Nanoparticle Production, Characterization and its Application to Horticultural Crops

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Introduction

Nanotechnology, which deals with the matter at nanoscale (1-100 nm), is commonly referred to as a generic technology that offers better-built, safer, longer-lasting, cost-effective and smart products that will find wide applications in household, communications, medicine, agriculture and food industry, amongst other. Nanotechnology-based products and its applications in agriculture include nano-fertilizers, nano-herbicides, nano-pesticides, recalcitrant contaminants from water, nano-scale carriers, nanosensors, veterinary care, fisheries and aquaculture, detection of nutrient deficiencies, preservation, photocatalysis, nanobarcode, quantum dots etc. This fast growing technology is already having a significant commercial impact, which will certainly increase in the future. While nanotechnologies offer many opportunities for innovation, the use of nanomaterials in food and agriculture has also raised a number of safety, environmental, ethical, policy and regulatory issues.

Nanotechnology is emerging out as the sixth revolutionary technology in the current era after the Industiral Revolution of Mid 1700s, Nuclear Energy Revolution of the 1940s, The Green Revolution of 1960s, Information Technology Revolution of 1980s and Biotechnology Revolution of the 1990s. It is an emerging and fast growing field of science which is being exploited a wide spectrum of disciplines such as physics, chemistry, biology, material science, electronics, medicine, energy, environment and health sectors. Materials reduced to the nanoscale show some unusal properties which are different from what they exhibit on a macro scale, enabling unique systematic applicaions. Nanoscience has revolutionized in different fields in achieving the processes and products that are hardly possible to evolve through conventional systems. The nanotechnology-aided applications have the potential to change agricultural production by allowing better management and conservation of inputs of plant and animal production. Currently, such nanotechnologies have been reported to be in use in the form of (i) Nanosensors, nanopesticides (Mukal *et al.*, 2009) (ii) Nanoscale adjuvants for pesticides (Bio-based, 2010) (iii) Bio synthesized nanoparticles for agricultural use (Tarafdar *et al.*, 2012 a, b, c; Mahajan *et al.*, 2011) (iv) Smart delivery systems for nanoscale pesticides and fertilizers (Mukal *et al.*, 2009) (v) Feed additives (Shi *et al.*, 2006; Spriull 2006) (vi) Veterinary medicines (Ochoa *et al.*, 2007) (vii) Aqua culture (Kumar *et al.*, 2008) as bio sensors (FSA, 2008) (viii) Plant growth regulators (Choy *et al.* 2006) and (ix) Plants to synthesize nanoparticles (Gardea-Torresday *et al.*, 2002, 2003). In order to gain the fruition of such development, more and more countries join the club of nanotechnology. A survey by Salamanca – Buentella *et al.* (2005) predicts availability of several nanotechnology applications for agricultural production for developing countries within next 10 years. These included - (i) Nanoforms zeolites for slow release and efficient dosage of water and fertilizers for plants; drugs for livestock; nanocapsules and herbicide delivery (ii) Nanosensors for soil quality and for plant health monitoring; nanosensors for pests detection (iii) Nanomagnets for removal of soil contaminants and (iv) Nano-particles for new pesticides, insecticides, and insect repellents.

Synthesis of Nanoparticles

The nanoparticles may be synthesized by physical, chemical, biological and aerosol technique. Physical synthesis method includes sedimentation process, rotor speed mill, high energy ball mill and pot mill. In general, phosphorus (P) nanoparticles are prepared by purifying rock phosphate and grinding with high energy ball mill or pot mill (Table 1).

Table 1. Synthesis of P nanoparticles by physical means

Method used	Average particle size (nm)
High energy ball mill	28.0
High energy pot mill	70.0

Mainly chemical synthesis of nanoparticles involved precipitation technique and Poly Vinyl Pyrrolidene (PVP) technique. Preparation of Zn nanoparticles from zinc acetate with potassium hydroxide in methanol is a perfect example of chemical precipitation of nanoparticles. It is possible to prepare nanoparticle size ranges between 18-22 nm (Fig. 1).

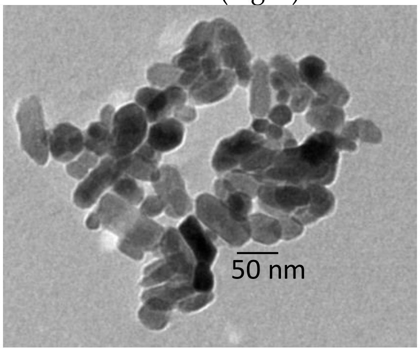

Fig. 1. TEM images of Zn nanoparticles prepared by chemical precipitation method

PVP technique is a solution phase synthesis of many nanoparticles. The hydroxyl end groups of PVP are a well-suited reductant for the aqueous synthesis of circular, triangular, and hexagonal nanoparticles. The morphology can be change by adjusting the molar ratio of PVP to the salt precursor and by altering the molecular weight of PVP. For example, the preparation of palladium (Pd) nanoparticles, sodium paladium (II) tetrachloride (Na_2PdCl_4) is used with the molecular weight of PVP ranges between 10,000 and 55,000 (Table 2).

Table 2. Production of different size and shapes of Pd nanoparticles by PVP technique take from slides

Metal precursor	MW of PVP	Molar ratio, PVP: metal precursor	Temp. ˚C	Time	Shape, average size, and yield
Na_2PdCl_4	55000	1.5	80	5 h	Hexagonal, 40 nm, 10%
Na_2PdCl_4	55000	5	80	5 h	Hexagonal, 45 nm, 70%
Na_2PdCl_4	55000	15	80	5 h	Triangular, 25 nm, 30%
Na_2PdCl_4	29000	15	80	5 h	Triangular, 10 nm, 40%
Na_2PdCl_4	10000	15	80	5 h	Triangular, 50 nm, 70%
Na_2PdCl_4	55000	5	95	5 h	Hexagonal, 30 nm, 30%

Biological nanoparticles can be prepared by selecting microorganisms to grow in a particular salt solution after preparation of microbial balls. Biosynthesized nanoparticles are relatively stable and ecofriendly as they are naturally encapsulated by fungal protein. Lists of some organisms are given (Table 3), which can successfully produce 100% nanoparticles from the respective salt solutions.

Table 3. A list of fungi efficient in producing (100%) biological nanoparticles

S. No.	Name of the microorganisms	NCBI Gene Bank Accession Number	Utility
1.	*Aspergillus terrus* Strain CZR1	JF 681300	Producing Zn & Mg nanoparticles
2.	*Aspergillus flavus* Strain CZR2	JF681301	Producing Zn, Fe, Ag & Au nanoparticles
3.	*Aspergillus flavus* Strain TFR1	JN 194185	Producing Zn, Mg, P, Ti & Ag nanoparticles
4.	*Aspergillus terreus* Strain TFR2	JN 194186	Producing Zn, Mg, P, Ti & Ag nanoparticle
5.	*Aspergillus tubengensis* Strain TFR3	JF126255	Producing Fe nanoparticles
6.	*Aspergillus japonicas* Strain AJP01	JF770435	Producing Fe nanoparticles

There are five different aerosol methods in use for nanoparticles synthesis. They are (i) furnace method (ii) flame method (iii) electro spray (iv) chemical vapor deposition and (v) physical vapor deposition method. By using furnace method it is very difficult to produce the particle size less than 100 nm. Flame method can be used only with suitable precaution and can successfully produce TiO_2 nanoparticles. Electro-spray is a very useful method where exact size and shape of nanoparticles can be produced but yield is very low (1 g per year). Chemical vapor deposition and physical vapour deposition methods are very popular for production of nanoparticles. To produce nanoparticles by aerosol technique one has to accurately control the gas flow rate, heater size and diffusion drier size. The instrumental set up for aerosol synthesis of nanoparticles is presented as Figure 2.

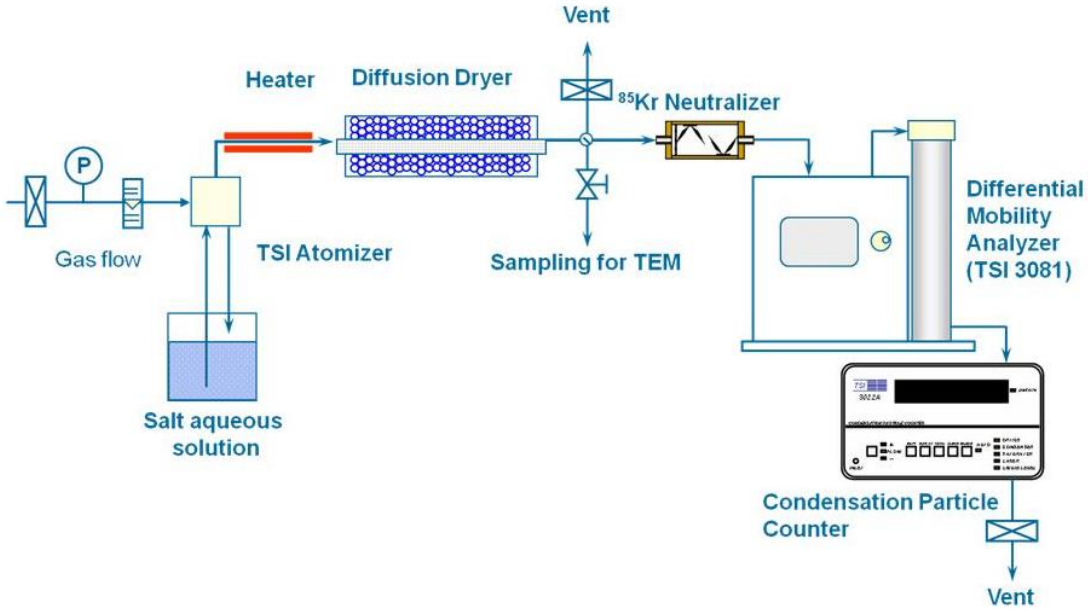

Fig. 2. Instrumental set-up for aerosol synthesis of nanoparticles

Nanoparticles may be of different shapes, broadly it can be classified as cube shape, plate-shape, wire-shape and cage-shape.

Application of Nanoparticles

Aerosol spray (with the help of nebulizer) is much superior than traditional spray for application of nanoparticles to plants and microorganisms. Lower concentration (5 ppm or less) can absorb and penetrate better through plants. Nanoparticle size 20 nm or less may be better to apply. Nanotube is found to be the better shape for more penetration both in plants and microorganisms.

Uptake of nanoparticles in plant systems

Nanoparticles are adsorbed to plant surfaces and taken up through natural nano-or micrometer-scale plant openings. Several pathways exist or are predicted for nanoparticle association and uptake in plants (Fig. 3).

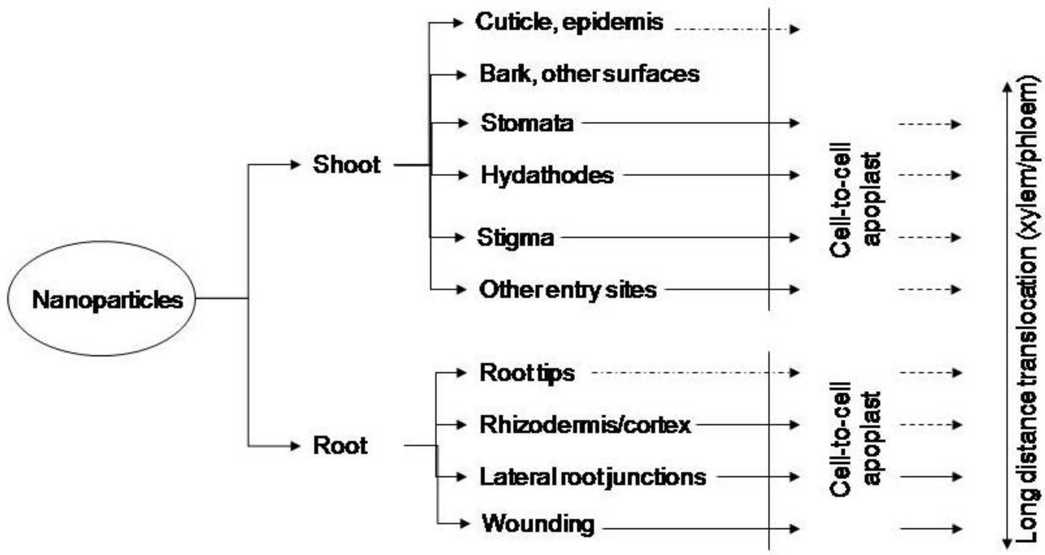

Fig. 3. Path ways of nanoparticle association, uptake and translocation in plants. Broken lines indicate the assumption of very low rates of transport.

Nanoparticle uptake into the plant body can use different paths. Uptake rates will depend on the size and surface properties of the nanoparticles. Very small sizes nanoparticles can be penetrate through cuticle. Larger nanoparticles can penetrate through cuticle-free areas, such as hydathodes, the stigma of flowers and stomata's. Nanoparticles must traverse the cell wall before entering the intact plant cell protoplast. Result suggests that only nanoparticle less than 5 nm in diameter will be able to traverse the cell wall of undamaged cell efficiently.

Application of Nanotechnology in Seed Science

Seed is a basic input deciding the fate of productivity of any crop. Conventionally, seeds are analyzed for their germination and distributed to farmers for sowing. Despite the fact that the germination percentage registered in the seed testing laboratory is about 80-90%, it hardly happens in the field due to the inadequacy or non-availability of sufficient moisture under rainfed system. In India, more than 60% of the net area sown is under rainfed system, it is quite appropriate to develop technologies for rainfed agriculture. A group of research worker is currently working on metal oxide nano-particles and carbon nanotube to improve the germination of rainfed crops. Khodakovskaya *et al.* (2009) at the University of Arkanasas, USA, have reported the use of carbon nano-tube for improving the germination of tomato seeds through permeation of moisture.Their data have vividly shown that carbon nanotubes (CNTs) serve as new pores for water permeation by penetration of seed coat and act as a gate to channelize the water from the substrate in to the seeds. These processes facilitate germination which can be exploited rainfed agriculture system.

Nano-fertilizers for balanced crop nutrition

In India, fertilizers are squarely responsible for enhanced food grain production from 1960s (55 mt) to 2011 (254 mt) that coincides with the exponential increase in fertilizer consumptions from 0.5 mt to 23 mt, respectively. It has been unequivocally demonstrated that fertilizer contributes to the tune of 35-40% of the productivity of any crops. Cosidering its importance, the Government of India is heavily subsidising the cost of fertilizers particularly urea to encourage farmers to use them to promote productivity of crops. This resulted in imbalanced fertilization and occurance of nitrate pollution in ground waters. In the past few decades, use efficiencies of N, P and K fertilizers remained constant as 30-35%, 18-20% and 35-40%, respectively, leaving a major portion of added fertilizers stay in the soil or enter into aquatic system causing eutrophication. In order to address a number of issues such as low fertilizer use efficiency, imbalanced fertilization, multi-nutrient deficiencies and decline of organic matter, it is quite pertinent to evolve a nano based fertilizer formulation with multiple function.

Nano-fertilizer technology is very innovative and scanty reported literature is available in the scientific journals. However, some of the reports and patented products strongly suggest that there is a vast scope for the formulation of nano-fertilizers. An enhanced production has been observed by foliar application of nano particles as fertilizer (Raliya, 2012; Tarafdar, 2012). It has been found that 640 mg ha^{-1} foliar application (40 ppm concentration) of nanophosphorus gives 80 kg ha^{-1} P equivalent yield under arid environment of clusterbean and pearl millet. Currently, research is underway to develop nano-composite to supply all the required essential nutrients in suitable proportion through smart delivery system. Preliminary result suggest that balanced fertilization may be achieved through nanotechnology. The impact of nano-fertilizer products on physiological, biochemical, nutritional and morphological changes in plants and the fate of nano-products in soil and plant systems have to be studied. In addition, the effects of nano-fertilizer products on rhizosphere microorganisms and biogeocycling of nutrients have to be explored under natural field conditions.

Nanofertilizers have the opportunity to profoundly impact energy, the economy, and the environment by reducing nitrogen loss due to leaching, emissions, and long-term incorporation by soil microorganisms (DeRosa *et al.* 2010). Currently, the nitrogen use efficiency of plants is low due to the loss of 50% and 70% of the nitrogen supplied in conventional fertilizers. New nutrient delivery systems that exploit the porous nanoscale parts of plants could reduce nitrogen loss. Fertilizers encapsulated in nanoparticles will increase the uptake of nutrients. In the next generation of nanofertilizers, the release of the fertilizer can be triggered by an environmental condition or simply be time released. Slow, controlled-release fertilizers have the potential to increase the efficiency of nutreint uptake. Nanofertilizers that utilize natural materials for coating and cementing granules of soluble fertilizer have the advantage of being less expensive to produce than those fertilizer that rely upon manufactured coating materials. Slow, controlled – released fertilizers may also improve soil by decreasing toxic effects associated with over application of fertilizer. Zeolites have been used as fertilizer delivery mechanism.

Nano-herbicide for effective weed control

Weeds are menace in agricultural production system. Since two-third of Indian agriculture is rainfed farming where usage of herbicide is very limited, weeds have the potential to jeopardize the total harvest in the delicate agro-ecosystems. Herbicides available in the market are designed to control or kill the growing above ground part of the weed plants. None of the herbicides inhibits activity of viable belowground plant parts like rhizomes or tubers, which act as a source for new weeds in the ensuing season. Soils infested with weeds and weed seeds are likely to produce lower agricultural yields than soils where weeds are controlled. Improvments in the efficacy of herbicides through the use of nanotechnology could result in greater production of crops and less injury to agricultural workers who must physically remove weeds if herbicides are not used. With a view to design and fabricate a nano-herbicide that is protected under natural environment and act only when there is a spell of rainfall that truly mimic the rainfed system, encapsulated nano-herbicides are relevant.

Developing a target specific herbicide molecule encapsulated with nanoparticle are aimed for specific receptor in the roots of target weeds, which enter into system and translocated to parts that inhibit glycosis of food reserve in the root system. This will make the specific weed plant to starve for food and gets killed (Chinnamuthu and Kokiladevei, 2007). Adjuvants for herbicide application are currently avaialble that claim to include nanomaterials. One nanosurfactant based on soybean micelles claims to make glyphosate-resistant crops susceptible to glyphosate when it is applied with the 'nanotechnology-derived surfactant' (Bio-Based, 2010). The regulatory structure in developed countries is driving development of nanoscale herbicides in the direction of nanoscale adjuvants rather than nanoscale-active ingredients (Observatory NANO 2010). Whether the nanoapplication is due to a nanosized active ingredient or the creation of a nanosized formulation through the use of an adjuvant, the benefits of nanoapplication are similar: Less herbicide is required to achive the weed reduction effects desired. If the active ingredient is combined with a smart delivery system, herbicide will applied only when necessary according to the conditions present in the field.

Nano pesticide

Persistence of pesticides in the initial stage of crop growth helps in bringing down the pest population below economic threshold level and to have an effective control for a longer period. Hence, the use of residues in the applied surface remains one of the most cost-effective and versatile means of controlling insect pests. In order to protect the active ingredient from the environmental conditions and to promote persistence, a nanotechnology approach "nano-encapsulation" can be used to improve the insecticidal value. Nano-encapsulation comprises nano-sized particles of the active ingredients being sealed by a thin-walled sac or shell (protective coating). Recently, several research papers have been published on the encapsulation of insecticides. Nano-encapsulation of insecticides, fungicides or nematicides will help in producing a formulation which offers effective control of pests while preventing residues in soil. In order to protect the active ingredient from the environmental conditions and to promote persistence, a nanotechnology approach "controlled release of the active ingredient" can be used to improve effectiveness of the formulation that may greatly decrease amount of pesticide input and associated environmental hazards. Nanopesticides shall reduce the rate of application because the quantity of product actually being effective is at least 10-15 times smaller than that applied with classical formulations, hence a much smaller than the normal amount could be required to have much better and prolonged management.

Clay nanotubes (Halloysite) have been developed as carriers of pesticides for low cost, extended release and better contact with plants, and they will reduce the amount of pesticides by 70-80%, hence reducing the cost of pesticide and also the impact on water streams.

Nanotechnology in Water Management

Nanotechology, the engineering and art of manipulating matter at the nanoscale, offers the potential of novel nanomaterials for the treatment of surface water, groundwater and wastewater contaminated by toxic metal ions, organic and inorganic solutes and microorganisms. Due to their unique activity towards recalcitrant contaminants many nanomaterials are under research and development for use in the treatment of water.

To maintain public health, pathogens in water need to be identified rapidly and reliably. Unfortunately, traditional laboratory culture tests take days to complete. Faster method involving enzymes, immunological or genetic tests are under development. Water filtration may be improved with the use of nanofiber membranes and the use of nanobiocides, which appear promisingly effective. Biofilms are mats of bacteria wrapped in natural polymers. These can be difficult to treat with antimicrobials or other chemicals. They can be cleaned up mechanically, but all the cost of substantial down-time and labour. Work is in progress to develop enzyme treatments that may be able to break down such biofilms.

Biosensors to detect nutrients and contaminants

Protection of the soil health and the environment requires the rapid, sensititve detection of pollutants and pathogens with molecular precision. Soil fertility evaluation is being carried out for the past sixty years with the same set of protocols which may be obsolete for the current production systems and in the context of precision farming approaches. Accurate sensors are needed for *in situ* detection, as miniaturized portable devices, and as remote sensors, for the real-time monitoring of large areas in the field. Generally speaking, a sensor is a device built to detect a specific biological or chemical compound, usually producing a digital electronic signal upon detection. Sensors are now used for the identification of toxic chemical compounds at ultra low levels (ppm and ppb) in industrial products, chemical substances, water, air and soil samples, or in biological systems.

A variety of characteristic volatile compound are produced by microorganisms that are useful as well as harmful to human beings, e.g. fermentation makes use of yeasts while alcohol is produced as a by-product when bacteria eat sugar. For rapid growth of a wide range of microorganisms, dairy products, bakery products and other food products represent ideal media. The most common causal organisms of food rotting are bacteria. Foul odor is a clear indication of food rotting. The human nose can detect and distinguish a large number of odors, but sometimes it may be impractical and a further cause of poisoning. Therefore, it is more sensible to use an instrument like rapid detection biosensors for the detection of these odors.

These instruments are able to reduce the time required for lengthy microbial testing and immunoassays. Application of these instrucments include detection of contaminants in different bodies such as water supplies, raw food materials and food products. Enzymes can act as a sensing element as these are very specific in attachment to certain biomolecules. According to, enzymatic biosensors on the basis of immobilization surface are classified into four grops (i) controlled-pore glass beads with optical transducer element, (ii) polyurethane foam with photo-thermal transducer element, (iii) ion-selective membrane with either potentiometric or amperometric transducer element and (iv) screen-printed electrode with amperometric transducer element.

Electronic nose (E-nose) is used to identify different types of odors; it uses a pattern of response across an array of gas sensors. It can identify the odorant, estimate the concentration of the odorant and find characteristic properties of the odor in the same way as might be perceived by the human nose. It mainly consists of gas sensors which are composed of nanoparticles e.g. ZnO nanowires. Their resistance changes with the passage of a certain gas and generates a change in electrical signal that forms the fingerprint pattern for gas detection.

Smart delivery system

Nanoscale devices are envisioned that would have the capability to detect and treat diseases, nutrient deficiencies or any other maladies in crops long before symptoms were visually exhibited. "Smart Delivery Systems" for agriculture can posses timely controlled, spatially targeted, self-regulated, remotely regulated, pre-programmed, or multi-functional characteristics to avoid biological barriers to successful targeting. Smart delivery systems can monitor the effects of delivery of nutrients or bioactive molecules or any pesticide molecules. This is widely used in health sciences wherein nanoparticles are exploited to deliver required quantities of medicine to the place of need in human system. In the smart delivery system, a small sealed package carries the drug which opens up only when the desirable location or infection site of the human or animal system is reached. This would allow judicious use of antibiotics than otherwise would be possible.

Nano-food industry

During last three years, food industries have witnessed that the nanotechnology has been really integrated in a number of food and food packaging products. There are now over 300 nanofood products available on the market worldwide. These exciting achievements have encouraged a large increase of R & D investments in nanofood. Today, the Nanotechnology is no longer an empty buzzword, but an indispensable reality in the food industry. Any food company who wants keep its leadership in food industries must begin to work with nanotechnology right now. The impact of nanotechnology is huge, ranging from basic food to food processing, from nutrition delivery to intelligent packaging. It is estimated that the nanotechnology and nano-bio-info convergence will influence over 40% of the food industries up to 2015. There is a strong need to develop nanofood through nano-engineering of food ingredients. Under this, texture, taste, flavour and color of food ingredients can be modified using nanoengineering without losing their nutritional value or with improved nutritional quality. For example, plant proteins may be given meat flavour by modifications. Waste utilization for nanoproducts development. Efforts are required to develop smart nanocarrier out of fruits and vegetables wastes of Industries. This has two fold application; (i) waste can be a continuous source of nanotechnology produce and large scale demand of substrate for nanoproducts development can be met. (2) Utilization of waste protects environmental pollution. Nanofilms may be developed for extending shelf life of perishables like fruits, vegetables, and flowers during transportation to prevent loss.

Health & Environmental Concerns

Researchers noted that rats breathing in Nano particles generally have those particles settle in the brain and lungs, which led to significant increases in biomarkers for inflammation and stress response (Elder, 2006) and that Nano-particles include induce skin aging through oxidative stress in hairless mice.

A two-year study at UCLA's School of Public Health found lab mice consuming Nano-titanium dioxide showed DNA and chromosome damage to a degree "linked to all the big killers of man, namely cancer, heart disease, neurological disease and aging (Schneider, 2010).

Extremely small fibres, so called Nano-fibres, can be as harmful for the lung as asbestos is (http://www.bbc.co.uk/news/health-193551960, 2012-08-24). The Royal Society report identified a risk of Nano-particles or Nano-tubes being released during disposal, destruction and recycling, and recommended that "manufacturers of products that fall under extended producer responsibility regimes such as end-of-life regulations publish procedures outlining how these materials will be managed to minimize possible human and environmental exposure". Reflecting the challenged for ensuring responsible life cycle regulation, the Institute for Food and Agricultural Standards (http://www.msu.edu/~ ifas/) has proposed that standards for Nanotechnology research and development be integrate across consumer, worker and environmental standards. They also propose that the NGOs and other citizen groups play a meaningful role in the development of these standards.

The ultra-small sizes that make the nanoparticles of immense usefulness, unfortunately the same characteristic is a basic cause of several adverse effects and may also represent significant hazards to environment, animals, human beings and plants when use non- judiciously.

- Nanoparticles as pesticides, fertilizers or in other formulations, when air borne may deposit on leaves and floral parts of plants. They may plug stomata and create a fine physical and toxic barrier layer on stigma preventing pollen tube penetration. They may also enter the vascular tissue and impair translocation of water, minerals and photosynthates.
- Animals may inhale nanoparticles deep into lungs resulting into various ill effects and disorders. The particles may enter the bloodstream.
- Nanopesticides may reduce environmental contamination through the reduction in pesticide application rates, but they may also create new kinds of contamination of soils and waterways due to enhanced transport, longer persistence and higher toxicity.
- Airborne nanoparticles, represents some specific hazards for human health, they may enter the body through the respiratory system. Due to entry of nanoparticles in to lungs and blood stream, there is possibility of pro-inflammatory effects on the basis of their ability to cause inflammation; protein fibrillation (caused by specific types of carbon nanotubes); induction of genotoxicity, and

side effects in terms of biodistribution. The particles may also translocate to other organs, especially to lungs and kidneys and may cause plugging of veinlets, Bowmans capsule of nephron etc. The nature of chemicals loaded on the nanoparticles would further aggravate the associated adverse effects.

Because of these risks, the use of nanotechnology in fertilizer and pesticide formulations has to be met very cautiously, and warrants mandatory need to critically analyze and examine the risks involved with the nano formulations. Recommended doses should be fixed for each and every product of safe limit before universal application.

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