Nano Fertilisers on Plant Nutrition and Soil Microbial Safety - A Review

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Abstract

In the past four decades, nutrient use efficiency (NUE) of crops remained constant despite our relentless efforts. Nano-fertilizers can be used to control the release of nutrients from the fertilizer granules so as to improve the NUE while preventing the nutrient ions from either getting fixed or lost in the environment. Nano-particles are nutrient carriers of Nano-dimensions ranging from 10-100 nm and capable of holding bountiful of nutrient ions due to their high surface area and release it slowly and steadily that commensurate with crop demand. Their role in crop nutrition either through soil or foliar application have a bigger impact in crop productivity and sustainable agriculture. However, application of these nano fertilizer directly to soil has a negative impact on soil microbial communities in terms of interfering in biological processes, microbes' life cycle and more. The aim of this review is to explore the possible threats posed by toxicity of nano particles on plants and microbial diversity.

Keywords : Nano particles, Nano fertilizer, Plant nutrition, Soil microbes, Toxicity

TERTILIZERS are inevitable factor in enhancing soil Γ fertility and productivity of crops regardless of the nature of cropping pattern or environmental conditions. It has been unequivocally demonstrated that one third of crop productivity is obtained by fertilizers besides influencing use efficiencies of other Agri-inputs (Uzu et al., 2010). In the past four decades, nutrient use efficiency (NUE) of fertilizer by crops remained low and constant despite our relentless efforts (Lv et al., 2019). In addition to the low nutrient efficiencies, agriculture in developing countries including India is facing a problem of low organic matter, imbalanced fertilization and low fertilizer response that eventually caused crop yield stagnation (Biswas and Sharma 2008). The optimal NPK fertilizer ratio of 4:2:1 is ideal for crop productivity, while the current ratio is being maintained at 6.7:3.1:1 in India due to the excessive use of nitrogenous fertilizers (Fernandez and Eichert,

2009). The extent of nutrient deficiencies in the country is of the order of 90, 80, 50, 41, 49 and 33 per cent for N,P, K, S, Zn and B, respectively. Thus, from all sources, the country will be required to arrange for the supply of about 40-45 Mt of nutrients by 2025 (Subramanian and Tarafdar, 2009).

Nanotechnology deals with particles measuring a dimension of one-billionth of a meter or onemillionth of a millimetre (Lv *et al.*, 2019). This enables atom-by-atom manipulation and thus processes or products evolved from nanotechnology are very precise and hardly possible to achieve through conventional methods (Uzu *et al.*, 2010). This fascinating field of science of manipulating te matter to Nano scale has been exploited widely in engineering, health, electronics, material sciences and agricultural scientists have begun to use it as a tool to improve the input use efficiencies by integrating Nano technological approaches in the conventional production system (Fernandez and Eichert, 2009). In this context, there would be greater importance of the information about how to increase the NUE of fertilizers by nanotechnology in the coming years (Subramanian *et al.*, 2008).

Nano-Fertilizers

Nano-fertilizers are nutrient carriers of Nanodimensions ranging from 30 to 40 nm (one-billionth of a meter) and Enable soil colloids to hold bountiful of nutrient ions due to their high surface area and release it slowly and steadily that commensurate with crop demand. Subramanian et al. (2008) reported that Nano-fertilizers and nanocomposites can be used to control the release of nutrients from the fertilizer granules so as to improve the NUE while preventing the nutrient ions from either getting fixed or lost in the environment (Fernandez and Eichert, 2009). Nano-fertilizers have high use efficiency and can be delivered in a timely manner to a rhizospheric target. There are slow-release and super sorbent nitrogenous and phosphatic fertilizers. Some new-generation fertilizers have applications to crop production on long-duration human missions to space exploration (Uzu et al., 2010 and Manikanta et al., 2023).

Use of Nano Fertilizers in Crop Nutrition

A large number of studies related to the use of NFs in crops have been reported, along with comprehensive lists of plant species tested and the NPs or NMs used. Mineral nutrients in the form of NFs can contribute to plant nutrition in two ways (Fig. 1) (Uzu et al., 2010). The first is to use Nano structured elements incorporated in a carrier complex that may or may not be a nanomaterial, as is the case of NPs of essential elements incorporated by absorption or adsorption in a matrix such as chitosan, polyacrylic acid, clay or zeolite (Subramanian and Tarafdar, 2009). The second is to use the element per se in a nanostructured form (in suspension or encapsulated), such as for the NPs of Fe or Zn for application to soil, substrate or by foliar spray (Fernandez and Eichert, 2009). Both types of Nano fertilizers contributions have certain advantages, such as greater solubility and rapid absorption or less leaching, compared with traditional fertilizers we use in the everyday agriculture. The first method is preferred because it provides greater control over the speed and timing of release of the nutrient element. (Subramanian et al., 2008 and Pruthiraj et al., 2022).

From a reductionist approach, it can be agreed that greater root growth is desirable, but in a natural environment, this response should be analysed in a broader context because on the one hand, it could be a possible indication of stress and on the other, increased root growth derived from blocking the perception and synthesis of ethylene could have a negative effect because it would hinder the normal interaction between plants and soil microorganisms, including mutualists as well as pathogens (Uzu *et al.*, 2010). In a recent study, it was found that the





Fig. 1 : Different ways of using of Nano fertilizers in crop nutrition

mobility of TiO₂, CeO₂ and Cu (OH)₂ NPs in the soil was limited because these NPs rapidly formed micrometric aggregates with steric hindrance promoted by organic matter (Fernandez and Eichert, 2009). Despite this delay in mobility and reactivity of the NPs, it was found that they changed the soil pH and the release of nutrients from the soil cation exchange matrix (Subramanian *et al.*, 2008). While the conclusion obtained is that NMs apparently do not move far from the point of emission, it would be remiss to ignore the participation of wind, precipitation and organisms as factors that can increase the distance and mobility rate (Lv *et al.*, 2019).

With the use of Nano fertilizer in crop production, enhanced efficiency of nutrient applied and up taken by plant, reduced level of application to soil, way easier method of handling and supply to plant, least effect on health of environment and soil can be expected by the properties of a Nano particle and its efficacy in supply of nutrients.

Mode of Entry of Nano Particles to Plants

Plant cell wall acts as a barrier for easy entry of any external agents including nanoparticles into the plant cells. The sieving properties are determined by pore diameter of cell wall ranging from 5 to 20 nm. Hence, only nanoparticles with diameter less than the pore diameter of the cell wall could easily pass through

and reach the plasma membrane (Subramanian et al., 2008). There is also a chance for enlargement of pores or induction of new cell wall pores upon interaction with engineered nanoparticles which in turn enhance nanoparticle uptake (Lv et al., 2019). Further inter nalization occurs during endocytosis with the help of a cavity-like structure that forms around the nanoparticles by plasma membrane (Melegari et al., 2013). They may also cross the membrane using embedded transport carrier proteins or through ion channels. In the cytoplasm, the nanoparticles are applied on leaf surfaces; they enter through the stomatal openings or through the base of the trichomes and then translocated to various tissues (Melegari et al., 2013). However, accumulation of nanoparticles on photosynthetic surface causes foliar heating which results in alterations of gas exchange due to stomatal obstructions that produce changes in various physiological and cellular functions of plants (Fernandez and Eichert, 2009).

Nano Capsules Entry to Plants through Foliar Mode

The crop protection agents (CPAs) are Nanoencapsulated and the resulted polymer Nano capsules are sprayed onto the leaf tissue. These Nano capsules enter the plant through the stomata orifices (Subramanian *et al.*, 2008). The Nano capsule's chemical bonds of the polymer wall can be weakened or broken by a critical amount of stress enzymes present. Plant cell stress enzymes are activated by mechanical, thermal, chemical or biological stress. This stress sensitizes the plant during an attack and infection from fungi and bacteria (Melegari *et al.*, 2013). These polymer-based CPA Nano capsules sprays are able to prevent this infection: in this case, the plant cell stress enzymes are the stimuli triggering the CPA release (Uzu *et al.*, 2010).

The supply of nutrients to plants through foliar mode is very efficient than any other method because of its easier entry and transportation in the plant system. Nano particle through foliar mode will have a 98 per cent of efficiency compared to any other mode of application of a nutrient to plant for its better growth and enhanced yield.

Microelements enter plant through root hairs and deliver nutrients

The step-by-step method includes the following : the microelements, such as Ca, Mg, Fe, S and Zn, are encapsulated into microspheres and these polymer microcapsules are with time, incorporated and dissolved into the soil (Subramanian *et al.*, 2008). Once close to the root, the chemical bonds of the microcapsule's wall polymer are broken down by the organic acids or phenolic substances from the root exudates (Fernandez and Eichert, 2009). These root

exudates are typically released to enhance plant feeding during the plant growth process and represent the stimuli activating CPA release (Chen *et al.*, 2017). The possibility of targeting the movement of nanoparticles to specific sites of an organism paves way for the use of Nano-biotechnology in the treatment of plant diseases that affect specific parts of the plant (Lv *et al.*, 2019). Different procedures have made use of nanoparticles in plants, such as the controlled release (Melegari *et al.*, 2013).

Effect of Nano Fertilizers on Soil Microorganisms

Soil microorganisms are involved in many biogeo chemical processes. They are a very important functional group of soil organisms. They are responsible for mineralisation of organic matter, element circulation, synthesis of proteins and nucleic acids, as well as transformation of phosphorus forms. Along with particle size, soil properties can also govern the behaviour of nanomaterials and therefore, influence their interaction with microorganisms and plants.

The factors such as soil pH, ionic strength, organic matter and phosphate concentration are known to influence the chemical properties of nanoparticles (Melegari *et al.*, 2013). A lower pH can lead to dissolution resulting in the formation of detrimental





reactive oxygen species. The stabilization of nanoparticles in soil due to their interaction with the natural organic matter may alter the surface chemistry which influences microorganisms and plants (Lv *et al.*, 2019). On the other hand, microorganisms and their activities can also change the behaviour of nanoparticles. In this section, we have discussed the effect of Nano fertilizers on the microbial community in context to physico chemical properties of nanomaterials and soil properties.

Effect of Nano Fertilizer on Rhizosphere Microorganisms

The rhizosphere is inhabited by a large number of microorganisms as compared to bulk soil and therefore, represents a key niche influencing the microbial community structure and functions involved in plant growth. A number of microbes mediate plant beneficial processes such as nitrogen fixation, solubilisation of nutrients and synthesis of bioactive metabolites that take place in this dynamic niche. The compounds released by the root exudates and rhizosphere microorganisms can form complexes with metal ions and also affect their bioavailability to both plants and microorganisms (Chen *et al.*, 2017). The bioavailability, particle size and other unique chemical properties of nanoparticles, can directly influence the microbial population and thereby microbe driven-biological processes in the rhizosphere. Interestingly, microorganisms can significantly contribute towards the biotransformation of metal nanoparticles which can subsequently alter the bioavailability of these ions (Lv *et al.*, 2019).

The Nano fertilizers may have both positive and negative impacts on rhizospheric microorganisms depending on their size and concentration, type of



soil and microbial diversity in the root zone (Guan et al., 2020). The carbon cycle, especially the processes involved in organic matter decomposition in the plant root zone is also improved by Nano fertilizer application. It has been reported that amine-modified polystyrene Nano spheres and titanium dioxide nanoparticles resulted in lower bacterial counts in the lettuce rhizosphere leading to the decreased root and stem growth (Kibbey and Strevett, 2019). On the other hand, sulfate-modified polystyrene nanospheres resulted in an increased rhizospheric bacterial population but had no effect on the growth of the lettuce plants (Kibbey and Strevett, 2019). This study indicated that the nanoparticles can significantly interfere with the attachment of bacteria to plant root surfaces.

The application of nanoparticles showed differential response between rhizosphere and bulk microbial community structure, particularly in a significant shift in the bacterial community than that of fungi. This show the beneficial effect and enhanced growth and development of microbes.

Effect of Nano Fertilizers on Mycorrhizal Fungi

About 80 per cent of all vascular plant species including bryophytes, pteridophytes, gymnosperms and angiosperms are known to have arbuscular mycorrhizal (AM) association with their roots (Brundrett and Tedersoo, 2018). To date, nearly 321 species of AM fungi belonging to 36 genera have been described (Nanjundappa et al., 2019). The mycorrhizal symbiosis helps plant to acquire soil nutrients that otherwise remain unavailable to roots (e.g. P and Zn), improved plant growth and tolerance against stresses Nanjundappa et al., 2019). These mycorrhizal fungi are also known to dynamically interact with other rhizospheric microorganisms to assist plant growth and survival (Nanjundappa et al., 2019). The interaction of nanoparticles with mycorrhizal fungi is diverse. The soil application of nanoparticles has been reported to be deleterious for the diversity of ectomycorrhizal fungi on the roots of Pinus muricata (Bishop pine) (Burke et al., 2015). The application of silver nanoparticles resulted in a reduction in AMF colonization and growth which was further associated with phosphorus mineralization and nutrition in maize. Similarly, Feng *et al.* (2013) reported that iron oxide nanoparticles (3.2 mg/kg) significantly reduced the mycorrhizal biomass and glomalin content in clover plants, however, no harmful effect of silver nanoparticles was observed at a concentration of 0.01 mg/kg. In contrary to adverse effects of soil application on mycorrhiza, foliar application of nanoparticles has been reported to be beneficial for ectomycorrhiza.

Effects of Nanoparticles on Soil Enzymes

Soil enzyme activities are important indicators of soil quality and health, as indicated earlier, effects of NPs on the activities of soil microbial enzymes have been noted. Studies by Wu et al. (2018) report on the inhibition by TiO₂ NPs (50 mg L⁻¹) of the activities of ammonia mono oxygenase and nitrite oxido reductase, enzymes potentially involved in the N₂ cycling process, following a 70-day exposure. However, no significant impact of TiO₂ NPs on the activities of exopoly phosphatase and polyphosphate kinase, nor on the transformation of intracellular polyhydroxy alkanoates and glycogen were found. The authors related these findings to the effects of TiO₂ NPs on biological N and P removal, as well as the depletion of NH₂-oxidizing bacterial population by the NPs. ZnO NP doses that were non-lethal to B. subtilis and P. aeruginosa completely inhibited or lowered the activities of enzymes involved in starch degradation, denitrification and urea degradation.

EL-Azeim *et al.* (2019) found inorganic fertilization system boosted urease (UR) activity reflecting the positive effects of this fertilization system on this particular enzyme activity (Allison *et al.*, 2006). On the contrary, organic and integrated treatments showed stronger effects upon dehydrogenase (DH), acid phosphatase (Ac-P) and β -glucosidase (β G), suggesting the availability of a higher quantity of biodegradable substrates and thus improvements in soil biomass and enzyme activities. In general, soil biochemical properties were markedly enhanced under integrated fertilization system in comparison



to inorganic system due to higher SOC soil contents. This suggests organic compost application in combination with inorganic NPK Nano or fertilizers even at lower rates has positive effect as demonstrated in literature by many researchers. These significant and positive correlations attributed to the role played by extracellular enzymes (dehydrogenase (DH), urease (UR), β -glucosidase (β G) and acid phosphatase (AcP) as the nitrogen fertilization affects the rate of SOC decomposition and the depolymerization of N-containing compounds by regulating extracellular enzyme activities (Burke *et al.*, 2015).

Inorganic fertilizers boosted urease (UR) activity having the positive effects. On the contrary, organic and integrated treatments showed improvement upon dehydrogenase (DH), acid phosphatase (Ac-P) and β -glucosidase (β G), indicating the availability of a higher quantity of biodegradable substrates as a food source.

Effect of Nano Fertilizers on Phyllosphere Microorganisms

The phyllosphere microbiome can be directly influenced by a multitude of factors like UV radiation, water scarcity, nutrient availability and metabolic activities of plants. The phyllospheric microbes are known to secrete EPS or other compounds to improve

the dissolution of nanoparticles and uptake of metal ions by leaves (Vitali et al., 2019). They often form a mucous layer to protect leaf surfaces against the uptake of nanoparticles (Lv et al., 2019). It yet remains unclear that how the phyllosphere microorganisms influence foliar uptake of nanoparticles by plants and how the nanoparticles influence the growth of the phyllospheric microorganisms (Lv et al., 2019). The application of silver nanoparticles (16 ppm) showed a significant reduction in the diversity of the bacterial community of Poplar leaves while the fungal diversity did not change substantially (Vitali et al., 2019). In comparison to the untreated leaves, the population of bacteria such as Rickettsia, Phenilobacterium and Sphingobium decreased significantly on treated poplar leaves while Novosphingobium and Sphingomonas were found to be present in higher numbers (Vitali et al., 2019).

It was suggested that AgNPs improved the nitrate reduction in bacteria like *Novosphingobium* and *Sphingomonas* which might have been an adaptive advantage for higher proliferation on leaves (Vitali *et al.*,2019). In the case of fungi, an unidentified species of the *Preussia*, was abundant in nanoparticle treated leaves while absent in untreated ones (Wang *et al.*, 2009). On the other hand, *Sawadaeapolyfida* had a relatively high abundance in untreated leaves

than in treated ones (Vitali et al., 2019). The titanium dioxide nanoparticles have also been shown to decrease the diversity and load of microorganisms on cucumber leaves (Wang et al., 2009). This decrease was, however, concentration-dependent. A low concentration of TiO, did not alter the microbial community significantly. It was suggested that a higher concentration of nanoparticles on leaf surfaces may result in the generation of ROS affecting the survival of microorganism (Wang et al., 2009). At the same time, it was found that phyllosphere bacteria can also modulate their antioxidant machineries for survival under such stress conditions (Wang et al., 2009). The foliar application of nanoparticles not only changes the microbial community on leaves, but they can also elicit plant immunity responses against phytopathogens.

The nanoparticles have been shown to enter the leaves through stomata, distribute within the spongy mesophyll and subsequently activate salicylic acid (SA)-dependent immune response against pathogens and building the resistance in the plant system.

Effect of Nano Fertilizer/Nanomaterial on Soil Invertebrates

The soil invertebrates include various arthropods, nematodes, platyhelminths which play important role in the maintenance of soil fertility, through their contribution to the carbon cycle, organic matter degradation which further helps in improving the growth and yield of crops and plant. The function and composition of the soil microbial community are strongly influenced by an extraordinarily diverse community of soil invertebrates (Phillips et al., 2020). The interactions between microorganisms and soil invertebrates may include predator-prey relations, competition for resources and habitat. It is known that nematodes may act as primary consumers of bacteria while collembolans and oribatid mites prey on saprophytic fungi Therefore, the effects of Nano fertilizers on soil invertebrates are expected to influence the microbial communities (Burke et al., 2015).

The nanomaterials can have serious ecotoxic effects on soil invertebrates. It has been reported that ROS forming silver nanoparticles were toxic to Caenorhabditis elegans at a concentration 1 mg/L (Kim et al., 2012). This study suggested that at 100 mg/L, a mortality rate of C. elegans went up to 60 per cent. The titaniumoxide nanoparticles (3000 mg/kg) showed no signs of toxicity to Porcelloscaber which indicated that the nanomaterial does not cause any hindrance to its enzymatic activity and feeding parameters (Noordhoek et al., 2018). The titanium dioxide nanoparticles with a size of 20 nm proved to be lethal to most of the nematodes and earthworms affecting their growth and reproduction potential. The silver nanoparticles at a concentration ranging 60.4-235.6 mg/kg were not sufficient for causing toxicity to soil worms such as Folsomia candida (Springtail) and Enchytraeus crypticus (Kim et al., 2012). However, it affected their reproductive potential and caused toxicity at higher concentrations. Kim et al. (2012) showed that ZnO nanoparticles (concentration>6400 mg Zn/kg dry weight) were toxic to springtail and the toxicity was not because of particle size, rather due to released Zn ions. Further, Noordhoek et al. (2018) reported that copper oxide and ferric oxide nanomaterials were non-toxic to springtail at a concentration up to 6400 mg/kg of dry soil while their chloride salts were toxic at a much lower concentration. Interestingly, CuO nanoparticles were toxic to springtails only in loamy soils with high clay contents.

Effect of Nano Fertilizer/Nano Material on Algae

Although algae are not a predominant group of soil flora, they play crucial roles in maintaining soil physical and biological health. Algae play a very significant role in soil aggregation (Noordhoek *et al.*, 2018). Their interaction with other soil microflora is also important in the maintenance of soil health. It has been reported that the development of indigenous soil microalgal crusts could promote overall microbial activity (Crouz *et al.*, 2019). The heterotrophic microbial communities have been reported to be induced by the release of EPS which can be utilized as readily available carbon sources (Mager and Thomas, 2011). Therefore, the influence of Nano fertilizers on soil algal populations will likely have impacts on the structure and function of soil microbial communities. However, such impacts have not been studied appropriately to date. In this section, we will discuss the effects of nanoparticles on various micro and macroalgae.

As soon as the nanoparticle passes through the cell membrane, it gets deposited in the periplasmic space. These nanomaterials after making their way through cytoplasm, interacts with cellular organelle either altering their function or damaging them (Crouzet et al., 2019). Many researchers have observed that nanomaterials may exert negative effects on chloroplast by damaging the grana and thylakoid (Wang et al., 2009). Certain heavy metals can accumulate in vacuoles of Chlamydomonas acidophila (Wang et al., 2019). Further, some studies have shown that nano tubes lead to swelling of endoplasmic reticulum and dysfunction of mitochondria. The additive effects can be observed when these nanomaterials enter the nucleus causing chromosomal abnormality which further leads to deterioration of genetic materials and cell division (Kim et al., 2012). Therefore, it can be presumed that as soon as nanomaterial enters the cell, it gets widely distributed in the cell and significantly affects the cellular and metabolic machinery of the cell (Melegari et al., 2013).

On exposure of algae to nanomaterial, exhibited partial inhibition of photosynthetic activity along with decreased biomass. When nanomaterials interacted with *Algal bloom*, it led to the formation of ROS and protein crystals which further damaged their cell membrane.

Factors	affecting	microbial	community	1
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With the growing popularity of precision agriculture, it is necessary to utilize nutrient sources in low quantity with high efficiency. Undoubtedly, it is quite difficult to achieve with conventional fertilizers. However, Nano fertilizers due to their unique physico-chemical properties can substantially overcome many of the drawbacks of conventional fertilizers. A large number of studies have already been conducted throughout the world to establish the effects of nanomaterials on plants. Incidentally, the majority of the initial studies created a notion that nanomaterials were generally 'phytotoxic'. There is no doubt that this inference was mainly drawn from the studies where higher doses of nanomaterial with short exposure times were used.

The available reports have shown both positive and negative effects of Nano fertilizers on soil and plant-associated microflora depending on the nature, dose and duration of exposure and soil properties. However, there are almost no reports available on the effect of Nano fertilizers on endophytes which play important ecological functions inplant growth and survival. It is also not clearly understood how theNano fertilizers alter the entire plant microbiomes of major cultivated crops? Further, their specific interactions with important growth promoting bacteria and beneficial fungi which are generally applied as bio agents under field conditions, need to be deciphered at the ecosystem.

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