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NANOTECHNOLOGY: SCOPE AND LIMITATIONS IN AGRICULTURE

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ABSTRACT

Nanotechnology is one of the fastest developing fields with potential to revolutionize industries such as pharmaceuticals, electronics, military, manufacturing, and agriculture. Nanomaterials have significant applications in food and agriculture systems as smart delivery mechanisms for agrochemicals, nano-formulations, nano-biosensors for precision farming and food packaging, nano-bioremediation, nanofibres for genetic manipulation etc. Besides direct applications of nanotechnology in agriculture, the engineered nanomaterials that are used in commercial products and industries (non-agricultural) may also affect agriculture indirectly. Many nano-based products are already in the market with or without proper labeling. Not much information is available on the interactions between nanomaterials and biological systems. Therefore, understanding the impact of nanomaterials and related technologies on soil and plant health is very important. The present review focuses on the application of nanotechnology in agriculture and its possible impact on plant growth and soil microflora. It emphasizes on more research to study the impact of nanotechnology on agriculture and develop regulatory protocols for safe production, use and release of nanomaterials to minimize environmental nanotoxicity.

KEY WORDS: Nanotechnology, Agriculture, Soil microorganisms, Nanotoxicity, Regulation.

INTRODUCTION

The fast development in the disciplines like biotechnology and bioengineering has transformed agricultural into a modern industry. Nanotechnology, another upcoming discipline has revolutionary applications in pharmaceuticals, electronics, military, manufacturing, and other life sciences. Nanotechnology is the understanding and manipulating matter at scales measurable in nanometers (1-100 nm) at least in one direction (NNI 2007). At nanoscale, the surface area of the particles is very large relative to their small size, which can make them very reactive. Due to the very small size and high reactivity, the fundamental properties of the matter at nano-scale may differ from that of corresponding bulk material. These novel properties may help in the development of revolutionary technologies having application in different fields. For example, carbon in the form of graphite is relatively soft but nano form of carbon nanotubes (made of carbon atoms) is 117 times stronger than steel and 30 times stronger than kevlar (Chang *et al.* 2010). Thermal behavior of nanoscale materials may also differ from bulk materials (Pivkina *et al.* 2004). Aluminum in its bulk form does not burn, however, aluminum nanoparticles combusts rapidly and are used as propellant in rocket fuel. Precise use of such novel materials can lead to enormous economic and societal benefits. Thousands of nanotechnology based products are already in the market in the form of medicines, cosmetics, food packaging, formulations, electronics etc.

The progressive development of novel nanoscale materials and related technologies has significant applications in food and agriculture systems (Joseph and Morrison 2006). Nanosensors and nanobased formulations of agricultural chemicals (pesticides, herbicides etc.) are some of the current applications of nanotechnology in agriculture. The role of nanoparticles (NPs) has been proposed as low cost technology for purification of drinking water (Yavuz *et al.* 2006) and mineralization of undesired organic pollutants (Mach 2004). Nanoparticles may be used for the remediation of polluted soil and groundwater (Zhang 2003). Thus through different means or applications, nanomaterial can come in contact with soil and waterbodies. Besides, the advancement in the use of various engineered nanoparticles in commercial products and industries like medicines, cosmetics, electronic appliances etc. is bound to impact agriculture directly indirectly or accidently. The nanomaterials entering water and soil ecological systems might affect soil and plant health and/or might be bio-accumulated through the food chain and finally accumulated in higher-level organisms. Although soil is a rich source of natural nanoparticles, little is known about the impact of engineered nanoparticles (ENPs) on food crops. Furthermore, there is lack of information on the effect/fate of these ENPs in the soil and food chain (Darlington *et al.* 2009). Accumulation of NPs may affect microbial communities which act as soil health indicators and also interact with plants in different ways. Plants play important role in ecological system and may serve as a potential pathway for NPs transport and a route for bioaccumulation into the food chain (Zhu *et al.* 2008). How these nanomaterials interact with biological systems at molecular level is not yet known (Maynard 2006). These interactions may be positive, negative or neutral (Fig. 1).

Although, advances in nanotechnology can help in using agricultural inputs more effectively, enhancing agricultural productivity in a sustainable manner, the nanomaterials used in agriculture may also become new environmental hazards themselves. Such technologies may also pose potential risks which may be hidden initially but may be realized at later stages. Asbestos is a current example of use of technology without knowing the consequences, disadvantages of which far outweighed the benefits. The toxic effects of nanoparticles on prokaryotic and eukaryotic organisms have been recently summarized (Ostroumov and Kotelevtsev 2011). It is necessary to review effects and possible consequences of nanomaterials related technologies on soil and plant health before expanding the application of this technology in different dimensions.

1. APPLICATION OF NANOTECHNOLOGY IN AGRICULTURE

Nano-agriculture involves the employment of nanomaterials or nano-based technologies in agriculture, aiming to get some beneficial effect on the crops in terms of productivity or quality. At present, the work on application of nanotechnology in agriculture is at its preliminary stage, worldwide. But in coming years we will witness more applications of nanotechnology in food and agriculture sector. The Government of India initiated a Nano Science and Technology Mission in 2007 through the Department of Science and Technology with an allocation of Rupees 1,000 crores (US$ 200 million) for a period of five years and continues to strengthen it (DST 2009). The Department of Biotechnology (DBT), Government of India launched the Nanotechnology Initiative in Agriculture and allied sectors (Sastry 2007). Indian council on Agricultural Research ICAR has also initiated work on application of nanotechnology in agriculture.

1.1. Nanobiosensors for agricultural applications

The work on the development of nanotechnology-based biosensors to monitor soil health, plant growth, and disease onset is in progress. Biosensors have a biological component that reacts to changes in surrounding environment, and then produce a signal in a linked transducer, that can be further processed to generate data. Compared to the conventional methods, biosensors are more sensitive and specific and can give real-time analysis in complex mixtures in very less time. These biosensors can be linked with GPS system and connected to a computer for real-time monitoring. Use of these biosensors in agriculture, can be very useful in precision farming where productivity can be optimized by judging the soil and plant health and nutritional status before the appearance of visible symptoms of any deficiency or disease and providing the required inputs and conditions, in a timely manner with precision (Day 2005, Joseph and Morrison 2006). Biosensors for livestock animals can be used to monitor changes in hormone levels or antibody profile, thereby helping in timely breeding practices and veterinary interventions (Scott 2005). Run *et al*. 2007 described an amperometric biosensor for the rapid detection of organophosphorus (OP) pesticides, by using carbon nanotubes for the surface modification of glassy carbon electrode, for the immobilization of acetylcholine esterase and bovine serum albumin. The degree of inhibition of the enzyme acteylcholinesterase (AChE) by OP compounds is determined by measuring the electrooxidation current of the thiocholine generated by the AChE catalyzed hydrolysis of acteylthiocholine (ATCh) (Joshi *et al.* 2005). The large surface area and electro-catalytic activity of carbon nanotubes increase the sensitivity and stability of electrode. However, such biosensors using inhibition of acetylcholine esterase (AChE) for the detection of OP compounds are not specific, and are more indirect and slow. A preferred direct biosensing route for detecting OP compounds involves the biocatalytic activity of organophosphorus hydrolase (OPH) as described by Deo *et al* (2005). A bilayer approach with the OPH layer atop of the carbon nanotube (CNT)-modified transducer (glassy carbon electrole) used for preparing the CNT/OPH biosensor lead to a highly sensitive and stable detection of the enzymatically (OPH) liberated *p*-nitrophenol thus offer great promise for rapid on-site screening of OP pesticides.

1.2 Nanosensors for detection of food pathogens

Nanotechnology applications in the food industry are contributing to the safety, quality and long shelf life of packed food. Many nano-based health drinks and foods containing nano-food supplements like iron have been manufactured. Nanocomposite, bio-degradable materials are being used for safe packaging and long shelf-life of food products. Composite materials with silicon nanoparticles used for packaging are found to be more airtight, thus preventing food decay (Moore 1999). Nanobiosensors are being used in the packaging material to detect microbiological and biochemical changes in food items, indicating food spoilage. Chip-based micro-arrays, Quantum dots and magnetic nanoparticles have been developed for rapid detection of biological pathogens like *E. coli*, *Salmonella*, *Staphylococcus* etc. in food (Su and Li 2004, Moraru *et al.* 2003; Yang *et al.* 2008). Nanobiosensors can also be designed to detect presence of pesticides and possibly genetically modified crops within the food system.

1.3. Control of pests and weeds using nano-based materials

Nanotechnology can play significant role in controlled and site targeted delivery of agrochemicals like pesticides and herbicides (Nair *et al.* 2010). These agrichemicals can be encapsulated in biogedradable, ecofriendly material under specific conditions. Their release can be controlled by structural manipulations, thereby requiring less dosage per application and minimizing runoff of unutilized excess chemicals. The use of nanocapsulated herbicides for the control of parasitic weeds also reduces the ecotoxicity of herbicides (Pérez-de-Luque and Rubiales 2009). Surface modified hydrophobic nanosilica has been successfully used to control a range of agricultural insect pests (Barik *et al.* 2008, Rahman *et al.* 2009). Photosensitive agrochemicals can be encapsulated in porous hollow silica nanoparticles (PHSN), with a shell thickness of nearly 15 nm and a pore diameter of 4–5 nm, which provide shielding protection from degradation by UV light (Li *et al.* 2007). Nanomaterial coated fertilizers have been found efficient in slow release of the fertilizers as compared to cemented fertilizers as well as safe for the germination and growth of wheat (Liu *et al.* 2006, Zhang *et al.* 2006). Certain agrochemical companies like Syngenta are using nanoemulsions in pesticide formulations. Primo MAXX®, a plant growth regulating product by Syngenta can impart tolerance to turfgrass against different stresses like heat, drought, disease etc. Encapsulated product “gutbuster” releases its contents under alkaline conditions, such as stomach of certain insects and exhibits broad spectrum insecticidal properties against insect pests of soybeans, rice, peanuts and cotton. Another microencapsulated, insecticidal product, Karate ® ZEON breaks open after coming in contact with leaves. (Joseph and Morrison 2006).

1.4. Agriculture based nanomaterials for industry use

Electrospinning techniques, where electric (high voltage) force instead of mechanical force is used, have been developed to produce nanofibres from cellulose, derived from scrap materials produced in huge quantity during conventional spinning of cotton (Frazer 2004). These cellulose nanofibres can find applications in filtration, clothing and in agriculture in the form of biodegradable cellulose mats that can absorb pesticides and fertilizers for their controlled release. Carbon nanotube-based composite fibers have been synthesized that are tougher than any natural or synthetic organic fibre. These extraordinary fibres can be woven into electronic textiles (Dalton et al. 2003).

Agriculture on one hand can benefit from nanotechnology and on the other hand, can support growth of nanotechnology. Many plants are known to biosynthesis nanoparticles which can be isolated/ extracted from different plant parts (Kalaugher 2002, Dubey *et al.* 2009). Many microorganisms including bacteria, fungi, actinomycetes and yeast also possess the ability to synthesize nanoparticles (Mohanpuria *et al.* 2008; Narayanan and Sakthivel 2010). Thus plants and microorganisms can be used for ‘nanoparticles farming’, wherein plants/microorganisms grown on specific medium/conditions can synthesize nanoparticles which can be harvested, rather than using the current conventional nanoparticles production techniques which are expensive and can have toxic effect on the environment.

1.5. Use of nanoparticles in bioremediation

Nanotechnology can also play important role in pollution sensing and remediation of contaminated agricultural lands, groundwater and drinking water by exploiting novel properties of nanomaterials. Nanosensors are capable of detecting microbes, moisture content and chemical pollutants at very minute levels. Photocatalysis using metal oxide semiconductor nanostructures can degrade organic pesticides and industrial pollutants into harmless and often useful components (Baruah and Dutta, 2009). This technology can help in the remediation of contaminated agricultural lands and water bodies. Efficiency of the nanoscale iron particles have been demonstrated for transformation and detoxification of a wide variety of common environmental contaminants, such as chlorinated organic solvents, organochlorine pesticides, and polychlorinated biphenyls (PCBs) (Zhang, 2003). Lanthanum nanoparticles can absorb phosphates in aqueous environments. Application of these nanoparticles in water bodies can absorb available phosphates thus preventing the algal growth (Joseph and Morrison, 2006). Nanofiltration (NF) has been shown to be an effective way of removing organic micropollutants from drinking water due to its size exclusion properties (Dixon *et al.* 2010).

1.6. Integration of nanotechnology and plant biotechnology

Nanotechnology offers efficient crop improvement through genetic manipulation by using nano-tools like, nanoparticles, nanofibres and nanocapsules. Among these, nano-fibre arrays which can deliver genetic material to cells quickly and efficiently have potential applications in crop engineering (Nair *et al.* 2010). Single walled carbon nanotubes (SWNTs) can traverse across both the plant cell wall and cell membrane (Liu *et al.* 2009 and can serve as effective nanotransporters to deliver DNA and small dye molecules into intact plant cells, thus can be used as small treatment delivery systems in plants (Gonzales–Melendi *et al.* 2008). Integration of carbon nanofibres surface modified with plasmid DNA, with viable cells has been reported for gene transfer in plant cells, resulting in controlled biochemical manipulations in the regenerated plant (McKnight *et al.* 2003; McKnight *et al.* 2004). Integration of the transferred DNA into host genome can be prevented by tethering it on carbon nanofibres. Due to this non-integration the expression of the tethered DNA can be restricted to one generation of cells and the trait does not pass to further generations. The fluorescent labelled starch-nanoparticles induce instantaneous pore channels in cell wall, cell membrane and nuclear membrane and can be used as transgenic vehicle to transports genes in the plant cells (Jun *et al.* 2008). Surface functionalized mesoporous silica nanoparticles (MSNs) can also penetrate plant cell walls, delivering DNA and its activators in a controlled fashion for precise manipulation of gene expression at single cell level. The MSNs are loaded with DNA and its chemical inducer and capped by gold nanoparticles to prevent the release of loaded molecules. After penetration into the plant cells the uncapping induced by chemical treatment, releases the DNA and its inducer thus resulting in controlled expression of the gene/s (Torney *et al*. 2007). With the advancement in imaging techniques movement of fluorescent labled nanoparticles carrying foreign DNA can be tracked across the cell wall, thus gene transfer mechanisms can be understood and improved further.

EFFECT OF NANOMATERIALS ON PLANT GROWTH

The effect of nanoparticles on plant growth varies greatly with the type of nano-particle, concentration used and the plant species being studied. Further different nano-particles affect different growth processes of plants. The nanomaterial can enter the plant by binding to carrier proteins, through aquaporin, ion channels, endocytosis, by creating new pores or by binding to the organic chemical in the environmental media (Rico *et al.* 2011). Interaction of nanoparticles with edible plants has been recently reviewed by Rico *et al.* 2011. Confocal fluorescence image studies have revealed the capacity of single walled carbon nanotubes to traverse across both the plant cell wall and cell membrane

(Liu *et al.* 2009). Compared to plant cell walls and membranes, the penetration of nanoparticles into seeds is expected to be difficult due to the significantly thick seed coat covering the whole seed. Khodakovskaya *et al.* (2009) demonstrated that CNTs could effectively penetrate seed coat, thereby influencing the seed germination and plant growth. Exposure of seeds to CNTs (40 µg/ml in MS medium) enhanced tomato seed germination and growth rate. The presence of CNTs inside the seeds was also confirmed by Raman spectrum and transmission electron microscope. The CNTs create pores in the cell wall thus enhancing water uptake thereby promoting germination. The seeds treated with CNTs showed higher moisture content as compared to control seeds (Srinivasan and Saraswathi 2010). The positive effect of multi-walled carbon nanotubes (MWCNTs) on seed germination and root growth of crops like radish (*Raphanus sativus*), rape (*Brassica napus*), rye grass (*Lolium perenne*), lettuce (*Lactuca sativa*), corn (*Zea mays*) and cucumber (*Cucumis sativus*) (Lin and Xing 2007) are reported. Rice seeds treated with SWCNTs and MWCNTs also showed improved germination (Stamphoulis *et al.* 2009).

Treatment of soybean (*Glycine max*) with a mixture of nanoscale SiO2 and TiO2 at low concentration resulted in improved germination and plant growth. The seedlings showed enhanced uptake of water and fertilizers and high nitrate reductase activity and better antioxidant system (Lu *et al.* 2002). Application of Nano-TiO2 (2.5 to 40 g/Kg soil) could promote growth of spinach by enhancing photosynthesis and nitrogen metabolism (Hong *et al.* 2005 a,b). The improvement in spinach growth under N-deficient conditions was related with N2 fixation by nano-anatase TiO2. Nano-anatase TiO2 on exposure to sunlight could chemisorb N2 directly or reduce N2 to NH3 in the spinach leaves, transforming into organic nitrogen and improving the growth of the spinach (Yang *et al.* 2007). Treatment with nano-anatase TiO2 promoted photosynthesis and growth of spinach under visible and ultraviolet illumination and accelerated electron transfer, photophosphorylation of chloroplast (Chl), water photolysis and oxygen evolution (Lie *et al* .2007). Xuming *et al.* (2008) further observed that, nano-anatase treatment resulted in enhancement of Rubisco mRNA amounts, the protein levels, and activity of Rubisco, thereby leading to the improvement of Rubisco carboxylation and high rate of photosynthetic carbon reaction. It could also promote antioxidant status of spinach chloroplast under UV-B radiation by removing ROS and activating SOD, CAT, GPX and APX and (Zheng *et al.* 2008). Similarly, nano-SiO_2 showed a corresponding positive effect on growth of Changbai larch (*Larix olgensis*) with increasing concentration up to 500mg/L (Lin *et al.* 2004).

Lin and Xing (Lin and Xing 2007) studied effects of five types of nanoparticles (multi-walled carbon nanotube, aluminum, alumina, zinc, and zinc oxide) on seed germination and root growth of six higher plant species (radish, rape, ryegrass, lettuce, corn, and cucumber) and reported significant inhibition of germination of ryegrass germination by nZnO (2000 mg/L) and of corn by nZnO and nAl_2O_3 (2000 mg/L). Inhibition on root growth varied greatly among nanoparticles and plants. Root elongation of the tested plant species practically terminated by 2000 mg/L of nano-Zn or nano-ZnO. Fifty percent inhibitory concentrations (IC50) of nano-Zn and nano-ZnO were estimated to be near 50 mg/L for radish, and about 20 mg/L for rape and ryegrass. In another study by Lee *et al.* (2010) the effect of three concentrations (400, 2000 and 4000 mg/L) of four metal oxide nano-particles, four metal oxide nanoparticles, aluminum oxide (nAl_2O_3), silicon dioxide ($nSiO_2$), magnetite (nFe_3O_4), and zinc oxide (nZnO) on the development of *Arabidopsis thaliana* using three toxicity indicators (seed germination, root elongation and number of leaves). Among these, nZnO was most phytotoxic, which (all concentrations) significantly inhibited development (seed germination, root elongation and number of leaves), followed by nFe_3O_4, $nSiO_2$, and nAl_2O_3, which was not toxic. Root elongation was significant improved with nAl_2O_3 (all tested concentrations) and $nSiO_2$ (400 mg/L) whereas $nSiO_2$ (2000 and 4000 mg/L) and nFe_3O_4 (all concentrations) showed significant inhibition of root elongation. The increasing concentrations (5, 10, and 20 µg ml^{-1}) of the cobalt and zinc oxide NPs severely inhibit root elongation of *Allium cepa* under hydroponic conditions due to massive adsorption into the root system (cobalt NPs) and accumulation in both the cellular and the chromosomal modules (Zn Nps) thus signifying their highly hazardous phytotoxic nature (Ghodake *et al*. 2011). Three desert plants, Parkinsonia florida (blue palo verde), Prosopis juliflora-velutina (velvet mesquite) and Salsola tragus (tumbleweed) responded differently to seed treatment with different concentrations of ZnO nanoparticles (0 to 4000 mg L $^{-1}$). Although germination was not significantly affected ($P < 0.05$) in any of the three plant species, root length in velvet mesquite was reduced at all ZnO NP concentrations used whereas root elongation in blue palo verde was reduced by16% (at 4000 mg ZnO NPs L^{-1}), and in Tumbleweed root size diminished by 14% and 16% (at 500 and 2000 mg ZnO NPs L^{-1} respectively. Further X-ray Absorption Spectroscopic (XAS) studies demonstrated the biotransformed of ZnO NPs on/within the root in all three plant species (Rosa *et al.* 2011).

Liu et al. (2010) attempted to study the effect of fullerence at cellular level in transgenic seedlings. The treatment with fullerence resulted in retarded root growth with shortened length and loss of root gravitropism. Fluorescence imaging revealed the abnormalities of root tips in hormone distribution, cell division, microtubule organization, and mitochondrial activity. Genotoxic effects of CeO2 NPs treatment in soybean plants have also been demonstrated (Lopez-Moreno et al. 2010). Use of synchrotron X-ray absorption spectroscopy revealed presence of CeO2 NPs in roots. Random amplified polymorphic DNA assay showed the appearance of four new bands at 2000 mg L−1 and three new bands at 4000 mg L−1 treatment of CeO2 NPs indicating the effect of NPs on DNA replication. Ma et al 2010 studied the effect of four rare earth oxide nanoparticles, nano-CeO2, nano-La2O3, nano-Gd2O3 and nano-Yb2O3, on seven plant species (radish, rape, tomato, lettuce, wheat, cabbage, cucumber) by means of root elongation experiments. Low concentrations of rare earth ions had a positive effect on plant growth. A suspension of 2000 mgL-1 nano-CeO_2 had no effect on root elongation of six plants except lettuce, whereas similar concentration of all other tested nano-particles severely inhibited the root elongation. The wheat plants were inhibited on seed incubation whereas lettuce and rape were inhibited on both seed soaking and incubation process. The fifty percent inhibitory concentrations (IC50) for rape were about 40 mg L−1 of nano-La_2O_3, 20 mg L−1 of nano-Gd_2O_3, and 70 mg L−1 of nano-Yb_2O_3, respectively. Calabrese and Baldwin (2002) explained the dose dependent effect of rare earth ions on plant growth by “hormesis effect” which is meant by a low-dose stimulation and high-dose inhibition. Further, it has also been reported that as compared to functionalized nano-particles, non-functionalized nano-particles have more inhibitory effect on plant growth (Canas et al. 2008). Thus, the inhibitory effect of nano-particles can be reduced by their functionalization. Moreover, functionalization also increases the specificity of nano-particles.

EFFECT OF NANOMATERIALS ON SOIL MICROORGANISMS

In soil, microbial communities play very important role in organic matter recycling and mineralization of nutrients thus play a crucial role in soil fertility and plant growth. Certain groups of bacteria form symbiotic relationships with legumes and fix atmospheric nitrogen, providing a major source of fixed nitrogen for host as well as other plants. Another group of rhizobacteria exert positive effect on plant growth and are called plant growth promoting rhizobacteria (Kloepper 1989). Denitrifying and nitrifying bacteria play an important role in nitrogen cycle. Many groups of bacteria form symbiotic relationships with animals from insects to humans. Some of these bacteria help in digestion process, others perform more unusual functions. There are groups of microorganisms which produce antibiotic against plants and animals pathogens. Microorganisms have been used as soil health indicators because of their intimate relationship with their surroundings owing to their high surface to volume ratio. Any factor affecting soil microflora also affects soil fertility and productivity thus causing imbalance in ecosystem. Population of soil microflora depends on physicochemical properties of soil, pH, moisture content, partial pressure of oxygen and composition of plant root exudates. Although the soil is rich in natural nanoparticles formed due to continuous weathering and re-arrangement of its geogenic constituents coupled with high biological activity. The extensive and uncontrolled use of engineered NPs may result in their accumulation in environment, agricultural lands and water bodies, affecting the physicochemical and biological properties of soil due to their very reactive nature. Therefore, it is very important to study the effect of released nanomaterial on the soil microflora (Mishra and Kumar 2009).

Many nanomaterials have been found to have anti-microbial properties, having application in the control of multi-drug resistant pathogenic microbes (Jones *et al.* 2008). Silver (Ag) NPs show broad spectrum antimicrobial activity against various plant pathogenic fungi. However their non-targeted effect on beneficial microflora may have negative consequences. Silver, known and being used since long for its anti-microbial properties, is a good example of technology application. Due to small size (1-50nm), silver nanoparticles have large surface area compared to volume, which increases their reactivity and toxicity against various microorganisms. The silver nanoparticles if accumulated in soil and water can adversely influence the ecosystem by affecting the beneficial microorganisms and related processes. Choi *et al.* (2008) studied susceptibility of nitrifying bacteria to silver nanoparticles and suggested that accumulation of AG NPs could have detrimental effects on the microorganisms in waste water treatment. Nitrifying microorganisms involved in nitrification are critical to biological nutrient removal in waste water treatment. Addition of AG^+ to a Swedish surface soil (100 µg g^{-1}) resulted in significant reduction in denitrification rate and in the copy number of copper-nitrate-reductase-encoding *nirK* gene (Throback *et al.* 2007). Kumar et al. (2011) studies the potential toxicity of 0.066% silver, copper or silica NPs on a high latitude (>78°N) soil of polar region, using community level physiological profiles (CLPP), fatty acid methyl ester (FAME) assays and DNA analysis, including sequencing and denaturing gradient gel electrophoresis (DGGE). Among the three NPs, Silver NPs were found to be highly toxic to the arctic consortia. Culture-based studies confirmed the high sensitivity of nitrogen fixing, plant-associating bacteria, *Bradyrhizobium canariense*, to silver NPs.

Fullerence a form of carbon (C60) is hydrophobic in nature and can act as adsorptive agents for different organic and inorganic matter in the soil, resulting in high concentration of these compounds at specific sites. Further adsorption of various chemical compounds (micronutrients and vitamins) by fullerence can deprive the soil organisms of nutrients, resulting in growth inhibition. Generation of reactive oxygen species by fullerence may also cause disruption of membrane lipids and DNA causing growth inhibition (Sayes *et al.* 2005, Foley *et al.* 2002). Fullerence have been found to inhibit the growth of commonly occurring soil and water bacteria (Fortner *et al.* 2005; Oberdorster *et al.* 2004). Tong *et al.* (2007) studied the impact of fullerence (C60) on soil microbial communities and microbial processes by treating the soils with C60 (1µg g^{-}1 soil in aqueous suspension or 1000 µg g^{-}1 soil in granular form) for 180 days. The introduction of fullerence did not show any significant effect on microbial processes like respiration and soil enzymes however, proportion of Gram-negative to Gram-positive microorganisms in treated soils was slightly higher as compared to untreated soils. Response of *Escherichia coli* and *Bacillus subtilis* exposed to different concentrations of fullerence varied under different growth conditions. Fullerence inhibited bacterial growth and respiration in minimal medium with low salt, whereas as in rich medium no effect was observed (Fonter *et al.* 2005, Lyon *et al.* 2005). Johansen *et al.* (2008) studied the effect of C_{60} fullerence on soil microbiota by measuring total respiration, biomass, number, and diversity of bacteria and total number and diversity of protozoa.

Fullerence had no effect on microbial respiration and biomass, whereas reduction in population of fast-growing bacteria was observed after the addition of C_{60}. Further, fullerence also showed some effect on diversity of microbial and protozoal communities in the soil. The inhibitory effects of fullerence on the soil microflora can have hazardous effects on the environment.

Besides, different nanoparticles like Cu, MgO, ZnO, TiO2, SiO2, Ag-topped TiO2, Pt(IV)-modified TiO2, C-doped TiO2, CNT, have been found to show antimicrobial activity against a number of microorganisms like *E. coli*, *Bacillus subtilis, B. megaterium, Pseudomonas aeruginosa, P. putida, Staphylococcus aureus, S. mutans, Micrococcus lylae*, *Pseudokirchneriella subcapitata* etc. some of which are agriculturally important (Fonter *et al.* 2005, Adams *et al.* 2006, Brayner *et al.* 2006, Neal 2008, Aruoja *et al.* 2009, Hoecke *et al.* 2008, Gajjar *et al.* 2009). We studied the effect of Au, Ag, CNT and Ag-CNT nanoparticles on two rhizospheric bacteria, *Pseudomonas putida* P7 and *Bacillus subtilis* RP24 and observed growth inhibition of both bacteria by Ag-CNT, whereas no inhibition was observed with Au, Ag, CNT treatments (Table 1). It is suggested that the nanoparticles damage microbial cells by destroying the enzymes that transport the cell nutrient and weakening the cell membrane or cell wall due to the production of reactive oxygen species. Sondi and Salopek-Sondi (2004) found that nanosilver damaged and pitted the bacterial cell walls, leading to increased cell permeability and ultimately cell death. Some researchers suggest that nanosilver interfere with bacterial DNA replication (Yang *et al.* 2009). Size and shape of the NPs play important role in nano-toxicity with smaller particles showing higher toxicity than the larger particles due to their easy passage in the microbial membrane (Pal *et al.* 2007). Further the toxicity of NPs is dose dependent indicating that certain concentrations can be risky for the environment (Gijjar *et al.* 2009).

NANOTOXICITY, ENVIRONMENTAL SAFETY AND REGULATORY MEASURES

The study on the toxic effects of nanomaterials on plants is an upcoming area of research. The plants have a tendency to absorb non-essencial elements along with essential elements, which when accumulated above a threshold level may have lethal effect on non-tolerant species (Ke *et al.* 2001, Bondada *et al.* 2004, Arias *et al.* 2010). Once accumulated in the plant tissue these toxic elements can enter the food chain and ultimately the higher organisms. The large quantities of different engineered nanomaterials being produced for application in wide range of functions thus open up the possibility of inevitable release of a considerable amount of these nanomaterials into the environment which may accumulate at specific sites in the geo and hydrosphere (e.g. soils, groundwater and water bodies) or in the biosphere. Once released in the soil or water directly in the form of nano-based agricultural chemicals, accidental release, or indirectly through contamination, these nanomaterials can be easily accessible to plants (Rico *et al.* 2011).

Although it is very clear that nanotechnology has the potential to revolutionize the fields of medicine, security and manufacturing. Applications of nanotechnology in Agriculture are also evident. But the hard fact about nanoscale materials is that they can be far more toxic to the biological systems than their bulk forms. Therefore, the unforeseen impacts and consequences of nano-toxics released directly or indirectly in the ecosystem, on agriculture are to be given due attention. A comprehensive study of the interaction of nanomaterials with their surrounding environment is needed. Total lifecycle of the nanomaterials and products containing them is a matter of serious consideration and need attention of researcher, manufacturers, consumers and policy makers.

Worldwide, there are no clear regulations on production, use, labeling and disposal of nanoparticles and the products and materials that contain them, thus exacerbating potential human and environmental health and safety issues associated with nanotechnology (Bowman and Hodge 2007, Faunce 2008). Serious concerns regarding the benefits and risks associated with nanotechnology are being raised (Hood 2005). Some countries have increased funding for evaluating the interactions of manufactured nanoscale materials with biological systems, to address health and safety issues associated with nanotechnology and to develop appropriate policies (Roco 2003). There is need to develop separate comprehensive regulation mechanisms for nanotechnology to ensure its safe development and applications (Bowman and Fitzharris 2007). Further public interest and expectations should also be considered while shaping the development of nanotechnology (Bowman and Hodge 2006).

Organics is considered as a food stream that do not include synthetic chemicals, additives and genetically modified organisms for food production. Nanomaterials clearly fall into the category of synthetic chemicals. At least three organic standards, namely, Australian National Standards for Organic and Bio Dynamic Produce, of 1 July 2007, The UK Soil Association Standards of 2008, The Demeter Standard, the only international labeling and standards scheme, have already excluded nanotechnology (Paull and Kristen 2008, Paull 2011). The Soil Association was also the first to declare organic standards free from genetic engineering.

The possible applications of nanotechnology in agriculture include smart delivery systems for agrochemicals, nano-formulations for plant nutrient and pest management, biosensors for precision farming, nano-bioprocessing for agriculture waste management, nanofibres for genetic manipulation etc. At present, application of nanotechnology in agriculture is at its nascent stage. Exposure of plant cells to nanomaterials may result in altered plant gene expression and modification in associated biochemical pathways which ultimately affect plant growth and development. Similarly NPs also exhibit inhibitory effect against beneficial soil microflora. Therefore consequences of interactions of nanomaterials with plants and microorganisms should be highlighted in future studies. Besides, stringent regulation of different NPs entering the environment is necessary. Success of nanotechnology in agricultural advancement will largely depends on the ability of researchers, technology developers, national and international policy makers to address the different challenges in the coming years.

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Table 1 : Effect of different nanoparticles (two concentrations) on growth of *B. subtilis* RP24 and *P. putida* P7 in terms of colony forming units

Treatments	***B. subtilis* RP24 (cfu/ml)**		***P. putida* P7 (cfu/ml)**	
	25µg/ml	**50µg/ml**	**25µg/ml**	**50µg/ml**
Ag	$1.4X10^8$	$2.5X10^8$	$4.0X10^9$	$2.0X10^9$
CNT	$1.6X10^8$	$1.8X10^8$	$6.8X10^9$	$3.0X10^9$
AgCNT	$6.4X10^2$	$5X10^2$	$4.8X10^2$	$1.0X10^2$
Antibiotic (standard)	$3.1X10^1$	$1.0X10^1$	0.0	0.0
Control	$1.2X10^8$	$1.0X10^8$	$3.8X10^9$	$1.8X10^9$

Figure Caption

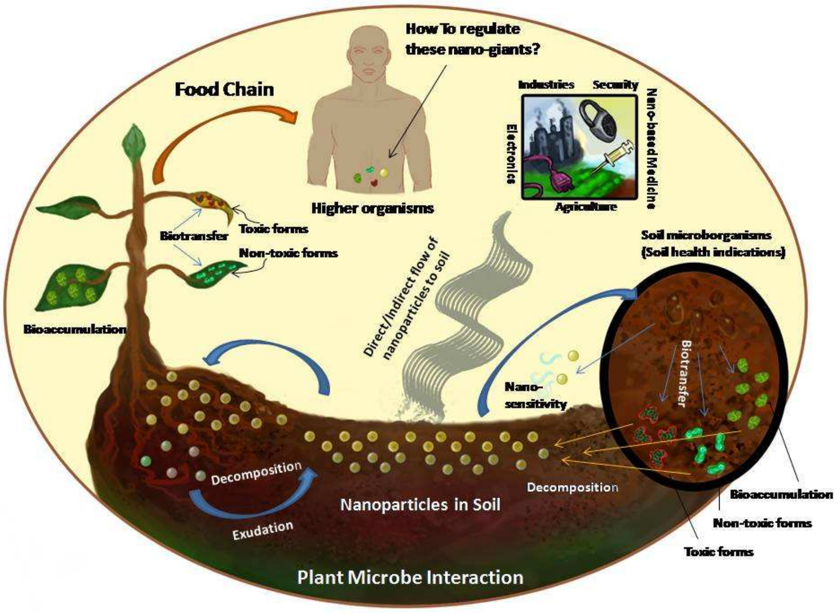

Figure 1. Interactions of Nano-materials with Biological Systems