Nanoparticles in Plant Growth and Development

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Sakshi Agrahari and Akhilesh Dubey

Abstract

Over time, nanotechnology has enabled a wide range of applications in the agricultural field due to the distinctive properties of nanoparticles, including high surface area, reactivity, agglomeration, penetration capability, size and structure. Nanoparticles have been by far advantageous for plant growth, development and protection. Nanoparticles bestow specificity in pesticide delivery, enhanced nutrient supply, managing pathogenicity, increasing photosynthetic capacity and germination rate. Apart from beneficial impacts on plants, there have been instances of toxicity and bioaccumulation of nanoparticles, which led to a few setbacks. Thus, it is necessary to have a complete knowledge of the positive and negative impacts of nanoparticles and to study all their characteristics in detail. This chapter highlights the impact of nanoparticles on the growth and development of plants.

Keywords

Nano-particles · Distinctive properties · Impact on plants · Bioaccumulation · Toxicity · Agriculture · Nanotechnology · Metals · Plant growth · Uptake and translocation · Carbon nanotubes · Graphene · Fertilizers · Phytotoxicity

2.1 Introduction

"Nanotechnology is a novel, innovative, interdisciplinary scientific approach of designing, developing, manipulating and application of materials at nano-scale" (Ali, Muhammad, et al. 2014). "Nano" signifies one-billionth unit, therefore

S. Agrahari · A. Dubey (✉)
Department of Biotechnology, Netaji Subhash Institute of Technology, New Delhi, India

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nanotechnology involves substances quantified as billionth of a meter. Ten hydrogen atoms placed side by side cover a distance equivalent to a nanometre. Although nanotechnology involves the science of minute components, it also encompasses a much wider range of disciplines, including expertise from physics, biology, chemistry and various other disciplines.

Nanoparticles (NPs) of sizes less than 100 nm belong to an intermediate zone between an atom and its bulk components, having the capability to alter a material's physicochemical properties, i.e. exceptional reactivity, sensitivity and conductivity. (Mishra and Kumar 2009). Nanotechnology has been widely applied in the agricultural field, as it is quite a challenge to feed the increasing population, beyond 7 billion, as well as to simultaneously provide adequate nutrients.

Among the discrete molecules and their respective bulk components, there exists a transitional zone where nanoparticles lie, with properties that are novel from those of its bulk as well as molecular equivalents (Singh et al. 2015). With the development of nanotechnology, apart from the classical agricultural methods, scientists have tried to make use of the advanced characteristics of nanoparticles to enhance the growth and development of plants.

Nanosized components can be engulfed by bacteria and can penetrate plant cells (Liu et al. 2009a, b), and at high levels of dosage can induce phytotoxicity (Stampoulis et al. 2009). Research on nanotechnology-based agrochemicals has influenced numerous scientists to ponder over the advantages that nanotechnology can bestow upon agricultural crops. Advantages offered by nanotechnology include treatment of plants by nanocides, nutrient maintenance through nano-fertilizers and prevention of diseases (Moraru Carment 2003; Priester et al. 2012). In different areas, the effect and usage of nanomaterial variants such as carbon nanotubes, polymers, metals and nonmetals, quantum dots, magnetic particles, etc. are being studied (Rico et al. 2015).

Nanoparticles have the capability to transform the food and agricultural industries due to their unique characteristics that enhance nutrient adsorption by plants, molecular level disease management and pathogen detection. It involves the operation at a similar level with those of the disease causing particles, which enables the instinctive detection and elimination of those particles (Prasad et al. 2014).

Nanoparticles with characteristics such as enhanced reactivity, small surface to volume ratio, surface structure, agglomeration, etc. have found application in various areas, like cancer therapy, nano-pharmacology, targeted drug delivery, nanomedicine and delivery of agrochemicals. These extraordinary characteristics of nanoparticles have enabled scientists to study their interaction with plants, both in vivo and in vitro.

Nanoparticles are specifically designed and engineered with unique surface and chemical properties. A varying class of nanoparticles have been produced, including metal oxide nanoparticles, magnetic nanoparticles, gold nanoparticle, mesoporous silica nanoparticle, quantum dots, carbon nanomaterials such as carbon nanotubes, fullerenes and graphene (Wang et al. 2016a). Scientists demonstrated that carbon nanotubes have the ability to penetrate seed husks, which enables faster germination of seeds (Zhang et al. 2015a).

Nanoparticles are designed such that they can favour usage of optimal concentrations, regulated release, decline in phytotoxicity and targeted delivery. Pesticides are loaded into the inner core of the mesoporous silica nanoparticles, leading to regulated release as well as protection from photodegradation (Wang et al. 2016a). Quantum dots have a diameter from 2 to 10 nm. They are minute semiconductor particles that are used for cellular imaging and labelling by the production of fluorescence.

Nanoparticles have found important perspective in targeting specific biotic life forms through their unique delivery system and are highly used in the medical field. A similar principle is applied in plants, specifically in tackling phytopathological infections, growth adjuvant and supplementation of nutrients.

Use of nanoparticles is still a fresh and new approach, which needs further study and research for proper understanding and implementation of their properties for the betterment of food and crop as well as in other fields of science. In this chapter, will discuss some of the widely studied effects of nanoparticles on agriculture, which specifically focus on plant growth promotion. Nanotechnology is a niche area which still needs a thorough understanding, but it is sure to expand its boundaries, including agriculture and allied sectors, providing immense benefits.

In food and agricultural fields, nanotechnology has found widespread applications, but still at a budding stage, and thus they require thorough knowledge and guidance so as to indulge in developing "green nanotechnologies" and take into account all the necessary precautions in order to minimize the prospective unfavourable impacts they can pose to the environment and to human health (Mishra and Kumar 2009).

Nanoparticles have gained demand in the agricultural and medical fields due to their unique physicochemical properties, including ability to penetrate, larger surface area and chemically active. With the increase in demand they have also become potential threats to the environment (Borm et al. 2006; Kreyling et al. 2006; Lam et al. 2006; Maynard 2004).

Nano-enabled products have been profoundly used globally due to their immense availability, which can be released into the environment in high quantities as engineered nanoparticles usually displaying properties different from their bulk components (Geisler-Lee et al. 2012).

Nanoparticles have aggregation tendencies and low solubility in water, which limits their access to most living organisms (Maynard et al. 2004; Brant et al. 2005). Certain experiments demonstrated penetration of nanoparticles through skin, food and water or air into animals and humans (Oberdörster et al. 2006; Jain et al. 2007). Nanoparticles can translocate along the food web and can accumulate in higher-level consumers, influencing the extent of toxicity on organisms which belong to distinct trophic levels (Krysanov et al. 2010).

Metal oxide nanoparticles, i.e. titanium oxide and carbon-based fullerene nanoparticles, show microbial toxicity, as well as fullerenes display low mobility in soil and aqueous medium (Lecoanet and Wiesner 2004; Brant et al. 2005). Nanoparticle toxicity is not only associated with size but also with their physicochemical properties.

It is presumed that biologically attainable nanoparticles will materialize in high quantities in the future and will be higher in organisms at higher trophic levels. Accumulation of nanoparticles in organs or tissues leads to increased effects on cells and cellular structures. The issue with translocation of nanoparticles within the food web is that it is inevitable.

Farmers used conventional chemicals for controlling pests and pathogens, which had a drastic impact on the environment as well as on the farmer's economy, because upon application 90% was lost as runoff or into the air (Thul and Sarangi 2015). Release of pesticides and insecticides through a nano-scaled delivery system led to the application of these chemicals only when the need arises (Gruère et al. 2011).

Toxicity and accumulation of nanoparticles have led to the necessity to search for biodegradation pathways for nanoparticles and their impacts on living species, including the natural structures and functions as well as artificial biocenoses (Krysanov et al. 2010).

At present, the knowledge on the providence of nanoparticles into the environment is scarce and their bioaccumulation by living beings and their tissues, is also sparse rather practically absent and specifically whether they have chronic impact or not (Fig. 2.1).

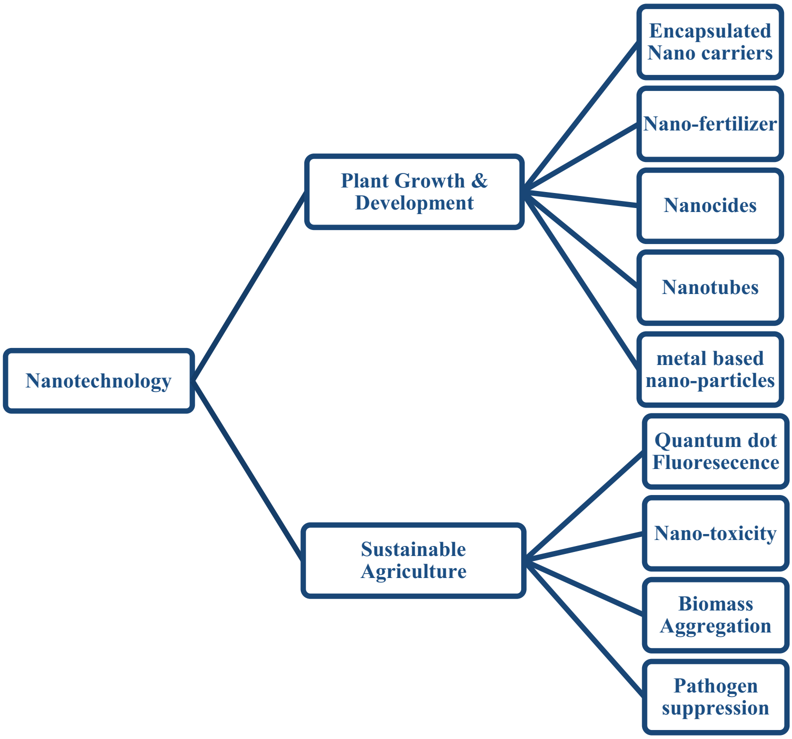

Fig. 2.1 Diversification in the application of nanotechnology (Modified from Ditta et al. 2015)

2.2 Molecular Mechanism of Nanoparticles in Plant Growth and Protection

2.2.1 Mode of Entry and Uptake

Plant transport pathways play a vital role in the entry of nanoparticles into plants as well as into the surrounding environment, which can lead to their accumulation. Engineered nanoparticles were transferred through protoplasts, intact plants and dissected organs (Wang et al. 2016a). Nanoparticle entry into plant cells largely depends on the species of plant and the nanoparticle properties and is obstructed due to its cell wall (Singh et al. 2015). Entry through the plant cell wall occurs either through engulfing, endosome formation or through sieving mechanism. Entry of ENPs occurs either by way of organs and tissues above the ground level, i.e. stomata, hydathodes, cuticles, stigma and trichomes, or by the root tissues, as well as through junctions and injuries (Wang et al. 2016a).

A plant cell wall has pores with diameters in the range of 5–20 nm. Usually, through the cell wall water molecule as well as solute accretion occurs, this is due to the porous polysaccharide fibre matrix of the cell wall (Tripathi et al. 2017). Thus, for efficient entry into the plant cell wall, the size of the nanoparticles should be less than the cell wall pore diameter. The efficient passage of these nanoparticles through the pores enables them to extend towards the plasma membrane. The pore size of the cell wall can even be enlarged upon association with engineered nanoparticles, thereby favouring the uptake of nanoparticles (Nair et al. 2010). Plants grown in soil as well as on sand depicted no uptake or minimal uptake of nanoparticles. Therefore plant cells are grown on growth medium for the uptake of nanoparticles. The low or no uptake of nanoparticles by soil- and sand-grown plants is due to the adhesion of metal oxide nanoparticles (Singh et al. 2015). The growth medium varies with different types of nanoparticle uptake.

The ion channels and carrier proteins which are embedded in the membrane also lead to the transport of nanoparticles across the membranes in the cell. Nanoparticle entry into the plant cells is therefore an active transport process which is regulated by various cellular mechanisms, including signal transfer, plasma membrane regulation and recycling (Tripathi et al. 2017). When applied on the surface of leaves, the entry of nanoparticles is governed either by the stomatal openings, by the trichomes or by cuticular routes which accumulate and are then transported to the varying plant tissues, which is described as a top-down movement.

After penetration into the root's epidermal cell wall and membrane, the nanoparticles enter the vascular bundles through a series of steps. To achieve the crossing of nanoparticles into the cell membrane, it is necessary for the nanoparticles to undergo passive integration from the endodermal apoplast (Tripathi et al. 2017). The penetration into the seed coat occurs by uptake through parenchymatous spaces and is regulated by the aquaporins present in the seed, which thereby enhances liquid diffusion into the cotyledons (Wang et al. 2016a). Entry of nanoparticles through the vascular system or lateral root sites occurs due to incomplete formation or breakdown of Casparian strips, respectively.

In comparison to plants, uptake of nanoparticles by animal cells occurs by the endocytic pathway which includes both clathrin-independent and clathrin-dependent pathways. Fluid phase and caveolae endocytosis and phagocytosis constitute the independent pathway whereas the synthesis of clathrin-coated forms by the formation of sheathed assembly on the plasma membrane constitutes the dependent pathway.

The uptake of zinc oxide nanoparticles was established by the electron microscopic images, which depicted the damage caused to the epidermal as well as cortical cells due to the uptake of nanoparticles. This even led to an injury on the vascular and endodermal cells, resulting in the inhibition of growth of ryegrass.

2.2.2 Nanoparticle-Plant Interactions

Engineered nanoparticles include:

- Carbon nanomaterials – carbon nanotubes (CNTs), single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), graphene and fullerenes
- Quantum dots – Cadmium-selenium (CdSe), Cadmium-telerium (CdTe), etc.
- Metal-based nanomaterials – metal oxide, i.e. zinc oxide (ZnO), titanium oxide (TiO_2), copper oxide (CuO) silicon dioxide (SiO_2), etc.; zero valent, i.e. iron (Fe), silver (Ag), gold (Au), etc.; and metal salts, i.e. silicates and ceramics
- Nanopolymers – latex, dendrimers, etc.

These particles have exceptional reactivity and surface areas in comparison to their bulk equivalents (Service 2003) which is the result of eccentric physical and chemical properties. The presence of nanoparticles in the environment and biotic surroundings leads to inevitable interactions with the biotic components, thereby causing physicochemical alterations, like dissolution, incidental coating by biomolecules and redox reactions (Rico et al. 2015).

Plants exhibit intense interactions with the external environment, thus exposure to nanoparticles affects plants strongly. These interactions lead to numerous changes, including anatomical and morphological, the alteration depends largely on the concentration and nature of nanoparticles.

López-Moreno et al. (2010) showed that roots of soybean when germinated upon treating with zinc oxide nanoparticles transformed from +2 to nitrite or acetate, whereas when treated with cerium oxide no such transformation occurred.

Dissolution is widely considered for the transformation of metal-based nanomaterials, which alters their properties and fate in the plant species. Plant roots excrete organic acids, which are necessary for biotransformation of nanoparticles as they promotes dissolution. Transformation of nanoparticles takes place outside the roots as well as after entering the roots of a plant. Metal nanoparticles with variable valences undergo redox reactions in the soil, transforming these particles by their interaction with plant's biogenic redox agents (Rico et al. 2015). Nanoparticles upon interacting with plant cells release reactive oxygen species (ROS) in large amounts due to stress which in turn affects the plant biomolecules. Among nanoparticles, carbon nanotubes specifically induce accumulation of ROS.

2.2.3 Translocation

Research on the uptake of nanoparticles led to the study of their absorption, translocation or transport and accumulation in plants, which is still not very clearly understood. Ongoing research on the transport of nanoparticles suggests that these particles have the ability to move across tissues either intra- or extracellular to the xylem.

Nanoparticles with small sizes are able to pass through the cell wall pores into the cell membrane whereas those larger in size than the pores accumulate outside the cell wall, unable to enter. For example, nano-conjugates of titanium oxide alizarin red (30 nm) can traverse through the cell wall and accumulate in the subcellular portions of roots and leaves of Arabidopsis plant (Kurepa et al. 2010); on the other hand the accumulation of 25 nm titanium oxide on the surface of roots of maize altered the hydraulic conductivity and availability of water, thus reducing transpiration rate and affecting development of plants (Asli and Neumann 2009).

Sabo-Attwood et al. (2012) demonstrated that the absorption of Au nanoparticles takes place in a size selective pattern in tomato seedlings, among which the 18 nm size particles are restricted from entering the pores and thus accumulate on the surface of roots whereas the 3.5 nm Au nanospheres easily traverse into the plant cells.

Zhu et al. (2012) depicted that roots easily take up gold nanoparticles (AuNPs) with positive charge whereas the negatively charged ones translocate from roots into the stems as well as leaves. The sequence of concentration of Au in the roots was AuNPs(+) > AuNPs(0) > AuNPs(−) whereas the reverse sequence was present in the shoots. Au concentration in rice roots followed the above order whereas reversed order for shoots, specifying favourable translocation of Au nanoparticles with negative charge.

Birbaum et al. (2010) suggested that treating maize plants with 37 nm cerium oxide nanoparticles either in the form of aerosols or in suspension form resulted in no internalization or translocation. Wang et al. (2012) provided evidence on the penetration of CuO nanoparticles into the root system of maize plants by the combination of energy dispersive spectroscopy (EDS) and transmission electron microscopy (TEM) of the xylem sap. Engineered nanomaterials upon reaching the xylem tissues are translocated towards the aerial segments. Through the experiments by split roots and observation by high-resolution TEM showed that the translocation of these nanoparticles can also occur from shoots into the roots by phloem, thus reducing nanoparticles from the copper (Cu) (II) to the copper (Cu) (I) state (Zhang et al. 2015b).

2.3 Effect of Nanoparticles

Nanoparticles with the emerging advancement have shown varying beneficial effects upon plants. They have been implemented for conversion of waste to energy, production of by-products via nano-bioprocessing, usage as nano-fertilizers and nano-pesticides for regulated delivery.

These nanoparticles play an important role in plant growth and development, which renders them widely used. Different nanoparticles bestow a different effect on plants as every particle interacts with plants in a different pattern, including alteration in morphology as well as physiology. The chemical composition, reactivity, size and other properties of these nanoparticles determine its function upon interaction with plants, which can be positive or negative (Rico et al. 2015).

Copper, gold, zinc oxide, cerium oxide, titanium oxide and silver nanoparticles are some of the widely used and synthesized metal nanoparticles. Apart from these, manganese, cobalt-ferric oxide and ferric oxide are also used. Among the engineered nanoparticles, both metal and carbon-based nanoparticles have the ability to accumulate ROS, which affects the macromolecules in plants and thus leads to stress.

Carbon-based nanoparticles have distinctive chemical, electrical, mechanical and thermal characteristics which render them their significant functions (Singh et al. 2015). Single-walled carbon nanotubes are responsible for the transport of DNA and other molecules, such as dye, across plant cells as well as from the outer surrounding into the plant cells. They are regarded as nanotransporters. On the other hand, MWCNTs play a vital role in the enhancement of water uptake with the uptake of nutrients, thereby augmenting germination of seeds and growth of plants. Studies reveal that carbon nanotubes promote accumulation of ROS and lead to peroxidation of lipid molecules in the root tips of seedling and cultures of cells (Siddiqui et al. 2015). The rate of germination of seeds is elevated due to the perforation of nanoparticles into the cell wall, which strengthens uptake of water.

2.3.1 Effect on Photosynthesis

For plant growth, hardly 2–4% of the radiation energy is used. Photosynthesis provides an easy detection parameter for the stress produced due to the living and non-living factors. Nanoparticles disturb the photochemical fluorescence, photosynthetic activity and efficiency and the quantum yield, causing oxidative stress to plants.

Govorov and Carmeli (2007) conducted an experiment in which nAg and nAu were bound to the chlorophyll of the reaction centre of PSI forming a unique hybrid system, leading to two contradictory effects on photosynthetic efficiency, i.e. the nanoparticles had plasmon resonance effect, which enhanced the chlorophyll's light absorption efficiency and hence led to a decrease in the photosystem's quantum yield which was due to a tenfold increase in the transfer of energy upon chlorophyll enhancement (Siddiqui et al. 2015).

Scientists observed that there was three times increase in the activity of photosynthesis upon supplementing single-walled carbon nanotubes into chloroplasts compared to those lacking nanoparticles (Giraldo et al. 2014). Treating with SWCNTs increased the transport of electrons and enhanced the ability of plants to recognize signal molecules, such as nitric oxide (Siddiqui et al. 2015). The enhancement of photosynthetic activity of plants by SWCNTs is due to its transport and irrevocable concentration within the chloroplast's lipid layer. The chloroplast-SWCNTs complex increases transport rate in leaves in vivo by a process supplemented with photo absorption.

Noji et al. (2011) deduced through his experiments that, the activity of reaction responsible for the generation of photosynthetic oxygen can be stabilized by the formation of a complex between the PSII and the nano-mesoporous silica compound (SBA), thus depicting the transport of electrons from water, due to light-mediated reaction, to quinine. This complex is expected to render properties needed for the development of artificial photosynthetic system and photo biosensors (Siddiqui et al. 2015).

The effect of Au nanoparticles upon fluorescence quenching of chlorophyll *a* of PSII in soybean leaves was analysed. The extracted chlorophyll was blended with varying concentrations and sizes of Au nanoparticles and their absorbance and fluorescence spectra at 538 nm and 625–800 nm was noted, respectively. This led to the conclusion that with increasing concentration of Au nanoparticles, the fluorescence quenching increased with increase in absorbance, whereas at the largest size of Au nanoparticles, the absorbance was lowest. Depicting that size of nanoparticles suppresses the fluorescence, thus lower the size of Au nanoparticles, higher will be the fluorescence quenching. The suppression of fluorescence was due to the high surface area which favours increase in adsorption of chlorophyll and enhancing electron transfer from chlorophyll to nanoparticles.

Metal nanoparticles, such as nano-anatase titanium oxide refine the rate of photosynthesis, transpiration rate and water conductivity in plants, upon their exogenous application. The photocatalysing feature of titanium oxide refines the absorption and transformation of light as well as encourages assimilation of carbon dioxide (Siddiqui et al. 2015). Carbon assimilation is enhanced by the activation of rubisco, the most abundant enzyme responsible for the carboxylation reaction occurring during the light-mediated photosynthesis pathway, and hence promotes plant growth.

The function of nano-anatase largely depends on its high thermal conductivity, high surface area and photocatalytic activity. The chloroplast of spinach upon treatment led to enhanced absorption of light by chlorophyll *a*, oxygen evolution rate, quantum yield and transfer of electrons in PSII. However, in *Ulmus elongata* the foliar application of 0.1–0.4% *n*TiO2-A when exposed to 800 and 1600 μmol $m^{-2}s^{-1}$light intensity led to decrease in quantum yield of photosystem II (PSII), electron transfer and fluorescence quenching but increase in water loss and non-photochemical quenching.

Similarly, the effect on photosynthesis by zinc oxide nanoparticles varied with the species of plant, though the concentration and stage of growth were among the other parameters upon which its activity depended. ZnO NPs (24 ± 3 nm) showed that after 20 days, there was reduction in photosynthetic activity, chlorophyll concentration and stomatal conductivity at 800 mg/kg in corn but no change in the other stages at 400 mg/kg (Du et al. 2017).

Among all the photosynthetic pigments, chlorophyll *a* has more sensitivity towards photodegradation and is used as an indicator of toxicity by nanoparticles, in comparison to other parameters. The photosynthetic efficiency can be determined by the chlorophyll *a*/*b* ratio. The ratio determines the availability of nutrients, specifically N and light to plants. Comparison of nanoparticle-treated plants with that of control elaborates that the ratio of chlorophyll in ferric oxide and cobalt ferric oxide nanoparticles decreased. These observations were in contrast to chloroplasts treated with cerium oxide nanoparticles, which have the ability to eliminate ROS, thereby protecting chloroplast and improving photosynthesis. However, these nanoparticles alter the stomatal openings and modify the microstructure of chloroplast, which adversely effects photosynthesis (Du et al. 2017).

Therefore, it is necessary to have detailed knowledge about the interaction of nanoparticles with plants' photosynthetic system, which will hence determine how nanoparticles promote anti-oxidant defence and oxidative stress in plants. It has been shown that ions released by the nanoparticles lead to stress generation and induce accumulation of ROS.

2.3.2 Effect on Seed Germination

The initial stages of growth and development of plants begins with seed germination, leading to elongation of roots and emergence of shoot. Depending on the concentration and on the plant species, nanoparticles have varying effects on seed germination. Metal oxide nanoparticles and carbon-based nanoparticles exhibit diversifying effects on seed germination, root elongation and shoot growth. Scientists have shown through their experiments that different nanoparticles augment both positive and negative effects upon different plant species. Upon exposure to metal oxide nanoparticles, the effect on seed germination as well as elongation can be inhibitory, neutral or promoting.

Among the metal-oxide nanoparticles, TiO_2 nanoparticles have the ability to enact as photo catalyst, thereby inducing redox reaction which enhances germination of seeds with the initiation of growth of plumule and radicle in the seedlings of canola (Crabtree 1998). TiO_2 NPs specifically favour vigour of aged seeds and formation of chlorophyll as well as enhance the activity of rubisco enzyme, which promotes photosynthesis and growth of plants (Siddiqui et al. 2015).

The effect of CuO nanoparticles in rice exhibited inhibitory patterns on elongation and germination of seeds specifically with size greater than 50 nm. This pattern was also seen in soybean, chickpea, maize, cucumber and Indian mustard seeds. These inhibitory patterns were studied by various scientists, including Da Costa and Sharma (2016), Wang et al. (2015), Adhikari et al. (2012).

Similarly, ZnO nanoparticles at lower concentrations exhibited neutral or promoting effects on seed germination of soybean seeds and inhibitory effects at higher concentrations, depicting a dosage-dependent pattern (Stampoulis et al. 2009; Ghodake et al. 2011; Lee et al. 2010; Yang et al. 2015). In a case studied by Helaly et al. (2014), supplementing ZnO nanoparticles with MS media stimulated plantlet regeneration and somatic embryogenesis, and enhanced the activity of superoxide, peroxidase, dismutase and synthesis of proline and catalase, which led to an increase in biotic stress tolerance. Wang et al. (2012) showed that CeO_2 nanoparticles led to no change in the germination of rice and tomato seeds, whereas there was significant reduction in the germination of seeds of cucumber and corn (Du et al. 2017).

Ag and Fe nanoparticles with zero valence at varying concentrations inhibit germination of seeds, which is observed at the incubation process of seeds instead of the soaking process. AgNPs had no effect on germination but caused alteration in the cell wall's chemical composition, which confirmed that the impact of nanoparticles was up to cellular and molecular levels. Among metal-based nanoparticles, AuNPs have shown no impact on germination of barley seeds, rather they led to lower biomass production and stimulating impact on growth. However, a contradictory observation by Savithramma et al. (2012) and Gopinath et al. (2014) in *Boswellia ovalifoliolata* and in *Gloriosa superb*, respectively, claimed that AuNPs did improve the germination of seeds.

When silicon oxide nanoparticles (up to $8gL^{-1}$) were applied exogenously to seedlings, it was observed that they enhanced growth of the seedlings as well as the quality, including diameter of root collar, quantity of seedling's lateral roots, mean height and root length. This exogenous application led to improved germination of tomato seeds, germination index, fresh and dry weight of seedlings, seed vigour index and utilization of nutrients which increases the parameters for germination making them available for the seeds thereby maintaining the growth medium's pH and conductivity.

Apart from enhancing germination and quality of seeds, SiO_2 nanoparticles also stimulated chlorophyll synthesis, which is effective for crop growth and yield (Haghighi et al. 2012; Li et al. 2012). Under salinity constraints, silicon oxide nanoparticles improve accumulation of proline, antioxidant enzymes, free amino acids which improve abiotic stress tolerance by plants (Kalteh et al. 2014; Shalaby et al. 2016).

Apart from metal oxide nanoparticles, multi-walled carbon nanotubes have been found to enhance seed germination. They specifically prompt the uptake efficiency of macronutrients especially Ca and Fe and water, which will in turn enhance the growth and development of plants. Like other nanoparticles, even MWCNTs have the ability to penetrate the seed coat, which triggers the germination of barley, corn and soybean seeds when added to a sterilized agar medium. This penetration was due to the regulatory effects of MWCNTs on the expression of genes encoding the proteins of the water channel.

Khodakovskaya et al. (2012) showed that upon upregulating the marker genes responsible for formation of cell wall (NtLRX1), cell division (CycB) and transport of water (aquaporin, NNtPIP1), they accelerated the tobacco cell growth in culture.

This proved that MWCNTs play a vital role as regulators of the growth and germination of seeds (Ditta and Arshad 2016). MWCNTs upon primary uptake and aggregation in roots, can improve the activity of peroxidase and dehydrogenase enzymes, which stimulate the growth of roots and shoots. Followed by accumulation, these MWCNTs translocate from the roots towards the leaves, which induces expression of genes (Smirnova et al. 2012).

Similarly, Graphene accelerates germination of seeds and specifically decreases the time duration of germination. The rate of germination of seeds treated with graphene showed exceptional increase for the first few days as compared to untreated seeds (Zhang et al. 2015a).

2.3.3 Root and Shoot Growth

Nanoparticles not only affect photosynthesis or seed germination, they also affect root and shoot growth. They have the ability to enhance or inhibit the root and shoot length. Different nanoparticles have differing impacts on root and shoot growth, including those discussed so far.

Roots of *Vigna radiata* and *Cicer arietinum* seedlings, upon absorbing zinc oxide nanoparticles, boosted the length of roots and shoots and their biomass as well (Mahajan et al. 2011). Gruyer et al. (2013) found that depending on the species of plant, Ag nanoparticles can induce and inhibit the elongation of roots. In case of barley, the length of roots increased whereas in lettuce it was inhibited. The enhancement of root growth in *Crocus sativus* occurs due to blockage of ethylene signalling. As compared to $AgNO_3$, AgNPs increased the length of roots in maize, barley and cabbage (Siddiqui et al. 2015).

Apart from the plant species, the morphology of nanoparticles also plays a vital role in root growth. Syu et al. (2014) demonstrated the effect on root growth and the physiological changes in Arabidopsis seedlings when subjected to Ag nanoparticles with three different morphologies, among which the decahedral morphology exhibited the highest level of promotion of root growth, whereas there was no effect on root growth in the case of spherical one, rather stimulated accumulation of anthocyanin at high levels. Ag nanoparticles also inhibited elongation of root by activating aminocyclopropane-1-carboxylic acid (ACC) in Arabidopsis seedlings and lowered the expression of ACC oxidase 2 as well as ACC oxidase 7, implicating that perception and synthesis of ethylene was inhibited by Ag nanoparticles (Siddiqui et al. 2015).

Metal-based nano-particles including silicon, palladium, high levels of copper, low levels of gold and mixture of gold and copper led to a positive impact on seedlings growth and ratio of shoot to root, while cerium oxide nanoparticles effected only root elongation of lettuce at 2000 mg/L concentration. Seeds of parsley when treated with nano-anatase had stimulated root and shoot length, germination and chlorophyll content of seeds. In pumpkin, elongation of roots occurs when exposed to iron oxide nanoparticles.

ZnO nanoparticles promote elongation of roots in soybean. In *Cyamopsis tetragonoloba*, the biomass of plant, root and shoot length, synthesis of protein and chlorophyll and other parameters of growth improved on exposure with ZnO nanoparticles (Singh et al. 2015). In radish and rape plant, the growth of roots decreased upon incubation in a suspension of Zn nanoparticles. However, this kind of inhibition was not seen in suspensions of ZnO nanoparticles because of seed coat's selective permeability.

In a range of plants, carrot, cucumber, cabbage and corn growth declined as pure alumina nanoparticles (13 nm) reduced the elongation of roots without causing any modifications. In the presence of Cu nanoparticles, seed germination in lettuce led to an increase in the ratio of shoot to root in comparison to plants in the absence of nanoparticles (Nair et al. 2010).

Supplementation of nutrient medium having protein in which infusorium *Tetrahymena pyriformis* was cultured with nanotubes, it had unexpected growth simulation and increased nanotube concentration (Zhu et al. 2006). This unexpected simulation was presumed to be due the binding between protein and nanotube supplements, thus increasing protein penetration into cells and enhancing growth (Krysanov et al. 2010).

Carbon-based nanoparticles, like carbon nanotubes and graphene, have also shown varying impact on roots and shoots of plants. In onion and cucumber, elongation of roots was induced by carbon nanotubes, as well as formation of nanotube sheets on the surface of roots of cucumber upon interaction with fCNTs and CNTs. But these nanotubes were unable to enter the roots. These nanotubes had no effect on cabbage and carrot plants. Elongation of roots was inhibited by fCNTs in lettuce and by CNTs in tomato, while tomato being highly sensitive to CNTs. Scientists demonstrated that at concentrations of 0.5, 0.9 and 0.16 gL^{-1}, SWCNTs enhanced the growth of roots in onion and cucumber seeds.

Among, carbon-based nanoparticles, graphene showed exceptional effects on seeds. In an experiment on tomato seedlings exposed to graphene, it was observed that on the 19th day, the seedlings exposed to graphene had stems up to 17% longer compared to the control seedlings and longer length roots of up to 12.5% compared to control (Zhang et al. 2015a).

2.3.4 Effect on Nutrient Delivery

In agriculture, the requirement for nutrients is fulfilled by the use of fertilizers as the soil lacks most of the macro- and micronutrients. Almost 35–40% of the overall productivity of crops is dependent on fertilizers and most fertilizers directly affect the growth of plants. At present, the nutrient utilization efficiency is quiet low, as approx. 50–70% of nitrogen provided through conventional fertilizers is lost. In the continually increasing population, the demand for food is increasing, which increases the need for macronutrients by crops.

To decrease the loss of nutrients, new systems were exploited for the delivery of nutrients which involved porous nanoscale plant parts that led to the reduction in nitrogen loss. Upon encapsulating fertilizers into nanoparticles the uptake of nutrients can be increased. Use of nano-fertilizers and nano-composites instead of the conventional fertilizers is an exquisite breakthrough in science as these have a slow nutrient release rate, which continues throughout the growth of crops, enabling the crops to utilize nutrients without wasting them and prevents water pollution (Singh et al. 2015).

Nano-fertilizers are composed of nanosized macronutrients such as N, P, K, Ca, Mg and S, which are needed in high demand by crops or to supplement the activities of chemical fertilizers. N is the chief nutrient for the growth of all plants, which is released slowly through urea-coated zeolite chips. Similarly, in the soft wood cavities of *Gliricidia sepium* nanoparticles of hydroxyapatite, a derivative of urea was encapsulated and observed for the slow and feasible release of nitrogen. Zeolites have a crystal structure with honeycomb-like layers and occur as natural mineral groups, supplying nutrients slowly on demand (Manjunatha et al. 2016).

Fertilizers are encapsulated within nanoparticles through three ways (Naderi and Danesh-Shahraki 2013).

1. Encapsulation of nutrients within nanoporous components.
2. Thin coating of polymer film.
3. Delivering nanoscale dimensions in the form of particles or emulsifiers.

Phosphorus is one of the essential components in most metabolites and is involved in almost all the metabolic processes, which is supplied through conventional fertilizers. Crops take up only 20% of the available phosphorus while the rest 80% accumulates in soil and water bodies due to runoff leading to eutrophication. Use of nanotechnology increases the efficiency of phosphorus utilization and eliminates environmental menace.

In greenhouse conditions, soybean (*Glycine max*) showed 33% increase in the rate of growth and 20% yield of seeds in comparison to chemical phosphatic fertilizers due to continuous Ca and P supply (Singh et al. 2015). Upon foliar application of nano-fertilizers, there was prominent increase in the yield of crops (Tarafdar et al. 2012a, b). Yield of 80 kg ha^{-1} of cluster bean and pearl millet was obtained through foliar application of 640 mg ha^{-1} (40 ppm concentration) nanophosphorus under an arid environment (Manjunatha et al. 2016).

Compared to chemical P fertilizers, administration of nanoparticles elevated the rate of growth and germination of seeds by 33% and 20%, respectively, indicating that soybean roots absorbed hydroxyapatite nanoparticles as an implicit P source. Nano- and sub-nano-composites control the release of nutrients from the fertilizer capsule (Singh et al. 2015).

The nutrient utilization efficiency (NUE) increases up to three times as well as provides ability to tolerate stress by the use of nano-fertilizers. Combining nano-fertilizers with nano-devices releases N and P fertilizer and the uptake by plants in a synchronized manner, thereby eliminating the undesirable loss of nutrients and preventing interaction with soil, air, microorganisms and water (Manjunatha et al. 2016). With respect to nutrient utilization, scientists demonstrated that nano-composites containing macronutrients, micronutrients, amino acids and mannose upon application influenced uptake and utilization (Ali et al. 2014). Iron chelated nano-fertilizers showed increased photosynthesis, adsorption and surface area expansion in leaves (Singh et al. 2015).

2.3.5 Effect on Rhizospheric Environment

Soil is an omnipresent habitat for a wide range of microbes interacting with the biotic components, specifically rhizosphere, and with each other. At the rhizospheric site, a complex association between root and associated microbes occurs with a high diversity of microbes. Microbes present in soil are involved in the productivity of crops, functions of the ecosystem and maintenance of soil health (Mishra and Kumar 2009).

Effective molecular techniques and certain biochemical processes were developed by microbes far before detected by plants to detoxify, efflux and accumulate metal ions. Microbes have the ability of volatilizing metal ions in order to eliminate acute toxicity (De Souza et al. 2000).

Rhizobacteria exhibiting propitious effects on growth of plants are termed as plant-growth-promoting rhizobacteria (PGPR). PGPR are soil-borne, free-living bacteria isolated from the rhizosphere, and upon application to seeds or crops enhance plant growth. PGPRs are involved in controlling plant pathogens, nutrient cycle, growth of seedlings and many other ecosystem functions. PGPR are associated with asymbiotic fixation of nitrogen, production of phytohormones, i.e. IAA (Indole-3-Acetic Acid), gibberellins, cytokinins, phosphate solubilization and production of siderophores, which help in the growth of plants (Mishra and Kumar 2009).

Bacterial taxa are altered in a dose-dependent manner in which some taxa increase in proportion while others decrease, resulting in reduction in diversity. The application of nanoparticles directly on land or through treated biosolids with mobile nanoparticles interacts with soil microbes. Microbes can absorb and accumulate nanomaterials effectively and initiate mobilization through food chain and altering taxa with diverse populations, i.e. bacteria, plants, fishes, within the food web (Holden et al. 2013). Soil bacteria and fungi help plants to take up nutrients easily from the soil (Thul and Sarangi 2015).

Uptake of cerium oxide nanoparticles into the roots and nodules led to the elimination of nitrogen fixation and impairing growth of soybean. Zinc oxide, titanium dioxide and silicon dioxide nanoparticles relay toxic impact upon bacteria, which intensifies in the presence of light (Thul and Sarangi 2015).

Fortner et al. (2005) demonstrated the inhibitory effect of C_{60} fullerene aggregates on *Escherichia coli* (gram negative) and *Bacillus subtilis* (gram positive) which were grown on rich and minimal media and under both aerobic and anaerobic conditions, respectively. It was observed that at concentrations above 0.4 mg/L, complete inhibition of bacterial growth occurred in both the cultures which were subjected to both the absence and presence of oxygen and light conditions, while at concentrations up to 2.5 mg/L it was observed that no inhibition occurred in rich media, which can be because of precipitation of C_{60} or by protein coating in the media (Mishra and Kumar 2009).

Nyberg et al. (2008) depicted that there was no effect of C_{60} fullerene nanoparticles on anaerobic microbes. Fullerenes inhibit growth of bacteria mostly found in soil and water, which can be due to the antioxidant property of fullerenes leading to generation of ROS which disrupts membrane lipids and other biomolecules specifically DNA. They adsorb vitamins, minerals and trace elements found in soil which limit the growth of bacteria indirectly, ultimately leading to adverse impacts of nanoparticles on the environment.

R. metallidurans cells grown in medium containing $AuCl^{4-}$ (50 mM) showed toxicity to gold as 90% of the cells died after 4 h however, increased after 72 h of inoculation, depicting that *R. metallidurans* possesses toxicity resistance to $AuCl^{4-}$ and can adapt to high concentrations of gold. Initially precipitation of gold was significant by *R. metallidurans* but after incubation of 8 h, 3 mM gold was precipitated.

Silver nanoparticles have size in the range of 1–50 nm, the surface area of such nanoparticles is larger as compared to their volume. The large surface area provides an increase in reactivity and toxicity towards various microorganisms and bacteria. In some cases, the usage of silver nanoparticles leads to antibiotic resistance amid toxic or lethal bacteria. Various ecosystem processes controlled by bacterial species are endangered due to the use of silver nanoparticles, as they act as a strong bactericide (Elechiguerra et al. 2005).

Antibacterial activity towards *Pseudomonas aeruginosa, Salmonella paratyphi*, *Shigella* strains and *Klebsiella pneumoniae* PGPs (Plant Growth Promoters) was experimented using copper oxide nanoparticles (80–160 nm) (Mahapatra et al. 2008). Apart from this, it was interesting to find that copper/copper oxide nanoparticles were synthesized by *Serratia*, a gram-negative bacterium which dies in the process (Mishra and Kumar 2009).

It is presumed that iron- and copper-based nanoparticles produce free radicals by reacting with peroxides found in the environment. These radicals are known to be highly toxic to microorganisms. ZnO and magnesium oxide nanoparticles are potent microbe killers and act as food preservative as well (Mishra and Kumar 2009).

Upon evaluation of the impact of various nanoparticles, including fullerenes, aluminium, silver, gold, etc. on PGPR, it was suggested that nanoparticles effectively degraded phytostimulatory bacteria found in soil and also caused ecotoxicity (Table 2.1).

2.3.6 Toxicity

Application of gentle nanoparticles has been commercially approved by the Food and Drug Administration (FDA) but accumulation of nanoparticles like heavy metal nanoparticles, nanopesticides, etc. can lead to mild as well as severe nanotoxicity, which is not rationalized with respect to its bulk counterparts (Dubey et al. 2018).

Table 2.1 Impact of various nanoparticles on different plant species (Siddiqui et al. 2015; Singh et al. 2015; Manjunatha et al. 2016)

S.No	Nanoparticles	Plant species	Optimal concentration	Impact
1	Silver	*Boswelia ovalifoliolata*	10–30 μg/ml	Enhancement in seed germination and growth
2	Gold	*Cucmis sativus, Lactuca sativa*	62 μg/ml	Germination index elevates considerably
3	Selenium	*Nicotinia tabacum*	0.1 mg/gm	Initiation of callus and micro-shoot formation
4	Aluminium	*Raphanus raphanistrum, Brassica napus*	2 mg/ml	Ameliorated root growth
5	Alumina	*Lemna minor*	0.3 mg/ml	Elongation of root length
6	Titanium oxide	*Lycopersicum esculantum*	0.05–0.2 mg/ml	Overall rate of photosynthesis and conductivity of water increased
		Triticum aestivum	1 mg/ml	Rise in amount of chlorophyll
7	Ferrous oxide	*Glycine max*	0.5–0.75 mg/ml	Quality and yield refinement
8	Cobalt (II,III) oxide	*Raphanus sativus*	5 mg/ml	Enhanced growth of root
9	Zinc oxide	*Gycine max*	0.5 mg/ml	Enhanced root growth
		Arachis hypogeal	1 mg/ml	Elevated shoot and root growth and increased yield
		Cicer arietinum L.	0.15 mg/ml	Considerable increase in shoot and dry weight
10	Multi-walled carbon nanotubes	*Lycopersicum esculantum*	50 μg/ml 200 μg/ml	Increase in flower number and boosted plant height
11	Carbon nanotubes	*Lycopersicum esculantum*	40 μg/ml	Improvement in seedling germination and growth
12	Silicon dioxide	*Arabidopsis thaliana*	0.4 mg/ml	Elevation in root length
			2 mg/ml and 4 mg/ml	Reduction in root length

Nanoparticles, upon direct application can have disastrous impact, whereas upon diffusing into the apoplectic intercellular space, results in membrane adsorption and incorporation (Nowack and Bucheli 2007). Transport of compounds with negative charge occurs due to a negatively charged plant cell surface. Transfer into apoplast is interfered because of the Casparian strips, which act as a barrier to the flow, thus favouring transport into xylem symplastically (Thul and Sarangi 2015).

The production and usage of nanoparticles is increasing, leading to increase in congregation into the environment. Toxicity and environmental harm due to nanoparticles, including both direct and indirect exposures, is an ongoing debate (Brayner 2008; Panda et al. 2011; Dubey et al. 2018).

2.3.6.1 Pathogen Suppression

Crop improvement and conservation was mainly achieved through pesticide application, plant breeding and maintaining regular sanitation. The yield and quality of crops decline due to pathogens, thus there was high usage of the traditional crop improvement techniques. The traditional techniques were expensive as well as led to increase in resistance with time. Scientists found that the use of nanoparticles not only led to preservation of crop quality but also enhanced the productivity of crops (Emamifar et al. 2010; Bouwmeester et al. 2009).

Toxicity cognate with nanoparticles was utilized to tackle microbial pathogenicity in plants. Suppression of pathogens due to the antimicrobial properties associated with nanoparticles has shown to increase product quality and yield.

Antimicrobial function of nanoparticles occurs through five general mechanisms. specifically of metal nanoparticles (Lemire et al. 2013; Zeng et al. 2007):

1. Alteration of membrane protein function and permeability due to liberation of toxic ions
2. Impairment in uptake of nutrients and transport system in membranes
3. Rise in genotoxicity and cell death due to the interaction of toxic ions with DNA
4. Oxidative stress and damage of cellular components as well as DNA due to the production of reactive oxygen species (ROS)
5. Interference in metabolic pathways, which alters energy generation, membrane properties and protein oxidation

The antimicrobial properties of nanoparticles are presumed to aid unique and improved antimicrobial actions which are dosage and temperature dependent. Macromolecule oxidation or microbial membrane interaction of nanoparticles exert antimicrobial functions, which either damage or alter the permeability of membranes. Upon entry into bacterial cell, nanoparticles produce reactive oxygen species (ROS), which oxidize macromolecules, hampering important processes of cell leading to cell apoptosis (Musee et al. 2011). Nanoparticles such as silver, titanium oxide, fullerene C_{60} and single-walled carbon nanotubes particularly depict antimicrobial functions on bacterial monocultures (Morones et al. 2005; Lyon et al. 2006).

Nanoparticle toxicity of Au, Ag, Fe and fullerene C_{60} towards bacterial pathogens including *E. coli*, *B. subtilis* and *A. tumefaciens* was evaluated, among which Ag nanoparticles exhibited vigorous bactericidal activity upon every tested strain while fullerenes also showed inhibitory action on growth of all the strains. Among all the pathogenic microbes, fungi has been shown to cause highly detrimental infections and diseases in plants. Zn, Si, Ti and Cu nanoparticles showed strong antifungal properties and it was seen that the growth of fungal pathogen *A. niger* was inhibited by ZnO and zinc titanate ($ZnTiO_3$) nanoparticles (Ruffolo et al. 2010). A comparative study of the antifungal impact of sulphur nanoparticles with their bulk counterpart against *A. niger* showed increase in growth inhibition due to sulphur nanoparticles (Choudhury et al. 2010).

Si nanoparticles organize themselves as biomineralized silicon dioxide, which imparts fungal resistance (Wang et al. 2001). Two rice varieties, the resistant Nongda

18 and the susceptible Mongolian, were tested for inhibitory effects when treated with and without Si nanoparticles showed inhibition of growth of fungal pathogen *M. grisea* while there was no effect on the control ones (Dubey et al. 2018).

Composition of Ag-Si nanoparticle as an effective alternative for expensive fungicides was sought to be environment friendly, feasible as well as advantageous to humans and offered resistance from pathogens, including *Pythium* spp*., Blumeria* spp., *Colletotrichum* spp*., Sphaerotheca* spp., *Botrytis* spp*., Magnaporthe* spp., *Rhizoctonia* spp. *and Phytophthora* spp. (O'Neill et al. 2003; Shankar et al. 2003; Yau et al. 2004).

2.3.6.2 Regulated Delivery of Pesticides

Application of agrochemicals on crops is usually done through suspensions or sprays. These agrochemicals are mostly leached or degraded by either microbes or by photolytic or hydrolytic mechanism, causing loss of chemicals as well as pollution of soil and ground water. In order to eliminate this problem, designing of nano-encapsulated agrochemicals was highly important as they have the necessary characteristics, including high effectiveness, stability and solubility, on demand release, target-specific activity and minimal toxicity (Boehm et al. 2003; Green and Beestman 2007; Thul and Sarangi 2015).

Silica nanoparticles with hydrophobic surface modification were used for controlling various agricultural pests (Rahman et al. 2009) while mesoporous silica nanoparticles with surface functions specifically manipulated the expression of genes at the cellular level through delivery of DNA and regulatory proteins in a controlled manner (Torney et al. 2007).

It was observed by Kaunisto et al. (2013) that using a polymer matrix, which has swelling and dissolution properties, influenced transport pathways, thereby altering the release conditions. The polymer matrix were prepared from polyethylene glycol or polyvinylpyrrolidone nanospheres. Liposome nanopolymers as regulated delivery units for monitored insecticide release were first prepared and used by Bang et al. (2009). Moreover, the haphazard use of pesticide increased the bioaccumulation and resistance in pathogens and pests with reduction in biodiversity of soil, leading to declining nitrogen fixation and decline in pollinators with diminishing bird habitats (Ghormade et al. 2011; Thul and Sarangi 2015).

Varying compositions of insecticides based on polyethylene glycol led to systemic release at rates lower than the commercially produced ones with imidacloprid, thiram and carbofuran (Adak et al. 2012; Pankaj et al. 2012; Kaushik et al. 2013). In insects, blockage of neurotransmission through avermectin, a pesticide which inhibits chloride ion channel, having 6 h half-life upon exposure to ultraviolet rays gets inactivated in the field, which can be avoided by the controlled release up to 30 days due to encapsulation of avermectin by NP carriers (Thul and Sarangi 2015).

Nanocrystals of polylactic acid and cellulose were organized as a network of nanofibers to which thiamethoxam at a concentration of 50% was added, resulting in the decline of whitefly within 9 days monitoring in a glass house experiment

Table 2.2 Toxicity caused by nanoparticles on diverse microbes (Thul and Sarangi 2015; Ditta et al. 2015)

S.No	Nanoparticles	Microbial species	Toxicity
1	Silver	*Escherichia coli*	Bactericidal activity inhibits growth of bacteria
		Staphylococcus aureus	
		Salmonella typhimurium	
2	Ferrous oxide	*Trifolium repens*	Decrease in biomass of mycorrhizal clover
3	Zinc oxide	*Rhizobiales, Bradyrhizobium.*	Reduction in bacterial communities
		Pseudomonas putida	Hinders growth of bacteria
		Bacillus subtilis	Synthesis of ROS causes mild toxicity
4	Aluminium oxide	*Bacillus cereus, Pseudomonas stutzeri*	Response to transcription by microbes decline
5	Titanium dioxide	*Bradyrhizobiaceae*	Bacterial community diminishes
		Escherichia coli	ROS production leads to amiable toxicity
6	Cerium oxide	*Rhizobium, Azorhizobium*	Decline in nitrogen fixation ability
7	Silicon dioxide	*Escherichia coli*	ROS production leads to toxicity
8	Single-walled carbon nanotubes	*Rhizobium leguminosarum*	Alters the cellular morphology of bacteria

(Xiang et al. 2013). Nano-formulations associated with active compounds were observed effective compared to the commercial compounds for use in agriculture (Thul and Sarangi 2015).

2.3.6.3 Physiological and Biochemical Changes in Plants

Generation of reactive oxygen species due to the application of nanoparticles leads to the peroxidation of lipid molecules (Cabiscol et al. 2000), which remarkably impacts the biochemical as well as molecular properties of the membrane, including permeability, fluidity, osmotic stress susceptibility and loss in uptake of nutrients. Osmotic stress was identified due to soil and water, which activates an array of metabolic functions, in turn alleviating metal stressors (Chinnusamy et al. 2004; Thul and Sarangi 2015) (Table 2.2).

2.3.7 Accumulation of Nanoparticles

Nanoparticles have been found to accumulate in plants and the surrounding environment, as plants bear large size, higher leaf area and are immobile in nature, rendering them highly susceptible to exposure from varied nanoparticles present in the environment (Dietz and Herth 2011).

The initiation of nanoparticle mobilization within the food chain due to the adsorption and accumulation of nanoparticles by soil microbes upon direct application onto land or biosolids having mobile nanoparticles can lead to alteration in the community's food web and multiple populations, including plant, microbes and fishes (Thul and Sarangi 2015).

The size of nanoparticles contributes to the contamination of the surrounding environment, depicting distinctive physicochemical characteristics, such as high surface area, energy and surface confinement, resulting in alteration in environmental behaviour and increasing toxicity drastically, compared to their bulk components. The surface properties are the main reason for toxicity of ENPs (Engineered Nano-Particles), which can be inhibited using surface functioning (Geisler-Lee et al. 2012).

Environmental conditions alter the surface coating of nanomaterials, which can amend or simulate toxicity in microbes (Suresh et al. 2013). Due to high demand for nanoparticle-derived consumer goods, soil and water are highly potent for contamination (Thul and Sarangi 2015).

2.3.7.1 In Plants

Accumulation in plants occurs mainly through the roots harbouring most of the nanoparticles they are exposed to and cause toxicity. In terrestrial plants, most of the soil is exposed to nanoparticles, leading to leaching of nanoparticles, release into subsurface, contamination of land due to biosolid applications and discharge into wastewaters (Pokhrel and Dubey 2013; Hai et al. 2013). Therefore plants can effectively decide the fate of nanoparticles and their environmental transport by aggregating them into their biomass (Navarro et al. 2008; Ma et al. 2010; Anjum et al. 2013).

The final consumers of products produced by plants in the ecosystem are humans and animals. Metal nanoparticles like AgNPs upon accumulation would be transported into humans and animals through plant products (Cheng et al. 2011; Kim et al. 2011; Yin et al. 2011). AgNPs-treated *Lemna paucicostata*, an aquatic plant, and *Loliummultiflorum*, common grass seedlings, led to toxicity whether they were placed in a petri plate or in an aquatic environment (Geisler-Lee et al. 2012).

The problem of concern is the transport of nanoparticles into the food chain, which happens through consumption of edible plants. Using synchrotron X-ray fluorescence (μ-XRF) and X-ray absorption near edge structure (μ-XANES) analysis of *Cucumis sativus,* a garden vegetable, was conducted, which demonstrated titanium dioxide nanoparticle translocation from roots to the fruit without biotransformation (Servin et al. 2013; Thul and Sarangi 2015).

It is difficult to completely explain the phytotoxicity caused due to AgNPs just by the release of Ag ions. Plants subjected to Ag NP and silver nitrate ($AgNO_3$) suspension indicated that higher accumulation of silver occurred in AgNP suspensions compared to $AgNO_3$ due to uptake of AgNP by plants. In the case of *Brassica juncea*, there was no accumulation of Ag when exposed to AgNP.

Ultra-small anatase TiO_2 nanoparticles upon entering plant cells get accumulated in the sub-cellular sections, including vacuoles and root nuclei, leading to reorientation and exclusion of microtubules, which thereby inhibit elongation of roots in

Arabidopsis (Kurepa et al. 2010; Wang et al. 2011). Similarly, effect of ZnO nanoparticles was also observed in *Arabidopsis*, which showed reduced accumulation of biomass in shoots and roots and decrease in chlorophyll content instead of carotenoid content (Wang et al. 2016b).

Through μ-XRF imaging, it was established that Ce and Zn traverse between tissues with water flow during transpiration, which led to their bioaccumulation (Zhao et al. 2013; Thul and Sarangi 2015).

Hu et al. (2014) suggested that upon exposure to ZnO nanoparticles upto 7 days, their accumulation and dissipation led to aggregation of zinc in leaves and roots of *Salvinia natans*. Whereas Zhai et al. (2014) demonstrated that in the cytoplasm, cellular organelles, root cells and leaf cells, there was accumulation of Au nanoparticles, which was confirmed by transmission electron microscopy and measured by inductively coupled plasma mass spectrometry (ICP-MS) (Thul and Sarangi 2015).

2.3.7.2 In Soil and Water Bodies

Aquatic invertebrates, including copepods (*Amphiascus tenuiremis*), amoebae (*Entamoeba histolytica*), cladocerans (*Daphnia magna*), infusoria (*Tetrahymena pyriformis* and *Stylonychia mytilus*), absorb carbon nanoparticles from food, but it is still unspecified if these nanoparticles penetrate internal organs (Oberdörster et al. 2006; Templeton et al. 2006; Zhu et al. 2006, 2009; Elías et al. 2007; Roberts et al. 2007).

It was experimentally observed that the existence of nanoparticles within aquatic organisms leads to reduced fertility, abnormalities in behaviour, physiological alterations and increased rate of mortality (Lovern and Klaper 2006; Templeton et al. 2006; Krysanov et al. 2010).

The penetration ability of carbon nanoparticles into aquatic organisms depends upon their structure and modification. In the embryos of zebrafish (*Danio rerio*), the unmodified C_{60} fullerenes penetrated through chorion, whereas hydroxylated derivatives ($C_{60}(OH)_{24}$) could not (Isaacson et al. 2007). Similarly, when subjected to copper nanoparticles, the NPs accumulated at a concentration of 1.9 fold in the gills after 48 h as compared to the controls (Griffitt et al. 2007).

Daphnias were subjected to titanium dioxide, aluminium oxide and zinc oxide nanoparticles for 48 h, which accumulated in the gut (Zhu et al. 2009). This was due to the fact that the nanoparticles aggregated within 12 h but their excretion was delayed due to which most of the nanoparticles were retained in the body for another 72 h.

Zhao et al. (2012) demonstrated the concurrence of zinc oxide nanoparticles with zinc-dissolved species which was liberated constantly into the soil, leading to replenishment of zinc ions, which were rummaged by roots in comparison to alginate-treated soil, thus promoting aggregation of zinc in the tissues of corn (Thul and Sarangi 2015).

Quantum dots have the ability to fluoresce, which makes them efficient in monitoring the penetration and translocation pattern within living organisms. Quantum dots have superseded in reaching not only to the gut but to various other parts of daphnia (*D. magna*) (Ingle et al. 2008; Thul and Sarangi 2015).

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