan et al. 2009). Because of their high surface-to-volume ratio, they are very reactive. They can pass even through the cell membrane. These fascinating characteristics make them unique and hence, overwhelming researches have been carried out to explore their possible roles in biotechnology and agriculture production (Singh et al. 2016; Mishra et al. 2017; Shweta et al. 2017, 2018; Arif et al. 2018;

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Vishwakarma et al. 2018; Kehri et al. 2019; Rastogi et al. 2019a, b; Singh et al. 2019). NPs have promising application in the field of nanofertilizer, nanopesticide, nanoherbicide, nanosensor and as a delivery system for the sustained release of agrochemicals (Mishra et al. 2017; Koul et al. 2018; Singh et al. 2018; Tripathi et al. 2018; Vishwakarma et al. 2018). It was of thought that NPs could be used for boosting the agricultural economic growth in the near future (Sabourin and Ayande 2015). But, unregulated use of NPs has threw the ecosystems at the brink of risk caused by toxicity of these NPs (Hong et al. 2014). Although NPs have gained the rapid attention of plant scientists during the recent few years, their fates in relation to sustainable agriculture are still unexplored.

Despite of achievements in the field of NPs in agronomy, their role is still in a topsy-turvy stage (Yang et al. 2017). Different types of plants behave NPs differently. Also, same plant behaves different NPs differently. The response of NPs on plants as well as microbes depends upon the nature and concentration of NPs, nature of coating material, nature of growth media, mode of application, part of the plants, and even stage of plant development (Yang et al. 2017). As most of the NPs are made up of heavy metals, their elevated concentration in the biological systems could be detrimental to both aboveground and belowground flora as well.

Yang et al. (2017) have concluded the interaction of NPs with plants and microbes in their review. They have discussed pros and cons of NPs in details. But unfortunately, no conclusion could be established about the effect of NPs on symbioses in plants. In this chapter, extensive literature mining has been carried out to collect all the reports on interactions where effects of NPs have been studied on plants in relation to the symbiotic bacteria and/or fungi. Although there are very limited reports on tripartite interaction, it has been tried to find out the nature of interaction. Also, the possible mechanism behind these interactions is elucidated with the help of artwork. Eventually, this chapter puts forward the future perspectives and direction of research to study the behavior of NPs with reference to plant-microbe symbioses in greater depth.

4.2 Nanoparticles Versus Plant Growth

First stage of NP and plant interaction is the uptake of NPs inside the plant cell. Usually, plants can accumulate NPs of 40–50 nm dimension (Sabo-Attwood et al. 2012; Taylor et al. 2014). Besides the size, morphology, chemical nature, and coating properties play crucial roles in the absorption of NPs in plants (Raliya et al. 2016).

After absorption of NPs through roots, they follow either apoplastic pathway (extracellular spaces and xylem vessels) or symplastic pathway (across the living cell and through plasmodesmata). On foliar application, most of NPs are absorbed through the stomata (Pérez-de-Luque 2017). After absorption NPs are transported to the sink and internalized into the cell by pore formation, carrier proteins, and plasmodesmata or through the ion channels (Pérez-de-Luque 2017).

Fe_3O_4, TiO_2, and carbon NPs were reported to inhibit the seed germination rate, root elongation, and germination index in cucumber plant (Mushtaq 2011). Cu NPs were found inhibitory on emerging root length, and Ag NPs decreased the plant biomass and transpiration (Stampoulis et al. 2009). ZnO NPs caused cytotoxic (lipid peroxidation) and genotoxic effects (decreasing mitotic index, increasing micronuclei and chromosomal aberration index) in *Allium cepa* (Kumari et al. 2011). CuO NPs have DNA-damaging effect in *Raphanus sativus*, *Lolium perenne*, and *Lolium rigidum* (Atha et al. 2012).

On the contrary to above findings, carbon nanotubes (CNTs) were reported to increase the seed germination and growth in tomato (Khodakovskaya et al. 2009). TiO_2 NPs improved the energy utilization and conversion efficiency in D1/D2/Cyt b559 complex in spinach (Su et al. 2009). Stampoulis et al. (2009) reported no negative effects of multi-walled carbon nanotubes (MWCNTs) and Ag, Cu, ZnO, and Si NPs on seed germination in *Cucurbita pepo*. Lee et al. (2010) reported no negative or positive effect of Al_2O_3 NPs on *Arabidopsis thaliana*. TiO_2 NPs were absorbed by the *Triticum aestivum*, *Brassica napus*, and *Arabidopsis thaliana* but did not affect their germination and root elongation (Larue et al. 2011).

Despite enormous researches carried out on NP-plant interaction, no clear conclusion has been made till date. NPs are reported to play positive, negative, as well as neutral roles on plant growth. Various fates of NPs in plants and their interaction have been reviewed in details by Pérez-de-Luque (2017). Accordingly, NPs alter the physiology, biochemistry, and genetics of plants. The interaction depends upon various factors, e.g., nature and concentration of NPs, nature of coating material, nature of growth media, mode of application, part of the plants, and even stage of plant development (Yang et al. 2017).

4.3 Nanoparticles Versus Soil Microorganisms

Plants are immobile organisms. They are dependent upon root system for water and nutrient absorption. In the vicinity of root systems, millions of microbes including plant growth-promoting bacteria (PGPR) dwell. They affect the root systems through their activity and themselves get affected by the exudates of roots (Philippot et al. 2013; Zoomi et al. 2017). More often, after continuous absorption, nutrient and water adjacent to the root systems get depleted. Because of the limited growth of roots, they cannot extract their need from soil from farther distance. In such conditions it is arbuscular mycorrhizal (AM) fungi and other microbes play key roles in the survival of plants (Zoomi et al. 2017; Kehri et al. 2018). NPs in the soil also interact with these microbes, and this interaction can control the growth and survival of aboveground plants. Therefore, the consequences of NPs on belowground biodiversity are of prime importance to understand.

The research study carried out on the effect of NPs on soil microbes is still in the beginning phase. NPs are reported as a good stimulator of soil microbes, but their harmful effect cannot be neglected (Rajput et al. 2018). Some research studies, however, report NPs as soil microbe neutral. The harmful aspect of NPs on soil microorganisms has been reviewed by Rajput et al. (2018) in details. The interaction of NPs with soil microbes depends upon particle configuration and coating (McKee and Filser 2016). McKee and Filser (2016) summarized the morphological nature of bacteria with reference to the NPs. In most of the reports, they have found gram-negative bacteria as less sensitive than gram-positive bacteria. However, any general conclusion could not be drawn. Further, toxicity may be related to specific nanoscale properties of the NPs.

McKee and Filser (2016) concluded that Ag- and Cu-based NPs exhibit antimicrobial activities. Suresh et al. (2013) described the toxic effects of Ag, Al_2O_3, TiO_2, CeO_2, CuO, CdSe, CdTe, FePt, and ZnO NPs. 1.0 g/l citrate-coated Ag NPs (c-Ag NPs) inhibited the growth of *E. coli* by 90 ± 5% (Doody et al. 2014). Simonin et al. (2016) reported very strong negative impact of TiO_2 NPs on nitrification enzyme activities and the abundances of ammonia-oxidizing microorganism just after 90 days of exposure to even the lowest, realistic concentration. High concentration of TiO_2 (Du et al. 2011; Ge et al. 2011; Simonin et al. 2015) and ZnO (Du et al. 2011; Ge et al. 2011) reported to inhibit microbial respiration and enzyme activity in the soil. CuO and Fe_2O_3 NPs were reported as potentially harmful to soil environment (Frenk et al. 2013). Besides the negative effects, NPs were also reported to pose positive effects on soil microorganisms. 1.0 g/l citrate-coated Ag NPs (c-Ag NPs) enhanced the growth of *Bacillus subtilis* by 127 ± 23% (Doody et al. 2014). Also, Si, Fe, Au, Pd, Ag_2S, and Pt NPs exhibit no or little effect on soil microbes (Suresh et al. 2013).

4.4 Nanoparticles Versus Symbioses

4.4.1 ZnO Nanoparticle Versus Symbioses

AM fungi reduced the Zn accumulation in plants (Wang et al. 2016; Li et al. 2015). Under low phosphorus condition, AM fungi reduced translocation, uptake, and accumulation of Zn (Jing et al. 2016; Wang et al. 2014, 2018) in plant. AM fungi-mediated reduction in the soil pH is responsible for decreased Zn bioavailability (Jing et al. 2016). Further, under low phosphorus conditions, AM fungi reduce the translocation and accumulation of Zn released from Zn NP (Jing et al. 2016). AM fungi secrete a group of glycoproteins called glomalin-related soil proteins (GRSPs) in the soil. GRSPs bind ZnO NP in the soil (Ghasemi Siani et al. 2017), making them immobilized. The efficiency of AM fungi is further increased under organic phosphorus (Wang et al. 2018) and low phosphorus conditions (Jing et al. 2016). Under low phosphorus condition, the efficiency of AM fungi is at maximum. High level of phosphorus in the soil inhibits the AM colonization, as plants get adequate phosphorus and the mutualism starts shifting toward parasitism. Under $ZnSO_4$

treatment the efficiency of AM fungi becomes more responsive toward the ZnO NP (Li et al. 2015). It is because AM fungi perceive heavy metal toxicity more quickly. Other mechanism of amelioration of ZnO NP toxicity in plants is the AM fungi-mediated increase in the accumulation of P, N, K, Fe, and Cu (Wang et al. 2014, 2016, 2018; Jing et al. 2016). Decrease in ZnO NP toxicity was confirmed by reduced ROS production in AM fungi-colonized plants (Wang et al. 2016).

ZnO NPs enhance the growth performance in plants inhabited by non-AM symbiotic microbes (Medina-Velo et al. 2017; Singhal et al. 2017; Bandyopadhyay et al. 2015). ZnO nanorods stimulate the number of fungal pellets, spore size, early sporulation, and thick hyphae in *Piriformospora indica*. This caused increased crop productivity (Singhal et al. 2017). ZnO NPs increased the growth performance in leguminous plants by accumulation of essential elements – sulfur and magnesium – assisted by higher nodule formation in the roots.

Contrary to above findings, ZnO NPs were found bactericidal to *Sinorhizobium meliloti* (Bandyopadhyay et al. 2012). However, it is less toxic than $ZnCl_2$ for *Medicago sativa* L. – *Sinorhizobium meliloti* association (Bandyopadhyay et al. 2015). Ultra-high-resolution scanning transmission electron microscopy (STEM) revealed that ZnO NPs were accumulated on the bacterial cell wall and internalized into the periplasmic space. ZnO NPs also altered the polysaccharide structures of extracellular polymeric substances on bacterial cell wall (Bandyopadhyay et al. 2012). ZnO NPs also reduced the root shoot biomass (Bandyopadhyay et al. 2015).

4.4.2 Ag Nanoparticle Versus Symbioses

Ag NPs over 0.01 mg/kg enhanced the growth and ecological behavior and also decreased the antioxidants' activity in AM fungi-associated plants by decreasing the Ag content in plants (Feng et al. 2013). They through X-ray microcomputed tomography found that AM fungi decreased the Ag content in plants.

Ag NPs in low concentration (50 ppm) improved growth parameters in leguminous plant (Pallavi et al. 2016). They have found that it increased the total bacteria, nitrogen fixer, and phosphate solubilizer count. Also, Ag NPs increase root nodulation in the roots of legumes. However, higher concentration (75 ppm) of Ag NPs was found toxic to nitrogen fixers and other bacteria (Pallavi et al. 2016).

4.4.3 CeO_2 Nanoparticle Versus Symbioses

CeO_2 NPs were found as bacteriostatic to the symbiotic N_2-fixer *Sinorhizobium meliloti*. It was accumulated on the surface of bacterial cell. CeO_2 NPs altered the protein and polysaccharide structures of extracellular polymeric substances on bacterial cell wall (Bandyopadhyay et al. 2012).

4.4.4 Fe_3O_4 Nanoparticle Versus Symbioses

Fe_3O_4 NPs in higher concentration reduced the biomass production in mycorrhizal clover (Feng et al. 2013). It also decreases the bacterial abundance and dissolved organic content (DOC) in maize plant rhizosphere (Cao et al. 2016) and shifted the community composition. At high concentration Fe_3O_4 NPs reduced the root mycorrhizal colonization, soil GRSP content, and alkaline phosphatase activity. This also decreased the availability of P nutrition in plants (Cao et al. 2017).

AM fungi reduced the negative effect of Fe_3O_4 NPs on the plant-microbe interaction (Cao et al. 2016). They have found that AM fungi enhanced the growth of plant and organic matter release from root (Cao et al. 2016).

4.5 Conclusions

On the account of controversial results obtained on the interaction of NPs with plants, soil microbes, and symbionts, it is hard to draw any straightforward mechanism of interaction. However, several common behaviors could be attributed to this interaction. NPs in higher concentration cause adverse effects on plants as well as their microbial symbionts including AM fungi. Higher concentration of NPs reduces AM colonization, soil GRSP content, and alkaline phosphatase activity (Fig. 4.1a). This decreases the availability of P nutrition in plants (Fig. 4.1a). NPs in higher concentration are bacteriostatic and sometimes toxic to nitrogen fixers and other symbiotic bacteria.

In symbiotically associated plants, the effect of NPs toxicity is significantly lower as compared to the plants without symbionts (Fig. 4.1b). AM fungal and bacterial symbionts reduce the NP toxicity to the plants by the mechanism yet to be much understood. AM fungi have more than one mechanism through which they dilute the NP toxicity in host plants (Fig. 4.1b). These include (1) immobilization and, hence, reduced accumulation of heavy metallic NP by secretion of GRSPs, (2) reduction in NP accumulation by altering the soil pH and production of more DOC, (3) accumulation of more nutrient resulting in more biomass accumulation, and (4) reduction in reactive oxygen species in plants (Fig. 4.1b).

Besides the negative roles, regulated dose of NPs has positive roles in plants, especially those inhabited by endophytic microbes and/or nitrogen fixers. The interaction among non-AM symbionts, NPs, and plants is also poorly understood. However, based on researches carried out, several conclusions could be drawn. Accordingly, NPs stimulate the plant growth by (1) increased accumulation of essential elements – sulfur and magnesium – assisted by higher nodule formation in the roots; (2) stimulation in the number of fungal pellets, spore size, early sporulation, and thick hyphae in endophytes; and (3) increasing the total bacteria, nitrogen fixer, and phosphate solubilizer count.

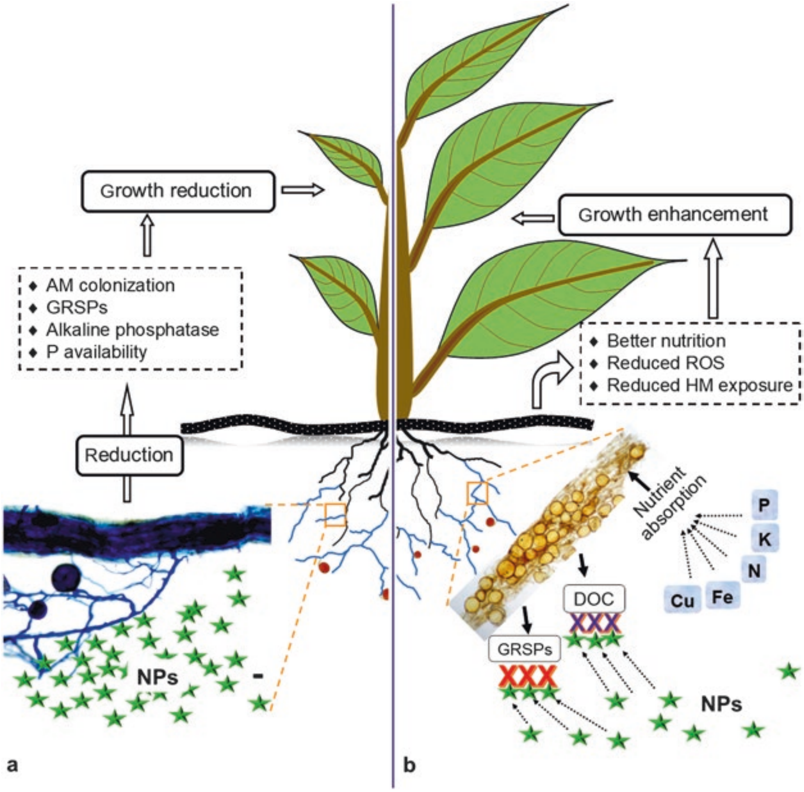

Fig. 4.1 Mechanism of tripartite interaction among NPs, AM fungi, and plants. (**a**) High concentration of NPs in soil. (**b**) Low concentration of NPs in soil

4.6 Future Perspectives

Use of engineered NPs in soil which is increasing day by day may pose detrimental effects on belowground biodiversity. Our understating on the interaction of NPs with belowground biodiversity is at its preliminary phase. For sustainable use of NPs in agriculture production, there is a need of extensive research to explore the effects of various NPs and their optimum dose, coating material, carrier, mode, time, and frequency of application on different symbiotic microbes with reference to the host plant. *Extensive research is also needed to trace and study the movement and localization of NPs in symbiotic microbes. Also, consequences of NPs internalization on gene expression and cellular functions should be studied.*

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