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Latest Developments and Applications of Nanotechnology in Agriculture Sector: A Review

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ABSTRACT

Nanotechnology, a term coined by Norio Taniguchi first time in 1974, pertains to controlling, building, designing, restructuring materials and devices on the scale of atoms and molecules. Till today nanotechnology is used in the entire spectrum of the agriculture sector, such as agriculture production, single-molecule detection for the determination of enzyme-substrate interactions, nanocapsules for efficient and timely delivery of agrochemicals and growth hormones in a controlled fashion, nanosensors for monitoring soil conditions, crop growth and quality and for detection of animals and plant pathogens, nanochips for the preservation of identity and tracking, nanocapsules for vaccine delivery and nanoparticles to deliver DNA (Deoxyribonucleic Acid) to plants, a process known as “targeted genetic engineering.” Especially in the agriculture sector, research and technical innovation are important, addressing the crucial global challenges like population growth, drastic climate change and the limited availability of prime plant elemental nutrients such as phosphorus and potassium. Despite these tremendous advantages, uses of nanotechnology are still comparably marginal in the agricultural sector and research has not been commercialized and reached to market to any larger extent in comparision to the other sectors of nanotechnology application. Major safety concerns and stringent laws related to human health and the environment were raised towords industrial-scale manufacturing and use of nanomaterials hinders its use in the agriculture sector as well.

Key words: Agriculture, Agri-nanotechnology, Nanotechnology.

Agriculture is an essential and a substantial sector as it yields raw components for food and feed industries. According to the Ministry of Finance report, Govt. of India, the substantial increase in agriculture growth was 3.59% between 2004-2014. It was proposed that the above-stated growth was lesser than the targeted 4% annual growth (Dasgupta *et al*., 2015). In the current scenario, intense efforts are being made to attain sufficient growth in the agriculture sector. One of the major concerns in agriculture is food grain production (Dasgupta *et al*., 2015). Due to the depletion of natural resources such as producible land, soil and water, there has been a great obligation in promoting development in the agriculture sector, which must be economically viable and eco-friendly (Duhan *et al*., 2017). Advanced technologies and their judicial use would undoubtedly promote agricultural growth by enhancing natural resources per unit productivity (Duhan *et al*., 2017). Despite numerous scientific advancements, nanotechnology has emerged as one of the most promising fields in the food and agriculture industry (Abbas *et al*., 2016). Nanotechnology has been identified as one of the six “Key Enabling Technologies” by the European Commission. Nanotechnology is the branch of science that deals with the technology of molecules measuring one of their dimensions in the nanoscale (1-100 nm) (Cheng *et al*., 2016). It involves thorough understanding of molecules, individual atoms and clusters of molecules and physical, chemical and mechanical properties associated with them. It also deals with fabricating devices at the nanoscale level (Ditta, 2012). Due to the rapid increase in the population in the underdeveloped and developing countries, their increasing demand for food supply triggers the need for agricultural advancements (Grillo, *et al*., 2016).

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The development of agriculture is a compulsory phenomenon for the purge of poverty and hunger, which must be removed from the present situation (Kumari and Yadav, 2014). Many potential techniques have been established to increase the farm yield and simultaneously reduce the environmental hazards associated with them (Pramanik *et al*., 2020). Nanotechnology has emerged as a promising field to handle critical issues related to health, energy, agriculture and water (Abobatta, 2018). Efforts have been made to leverage nanotechnology applications in the agricultural sector, emphasizing sustainable production (Abobatta, 2018). Novel and new agrochemical agents have been developed through nanotechnology to enhance crop productivity and the new delivery mechanisms (Chen and Yada, 2011). Some of the major applications of nanotechnology in the agricultural sector include: nanoformulations used in crop improvement as fertilizers and pesticides, nanodevices developed for genetic improvements of stocks in plants, nanosensors for determination of diseases and other techniques to enhance animal breeding, health and post-harvest management (Peralta *et al*., 2020). A humongous amount of agricultural wastes are generated every year due to their perishable nature and persistence of insects and pests (Sekhon, 2014). Thus, nanotechnology has made it possible to comprehend insecticide resistance mechanisms in real time and reduced the need for plant protection products. Nanocapsules have been developed to eradicate the occurrence of diseases and to help in the early detection of diseases (Sekhon, 2014). In the current scenario, there have need to develop reliable, cheap and fast systems to detect and monitor biological host molecules in agricultural sectors. In the modern world, there have been a great advancements in agriculture. Thus, there is a need for agrochemicals such as fertilizers and pesticides to be produced efficiently and sustainably (Servin and White, 2016). Nanobiosensors have been developed for biochemical analysis and detection of mycotoxins in food products. They have high sensitivity, stability and are compact to use.

Types of nanotechnology systems and their applications in agriculture

The nanotechnology systems that have been effectively and efficiently used in various agriculture fields are nanoparticles, nanoemulsions, nanosuspensions, nanocapsules, agri-based materials in nanocomposites, nanosensors, *etc*. Table 1 enlists the different nanosystems and their uses in the agriculture sector (Zaytseva and Neumann, 2016).

Nanoparticles

Nanoparticles are the solid colloidal particles ranging from 1-100 nm in size. They are classified into nanospheres [in which the material to be delivered is evenly dispersed], nanocapsules (in which the material to be delivered is encapsulated in a polymer membrane and confined to a cavity), solid lipid nanoparticles, polymeric nanoparticles, magnetic nanoparticles, polymeric micelles, ceramic nanoparticles, metallic nanoparticles, semiconductor nanoparticles, *etc*. (Chhipa, 2019). Nanoparticles can further be classified as metallic and non-metallic nanoparticles. Metal oxides nanoparticles such as TiO_2, Fe_2O_3, CuO show photocatalytic characteristics. They have the property of interacting with photons and when the incident energy is greater than the energy bandgap, an electron is excited to the conduction band (Fraceto *et al*., 2016). This creates a positive hole for hydroxyl radicles in the valance bond, thus leading to degradation reactions. Nanoparticles exhibiting such properties can be exploited to treat wastewater, agricultural waste and to remove persistent pollutants such as pharmaceutical compounds, dyes and recalcitrant (Fraceto *et al*., 2016). Plant diseases caused by bacteria, insects, fungi, nematodes and viruses are responsible for major devastations of agricultural products (Rizwan *et al*., 2014). Most of the inorganic nanoparticles like silver, Zinc oxide, TiO_2, Magnesium and Silicon have been utilized to

Table 1: Nanotechnology systems and their potential uses in agriculture.

Nanotechnology systems	Uses	References
Nanoparticles	Carbon nanotubes in agrochemicals, Silver nanoparticles in antimicrobial, Gold nanoparticles in nanomedicines, nanodiagnostics, Nanoclay in antimicrobial, filler for composites, Metal oxides [e.g., TiO_2, ZrO] in disease control, fertilizer, agrochemical applications, Biochar in sorption, filtration media, soil amendment	(Zaytseva and Neumann 2016)
Nanocapsules	Improved delivery, increased stability, controlled release of agrochemicals	(Walz *et al*., 2017)
Nanogels	Encapsulation of active ingredient	(Abd Elrahman and Mostafa 2015)
Nanoemulsions and suspensions	Increased solubility, potency, accessibility and controlled release of the active ingredient	(Mousavi and Rezaei 2011)
Nanosensors	Detection of agrochemicals, pathogens, moisture, pH, *etc*., detection of environmental pollutants	(El Beyrouthya and El Azzi 2014)
Nanocomposites	Nanoclay as fillers for food packaging film, Metal oxide fillers as antimicrobials	(Ashfaq *et al*., 2020)
Agri-based materials in nanocomposites	Starch and cellulosic derivatives as polymeric matrix, Nanocellulose as filler, Agri-based materials as compatibilizing agents	(Gupta 2018)
Nanobarcodes: Smart cards for plants and animals for identification and tracking	In information technology	(Pramanik *et al*., 2020)

act on the fungal diseases (Rizwan *et al*., 2014). Ag nanoparticles can cause suppression of anthracnose pathogen *Colletotrichum* spp. in field trials. Ag nanoparticles combined with the fungicide fluconazole had the greatest anti-fungal activity against *Pleospora herbarum*, *Trichoderma* sp., *Candida albicans, Phoma glomerata* and *Fusarium* (Rizwan *et al*., 2014). ZnO nanoparticles have proven to be a more appropriate choice for fungal pathogen control than Ag. ZnO nanoparticles demonstrated higher inhibition (26%) against *Fusarium graminearum* in comparison to bulk ZnO because of the high surface area exposure to the fungi (Singh *et al*., 2015). Nanotechnology has proved to be a compelling approach to control the ever-increasing viral strains affecting the plants (Prasad *et al*., 2017). Green synthesis of silver nanoparticles using the spore crystal mixture of *Bacillus thuringiensis* was effective in complete suppression of sun hemp rosette virus on cluster bean leaves (Prasad *et al*., 2017). Many different nanoparticles *i.e*., silver nanoparticles, aluminum oxide nanoparticles, zinc oxide nanoparticles, titanium oxide nanoparticles are used to control the *B. mori* nuclear polyhedrosis virus (Prasad *et al*., 2017). For nutrient release, biodegradable, polymeric chitosan nanoparticles (78 nm) have been used for controlled discharge of NPK (Nitrogen, Phosphorous, Potassium) fertilizer sources equivalent to urea, calcium phosphate and potassium chloride (Manjunatha *et al*., 2016). Soil amended with metallic CuNPs significantly increased 15-day lettuce seedling growth. They had the potential to reduce the toxicity of the soil and decrease the frequency with which the fertilizers are applied in the fields (Manjunatha *et al*., 2016).

Carbon nanotubes (CNTs)

CNTs are hexagonal networks of carbon atoms present as a layer of graphite rolled into a cylinder (Kizilbash *et al*., 2020) with a diameter of 1nm and a length ranging from 1-100nm. Two types of CNTs are: Multi walled CNTs (MWCNTs) and Single walled CNTs (SWCNTs). They have a distinct size, shape and have remarkable physical and physicochemical properties. These have an ordered structure, high electrical and thermal conductivity, a high aspect ratio, high mechanical strength, metallic or semi-metallic behavior and high surface area. A number of CNT-based nanomaterials have been developed due to their potential applications in regulating plant growth (Patel *et al*., 2020). CNTs have shown to enhance the biomass productivity and yield by inducing changes in metabolic functions of plants. It has been reported that MWCNTs bolstered plant growth and seed germination in lettuce, radish, rye, maize, cucumber, rapeseed, phaseolus mungo L. and *Brassica juncea L* (Patel *et al*., 2020). Similarly, it has been reported that SWCNTs induced seed germination in tomato, carrot, onion cucumber and lettuce. They have also shown a significant increase in the root length of ryegrass. Most of the CNTs are generally absorbed by plants (Patel *et al*., 2020). The factors affecting the absorption include the types of CNTs used and plant species (Patel *et al*., 2020). Depending upon the properties of the CNTs such as concentration, size, structure and chemical properties, their effect on plants can be neutral, positive or negative. Several cases have been reported that interaction with CNTs has led to change in gene expression and biological pathways. MWCNTs are used to enhance the fruit production in plants, however over absorption could lead to shrinkage of cytoplasm, generation of vacuoles and disappearance of nuclei (Peralta *et al*., 2020).

When citric acid molecules were mixed with MWCNT-designated pesticides such as Zineb and Mancozeb and entrapped in aqueous solution, it led to the encapsulation of the pesticides, which damaged the fungi *Alternaria alternate* (Mukherjee *et al*., 2016). Multi-walled Carbon nanotubes in conjugation with iron coated nanoparticles have been used to remove Chromium [III] (Rudakiya *et al*., 2019). MWCNTs have been used to adsorb Procion Red MX-5B dye from various industrial effluents. There has been an increment in seed germination rate and vegetative biomass of tomato seeds with the aid of MWCNTs (Rudakiya *et al*., 2019). Here, penetration of cell wall by nanoparticles led to an increase in uptake of water by seeds, which caused the enhancement in germination percentage (Rudakiya *et al*., 2019). Multiwalled Carbon nanotubes have been used to enhance water and nutrient (Ca, Fe) uptake to facilitate the germination of seeds and growth of plants. It has been reported that there is a great enhancement in root elongation in rape, ryegrass and corn by MWCNTs. SWCNTs exposure to cucumber seedlings and onion enhanced their root growth (Rudakiya *et al*., 2019).

Quantum dots (QDs)

Quantum Dots are semiconducting materials made up of a core, shell (to improve optical properties) and a cap (to enhance solubility in aqueous buffers). They are neither atomic, nor bulk material. Their size ranges from 10-100 Å (Mukhopadhyay, 2014). These have a bright fluorescence, narrow emission, broad UV excitation and high photo stability. ZnCdSe/ZnS core shell QDs, InP/ZnS core shell QDs, Mn/ZnSe QDs, gold nanorods, core shell QDs, *etc*. have been used in controlled release of trace elements (Mukhopadhyay, 2014). QDs exhibit several properties such as narrow and symmetric broad absorption spectra, high cell permeability, high hydrophobicity which are relevantly applicable in agricultural sector (Walz *et al*., 2017). It has been reported that Carbon QDs integrated with thionin increased the disease resistance ability of rice plant. There is a correlation between the structures of plant hormones and metabolic fate of CQDs. Thus, it leads to the promotion of plant growth (Walz *et al*., 2017). Nowadays, researches are being carried out to synthesize green engineered CQDs (Prasad *et al*., 2017). Semiconductors are used for the synthesis of QDs because of narrow and symmetric fluorescence spectra, high resistance to photobleaching, high quantum yield and large effective excitation, high molar extinction coefficients and exceptional resistance to photochemical degradation. QDs are widely used in the bioimaging field and fabricating nano biosensors. It has been shown that low concentration of QDs exhibit no detectable cytotoxicity for growth and seed germination. Thus, it has also been used as a delivery systems and live imaging of plant root systems to thoroughly understand the physiological processes (Prasad *et al*., 2017).

Nanocomposites

These are the composite materials with at least one dimension equal to 1nm. They are the materials with atoms arranged in nanosized clusters, which become the constituent grains or building blocks of the material. They have excellent mechanical strength, toughness and electrical or thermal conductivity. Nanocomposites are categorized according to their matrix material such as ceramic, metal or polymer nanocomposites. Some of the examples of nanocomposites used in agriculture are- ceramic matrix nanocomposites, elastomeric nanocomposites, bio nanocomposites, polymer- matrix nanocomposites and polymer- silicate nanocomposites *etc*. (Ashfaq *et al*., 2020). Some nanocomposites have shown to be 1000 times tougher than the bulk component materials. They have been used for controlled and timely release of nutrients from the fertilizer granules, which considerably reduces the loss of nutrients and simultaneously enhances the crop yield (Ashfaq *et al*., 2020).

Nanocomposites have been widely used as pesticides. Although the mechanism of nanocomposite is still elusive, it has been widely used to eradicate various pests such as fungal, bacteria and microorganisms (Gupta, 2018). Metal nanocomposites tend to bind with different cellular organelles such as lysosomes, endoplasmic reticulum, mitochondria, Golgi bodies and ribosomes after entering into the microbial cell. Nanocomposites disrupt the metabolic processes occurring in a metabolic cell affecting the biochemical processes after penetrating into specific cell organelles. They exhibit toxic behavior against mitochondria and induce oxidative stress by generating reactive oxygen species (Gupta, 2018). They further disrupt all other necessary processes such as ATP production, inhibition of protein synthesis and DNA replication, disrupt the permeability of the membrane by inhibiting the process of microbial respiration. Moreover, they also react with the H-bonding of DNA and disrupts mRNA synthesis leading to cell death (Gupta, 2018). It has been reported that nanocomposites have a profound effect on plant growth. Silver nanocomposites have a positive effect on enriching soil bacterial diversity and help to grow root and shoot parameters of wheat, *Brassica* and cowpea (Ashfaq *et al*., 2020). Gold nanocomposites have shown to enhance nutrient efficiency by promoting soil enzyme and microbial community. A report has shown that combination of silver, zinc, carbon and titanium nanocomposites help the growth of *Piriformospora indica*. Silver nanocomposites have shown to impart antifungal activity against *Bipolaris sorokiniana* in wheat plants along with *Serrati sp*. Copper nanocomposites showed antibacterial activity against *E. coli* and *Micrococcus luteus* (Ashfaq *et al*., 2020).

Zeolite

Zeolites are crystalline, microporous aluminosilicates which are found on earth's surface and in soils, seafloor deposits, hydrothermal alteration products, altered volcanic deposits, sediments, *etc*. (Nakhli *et al*., 2017). Their structure resembles a sponge but with a difference of being rigid, unlike the flexible sponge. Zeolites are classified on the basis of their pore diameter and ring size. Zeolites have been widely used in agriculture due to their exceptional properties such as desorption, special cation exchange and molecular sieving. They are widely used as fertilizers, chelators, stabilizers and as organic and inorganic fertilizers. Due to their large porosity, they have high affinity for potassium and ammonium cations. They can be exploited as carriers of nutrients and they have been widely employed in agriculture for storage, capture and slow release of nitrogen (Mahesh *et al*., 2018). They help to reduce the loss of nitrogen in agricultural field by trapping most of the ammonium in the fields. Zeolites with charged ammonium charged have shown to enhance the solubilization of phosphate minerals. They showed an increase uptake of phosphorous by sudangrass. They are also used as nitrogen fertilizer by increasing the uptake of nitrogen uptake in crop production systems (Mahesh *et al*., 2018). Zeolites have been successfully modified as a potting media in horticulture and used for the subsequent release of nutrients and substrates for growth of plants (Sangeetha and Baskar, 2016). However, some of the inorganic zeolites such as erionite have proved to be harmful for humans and cattle. Some of the zeolites are used to improve the availability of P from N-NH4+ *etc*. and increase the efficiency of water holding capacity of the plants. Zeolites have caused increased efficiency of nitrogen uptake in rice plants. Surfactant modified zeolites are widely used as an excellent sorbent for nitrate uptake which helps in the process of nitrification. Moreover, they have the capacity to enhance water retention and absorption of NPK fertilizers. They are used to control overuse of herbicides. Humic acid zeolites have proved to remove extra atrazine from soil and absorb phenylurea herbicides. Due to the crystalline nature of zeolites, they have high porous structure which holds more than half of their weight of water and improves soil physical properties. They can act as permanent water reservoir to the roots and promote rapid rewetting and keep the soil aerated and moist (Sangeetha and Baskar, 2016).

Nanobarcodes

Nanobarcodes are used as identification tags in agricultural biotechnology as they are small in size and can make a large number of combinations. The materials to be used to make a nano barcode should be easily encodable, machine-readable, durable, sub-micron sized taggant particles (Sertova, 2015). Nanobarcodes have been employed in various aspects of agriculture biotechnology, such as improvement in the plant resistance against various environmental stresses like drought, salinity, diseases, cost-effective detection of pathogens from food products, *etc*. (Sertova, 2015). In current scenario, it has become a practice to apply identification tags in livestock products and wholescale agriculture. Because of their miniature size, they have been widely used in multiplexed bioassays. An ideal nanobarcode should be- durable, easily encodable and machine readable (Oliveira *et al*., 2014). The nanobarcode particles are manufactured by a process which is highly scalable and semi-automated. It involves electroplated templates with inert materials such as silver and gold and then the release of resulting striped nanorods from the templates (Oliveira *et al*., 2014). Nanobarcodes have been extensively used as ID tags for intracellular histopathology and multiplexed analysis of gene expression (Srilatha, 2011). Nanobarcodes are used nowadays in early detection of various environmental stresses such as salinity, diseases and drought and help to increase plant resistance. Nanotechnology based gene sequencing has been developed to utilize and identify effectively plant gene trait resources. Thus, they can be helpful in cost-effective detection of pathogens from food products. Nanobarcodes are used as identifiable nanoscale tags for authentication in husbandry products and agricultural food and as substitutes for organic dyes used as biolabels in staining bacteria. QDs are used as substitutes for conventional organic fluorophores due to their capability of exhibiting more luminescence, photostability, tunability, narrow and symmetrical emission spectra as compared to the organic dyes. Due to their broad absorption spectra, they get excited to all colors of the quantum dots using a single excitation light source. *E. coli* can be detected by quantum dots coupled with immune magnetic separation (Srilatha 2011).

Applications of nanotechnology in the agriculture sector

Agri-nanotechnology is gaining a lot of attention in the areas of nanofertilizers, nanoparticles for gene delivery, controlled gene delivery, multiple gene delivery, nanobiosensors, nanofoods, pesticides, nanoformulations, nanoscale carriers, *etc*. Fig 1 below depicts a brief account of the applications of nanotechnology in agriculture.

Nanobiosensors

Biosensors are analytical devices that couples a biological recognition element to a transducer to convert a biological signal into an observable signal. Biosensors determine the presence and concentration of a specific substance in a biological analyte. Nanotechnology has a profound impact on the agriculture sector. Nanobiosensors are the sensors developed on a nanoscale on which the biological element is immobilized (Vishwakarma, *et al*., 2018). These analyze the analyte at the atomic level and are used to recognize glucose, urea, herbicides, pesticides, analyze the enzymes and metabolic products, detect pathogens, control soil nutrients and reduce environmental pollution (Ingle AP, *et al*., 2014). Nanosensors are highly specific, sensitive and hassle-free devices for detecting pathogens in agricultural fields, leading to the prevention, treatment and eradication of various diseases that a plant may encounter.

Various nanoparticles have been effectively applied as diagnostic tools for microorganisms causing diseases in plants.

The most common class of nanomaterials utilized for agricultural diagnostics is metal nanoparticles (Darr, *et al*., 2017). Fluorescent silica nanoparticle probes (FSNPs) in conjugation with a secondary antibody were developed to detect *Xanthomonas axonopodis pv. vesicatoria* (Xav), a Gram-negative bacterium causing spot disease in

APPLICATIONS OF NANOMATERIALS IN AGRICULTURE	Nanopesticides
	Crop improvement
	Plant protection ingredients
	Soil management
	Plant growth regulators
	Food technology
	Water management
	Monitoring the quality of agricultural produce
	Nanobiotechnology
	Biosensors for Aqua culture
	Bio-synthesized nanoparticles for agriculture
	Post-Harvest technology
	Nanofertilizer to balance crop nutrition
	Weed management

Fig 1: Applications of nanotechnology in various aspects of the agricultural sector.

Solanaceae plants. The SNPs were doped with a fluorescent dye- Rubpy, which allowed for the rapid and easy detection of Xav by fluorescence-linked immunosorbent assay (FLISA) (Darr, *et al*., 2017). Mishra, *et al*., exploited the property of diatom frustules to emit excellent photoluminescence to fabricate an immunosensor for the early detection of *Tilletia indica*, causative fungal agent of Karnal bunt (KB) disease in wheat. Amine-functionalized diatom, immobilized by *T. indica* antibody when subjected to UV excitation, emitted clear, strong PL whose intensity directly pointed towards the concentration of the fungal spores (Mishra, *et al*., 2020). A colorimetric AuNP-probe assay was developed by Khaledian *et al*., to facilitate on-field detection of a soil-borne, gram-negative bacterium, *Ralstonia solanacearum,* which causes potato bacterial wilt. AuNPs were functionalized with ss-oligonucleotide to allow its interaction with the complementary genomic DNA of *R. solanacearum*. In low pH conditions, a stable interaction between the oligonucleotide and complementary DNA was observed which caused a color change to red, while in the absence of *R. solanacearum* DNA, the sample turned purple, indicating aggregation of the AuNP probes. This nanobiosensor has a 15-minute readout and is very sensitive and specific; however, quantitative evaluation is warranted (Khaledian *et al*., 2017). Yüksel *et al*., came up with a label-free, SERS-based DNA hybridization platform using adenine on the target DNA as an endogenous marker for the specific detection of *Phytophthora ramorum*. Hybridization occurring between the adenine-free capture probes and the target DNA on a silver nanoparticle SERS-substrate pointed towards the plant pathogen's presence (Yüksel, *et al*., 2015).

Quantum Dots (QDs) are semiconducting NPs with superior fluorescence properties, allowing for better detection of the target than other conventional fluorophores. Safarpour *et al*., used antibody-functionalized QDs in a FRET-based biosensor against *Polymyxa betae,* vector of Beet necrotic yellow vein virus (BNYVV) (Safarpour, *et al*., 2012). Shojaei *et al*., also attempted to develop FRET sensor to probe *Citrus tristeza virus* (CTV). CdTe QDs coupled with CTV coat protein (CP) was used as a donor, whereas AuNP-labeled CP was the acceptor molecule. The donor-acceptor complex formed due to the proximity of QD-AuNP interaction led to an energy transfer quenching the fluorescence of QDs, reversing the decrease in the presence of free CPs (Shojaei, *et al*., 2016). A variety of DNA/RNA-based affinity sensors has also been used for detection purposes (Eun and Wong, 2000) (Eun, *et al*., 2002).

Some other techniques to carry out plant disease detection are classified into 2 types-direct and indirect. Direct methods include PCR, FISH, ELISA, Immunofluorescence, while indirect methods include monitoring plant parameters like temperature, transpiration rate, VOC release, morphological changes, *etc*. (Fang and Ramasamy, 2015). Precision farming is a process to maximize output (crop yields) while minimizing inputs (fertilizers, pesticides, herbicides) through monitoring environmental variables and applying targeted actions to reduce agricultural wastes. This keeps environmental pollution at the bar and enhancing productivity (Ghidan and Antary, 2020). Nano-based smart delivery systems can help in the efficient use of agricultural, natural resources like water, nutrients and chemicals through precision farming. Using nanomaterials and GPS (Global Positioning System) with satellite imaging of fields, remote detection of crop pests is possible, providing evidence of stress such as drought. Once pest or drought is detected, there will be an automatic adjustment of pesticide applications or irrigation levels. Nanosensors dispersed in the field can also detect plant viruses and the level of soil nutrients (Rai, *et al*., 2012).

Nanofertilizers and nanopesticides

Fertilizers are materials, natural or synthetic, required for plants' initial growth. The overall crop production and food security rely on the amount of fertilizers. They supplement the plants with nutrients, promote their growth and increase the crop yield (Srilatha, 2011). The three major constituents of fertilizer are nitrogen, phosphorous and potassium. Nitrogen plays a vital role in the plant's physiology, leaf growth (strong growth of plant foliage) and enhancement of protein content. Phosphorous plays a vital role in the storage and usage of energy (Srilatha 2011) in cell division and new tissues' development. Potassium is the most important component of a commercial fertilizer as it aids in sturdy stem growth, water movement and the promotion of fruiting and flowering. It also protects the plant from extreme cold or dry by strengthening its root system and preventing wilt (Naderi and Shahraki, 2013). However, there are certain problems associated with commercial fertilizers. The nutrients left unutilized are washed off from the crops from fields into the water bodies causing eutrophication (Naderi and Shahraki, 2013). This calls for an alternative to chemical fertilizers. Nanofertilizers (NFs) have been produced to promote environment-friendly and sustainable agriculture.

NFs are nanomaterials that assist the existing fertilizers in enhancing their efficiency. Their unique properties make

Table 2: Commercially available nanofertilizers and their merits.

Nanofertilizer	Merits
Nano- Gro™	Immunity enhancer and plant growth regulator
Nano max NPK fertilizer	Multiple organic acids chelated with macro and micronutrients.
Nano- Ag answer	Microorganism, mineral electrolyte, sea kelp
Master nano chitosan organic fertilizer	Organic acids, salisylic acids, phenolic compounds
Nano green	Extracts of corn, soybean, coconut, palm, potatoes

them a promising agent in the agriculture sector. They promote steady, controlled and targeted delivery of nutrients through site-directed delivery, enhance the fertilizer's effective nutrient utilization and reduce toxicity. They are known to enhance absorption capacity, rate of photosynthesis, prevent nitrogen leaching and plant growth (Prasad, *et al*., 2017). NFs had proven effects on plant growth, a number of reproductive tillers, spikelets, panickles and total chlorophyll content in rice crops (Jyothi and Hebsure, 2017). Various manufacturers have thus shown interest in manufacturing and commercializing fertilizers derived from various nanosystems. Table 2 enlists the commercially available nanofertilizers and their merits.

Owing to their controlled and steady release properties, urea-modified hydroxyapatite NPs were exploited as a rich source of nitrogen (Millan, *et al*., 2008). The seed yield in *Glycine max* was enhanced after treatment with Calcium and Phosphorous hydroxyapatite nanoparticles (Liu and Lal, 2014). The chlorophyll content in *G. max* was increased using Iron NPs in low concentrations (Ghafariyan, *et al*., 2013). Ag nanoparticles, ZnO nanoparticles and TiO nanoparticles have been used as an insecticide and have been successfully utilized in controlling silkworm disease and rice pests. Phenolic suspension of hydrophobic alumina-silicate nanoparticles is tremendously effective against grasserie disease in *Bombyx mori* leaves (Goswami, *et al*., 2010). TiO_2-SiO_2 combination nanoparticles accelerated the growth, germination, water-absorbing capacity, root, leaf activity and improved stress resistance in *Glycine max* (Lu, *et al*., 2002). Ag nanoparticles act as a pesticide against pathogenic fungi. Green synthesis of Ag nanoparticles by *Tinospora cordifolia* showed maximum pesticidal action towards the top louse *Pediculus humanus,* fourth instar larvae of *Anopheles subpictus and Culex quinquefasciatus* (Jayaseelan, *et al*., 2011). The overall crop productivity, stress tolerance, microflora of the soil was enhanced manifolds in *Cicer arietinum* L. after treatment with a combination of Molybdenum NPs and microbial preparation of nitrogen-fixing bacteria (Taran, *et al*., 2014). CNTs are also a good fertilizer, as proved by *Srinivasan and Saraswathi*, who observed a marked increase in seed germination, plant growth and water holding capacity (Srinivasan and Saraswathi, 2010). CNTs were also used in *in-vitro* cultures of date palm, where they increased the overall callus biomass, germinated number of embryos, shoot length, leaf number. The amount of N, P, K, total chlorophyll a and b were also significantly increased (Taha, *et al*., 2016). Nanoencapsulated slow-release fertilizers have also become a trend to save fertilizers consumption and minimize environmental pollution. A new nanosized silica-silver composition for the control of various plant diseases consisted of a nanosilver combined with silica molecules and water-soluble polymer prepared by exposing solution including silver salts, silicate and water-soluble polymer to radioactive rays (Park, *et al*., 2006). It showed antifungal activity and controlled powdery mildews of pumpkin at 0.3 ppm in both field and greenhouse tests. Fig 2 shows an example of precision farming.

Pesticides either make a plant resistant or kill unwanted substances. They may be insecticides, fungicides, herbicides, or disinfectants. But the disadvantages of chemical pesticides cannot be overlooked, including a reduction in nitrogen fixation, development of pathogen and pest resistance, contribution to bioaccumulation of pesticides, reduction in soil biodiversity, pollinator decline and destruction of habitats for birds (Ghormade *et al*., 2011). Nanoencapsulation of pesticides to confer protection against plant pathogens is the practical way to overcome the negative impacts and bring about environmental balance. Advantages of nanopesticides include the controlled release of the active ingredient, less toxicity, eco-friendliness, biodegradability, improved solubility, stability of active ingredient, control over eutrophication and residual pesticide accumulation.

Lately, the use of NPs to encapsulate the active agent against pests has been gaining much attention. The

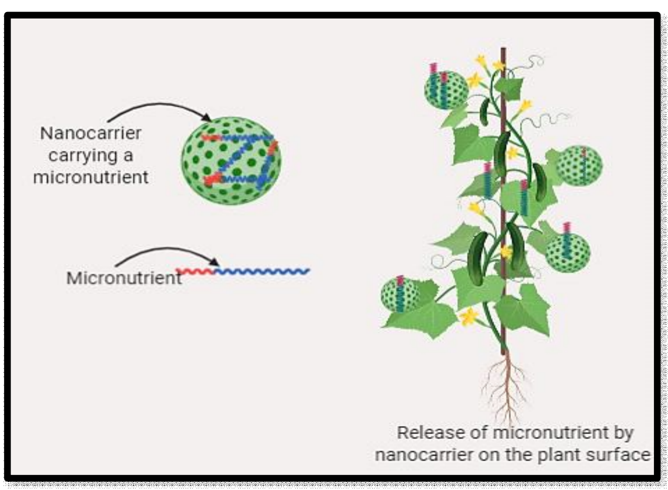

Fig 2: A model of a nanocarrier containing releasing its micronutrient to the specific plant part.

insecticidal properties of chlorfenapyr, a broad-spectrum insecticide, greatly improved in both field and lab testing when encapsulated inside silica NPs (Song, *et al*., 2012). Metal NPs from biological sources like Iron NPs from eucalyptus extract proved to have superior fungicidal and insecticidal properties against pathogens like *Fusarium oxysporum, Rhizoctonia solani and Botrytis cinerea*. The cidal activity increased with the increase in nanoparticle solution (Chhipa and Kaushik, 2015). Significantly less concentration of copper (10000 times lesser than recommended) in its nanoform was able to control the bacterial blight on pomegranate (Mondal and Mani, 2012). The antimicrobial nature of copper was also tested in combination with chitosan nanogels against a fungal pathogen, *Fusarium graminearum*. Copper release from this stable nanoformulation was dependent upon pH change. A decrease in pH triggered Cu (II) release initiating a biopesticidal action (Brunel, *et al*., 2013). Photocatalytic titanium dioxide nanoparticles doped with zinc [$T_iO(2)$/Zn] and silver [$T_iO(2)$/Ag] were effective against *Xanthomonas perforans*, which leads to the bacterial spot disease in Tomato (Paret, *et al*., 2013). The Copper-tolerant strain of *X. perforans* has also been targeted by multiple other nanoformulations, including Cu-Zn hybrid NPs (Carvalho, *et al*., 2019), copper composites core-shell copper (CS-Cu), fixed quaternary ammonium copper (FQ-Cu) and multivalent copper (MV-Cu) (Strayer-Scherer, *et al*., 2018), DNA-directed AgNPs grown on graphene oxide (GO), *etc*. (Ocsoy, *et al*., 2013). PEG NPs are another key nanomaterial for nanopesticide formulation. Culex quinquefasciatus' larvae were attacked by temephos and imidacloprid containing PEG. Slow and controlled release from PEG NPs led to a better insecticidal effect (Bhan, *et al*., 2014). Essential oils from *Geranium* and *Citrus reticulata*, encapsulated in PEG NPs, were potent insecticides against adult Tribolium castaneum, a store product pest (Yang, *et al*., 2009).

Despite the pros of using nanopesticidal formulations, their toxicity must be evaluated before their application on-field. They must fit well in the regulations put forth by the Ministry of agriculture of India or the federal agencies in other countries.

Plant protection and crop improvement

Nanotechnology in plant protection and crop improvement has improved sustainability, reduced waste generation and enhanced production efficiency. Bacteria, fungi, insects, nematodes, phytoplasmas and viruses are majorly responsible for plants' losses. Nanomaterials have been developed to eradicate perishes caused by plant pathogens (Worrall, *et al*., 2018). Agricultural nanotechnology is the new wave in improving and protecting the plant.

AgNP spray over guar leaves infested with sun hemp rosette virus had desirable viral inhibition (Jain and Kothari, 2014). Yellow mosaic virus-infected faba beans were also benefitted from AgNP spray 24 hours after infection (Elbeshehy, *et al*., 2015). AuNP application on Barley had destructive effects on barley yellow dwarf virus-PAV (BYDV-PAV) (Alkubaisi and Aref, 2016). Metallic NPs attacked Tobacco Mosaic Viruses (TMVs) like ZnONPs, SiO2NPs and Fe_3O_4NPs. In both in vitro and in vivo experiments, aggregation of TMV particles was observed, viral replication and accumulation were markedly decreased with regular NP spray. The NP formulation was well absorbed by the plants which were confirmed by the increase in the dry and fresh weight of tobacco crop post-treatment (Cai, *et al*., 2020) (Cai, *et al*., 2019). ZnNPs have also been employed for crop improvement applications in cucumber, where they increased the protein and starch content (Hong, *et al*., 2014); safflower, where it improved the drought tolerance; pomegranate, where it increased the yield and availability of nutrients to the plant (Cakmak, 2008) (Verma, *et al*., 2021). Chitosan NPs have been used an antiviral against mosaic virus in potato, peanut, alfalfa, Tobacco Mosaic Virus and Tobacco Necrosis Virus and as antimicrobial against *Cacopsylla pyricola*, *Meloidogyne javanica, Spodoptera littoralis, Aphis nerii*. (Chirkov, 2002) (Malerba and Cerana, 2016).

Genetic modification of organisms by RNA*i* to resist pests is the most recent advancement in agri-nanotechnology. In plants, ds-RNA triggers RNA*i*. Hence ds-RNA in naked or encapsulated form is used for crop protection (Li-Byarlay, *et al*., 2013) (Thairu, *et al*., 2017) (Das, *et al*., 2015) (Koch, *et al*., 2016). Fig 3 shows an example of the application of nanotechnology in plant protection.

Water quality management

Nanotechnology has improved the quality of agricultural produce and its role in the area of storage, packaging and water quality management is also registered. It has augmented the capabilities of conventional wastewater treatment technologies. Wastewater may create havoc if not

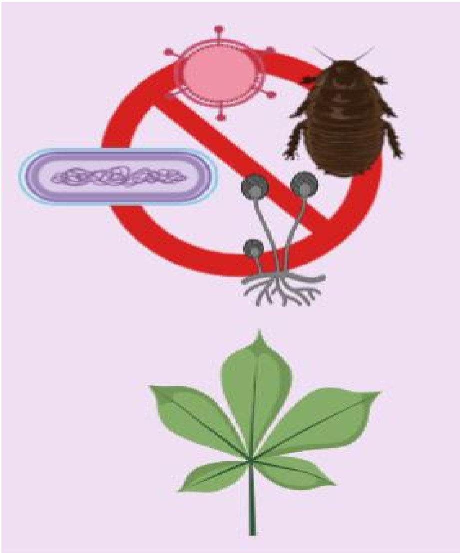

Fig 3: Figure depicting protection against plant pests, *i.e.,* Bacteria, Fungi, Insects and Virus.

treated properly. Semiconductor-sensitized photosynthetic and photocatalytic processes can be used to destroy unwanted microbes, remove organic matter, *etc*. (Hua, *et al*., 2012). Determining the rate-limiting step of the inactivation mechanism of various metals and metal oxides can help formulate appropriate disinfectants for water treatment. Nanoparticles such as oxides of iron, manganese, aluminum, titanium, magnesium and cerium removed heavy metals and metal oxides from water resources. They have a high overall surface area, high absorption rate, are highly reactive, cost-effective, have a high affinity towards heavy metals and can be quickly regenerated after treatment with a bifunctional, self-assembling ligand of the previously known nanocoating media (Kumar and Chawla, 2014). Nanoparticles can also be used for the nanobioremediation of those compounds, which are difficult to break down and can cause severe problems to the animal population if entered into the food chain. Organic pollutant removal was carried out by T_iO_2 ceramic filters, whose pores infused with a hyperbranched polymer and a dendrimer, giving rise to a hybrid polymer that acts as a water treatment agent (Guo, *et al*., 2012). Very low amounts of organic pollutants have also been eliminated using carbon-based nanomaterials, *e.g.,* by using CD-co-hexamethylene-/toluene-diisocyanate polyurethanes along with CNT (Rizwan, *et al*., 2014). Nanobioremediation can be used to remediate uranium, hydrocarbon, groundwater, wastewater, solid waste, heavy metal remediation. The nanomaterials used in nanobioremediation to date are nano iron and its derivatives, nanosized dendrimers, carbon nanotubes, single enzyme nanoparticles, engineered polymeric nanoparticles, *etc*. (Ingle, *et al*., 2014). Microorganisms are also exploited because of their property to accumulate heavy metals. Some of them are: *Gundelia tournefortii, Centaurea virgata, Reseda lutea, Scariola orientalis, Eleagnum angustifolia, Bacillus sp. and Noaea Mucronata* (Ingle, *et al*., 2014) (Rizwan, *et al*., 2014). Since the freshwater reserves are soon going to deplete, water desalination will be the only way to keep up with the population's water demands. The traditional ways of desalination like Reverse osmosis (RO) consume much energy and are not cost-effective. So, nanotechnology was used for the development of low-energy techniques for the same. Protein-polymer biomimetic membranes, aligned carbon nanotube membranes and thin film nanocomposite membranes have been proved to be 1000 times more efficient in desalination as compared to reverse osmosis as they use CNT membranes. These membranes can also couple other processes like disinfection, deodorizing, defouling and self-cleaning (Mukherjee *et al*., 2016). Nano-adsorbents are nanoscale particles with a high binding capacity for organic and inorganic pollutants (Kumar and Chawla, 2014). These nano-adsorbents have high reactivity, catalytic potential, a high surface area leading to the creation of more absorbing sites (Ingle, *et al*., 2014). Magnetic nano- adsorbents with superparamagnetism can be used to remove radionuclide and heavy metals from water. *Figure 4* shows the role of nanotechnology in water quality management.

Promoting plant growth

When nanomaterials interact with the plants, various morphological, physiological and functional changes occur depending upon nanomaterials' properties (Remédios, *et al*., 2012). Engineered nanomaterials modify the gene expression, related plant metabolism and physico-chemical properties affecting the overall plant growth and development. The nanomaterials' effect on plants depends on the growth stage, exposure duration, size, shade, chemical composition, concentration, aggregation and solubility of the nanomaterial (Khiew, *et al*., 2011). Engineered nanomaterials can reach plants through direct application, accidental release, contaminated soil/ sediments, or atmospheric fallouts, which results in a significant adverse effect on food crops and food chains. Nanoparticles are the most commonly used nanomaterial for plant growth and development. Their efficacy is dependent on size, surface coating, composition, effective dose and reactivity. Silver has been a potent plant growth-promotor; however, its applied concentration must be

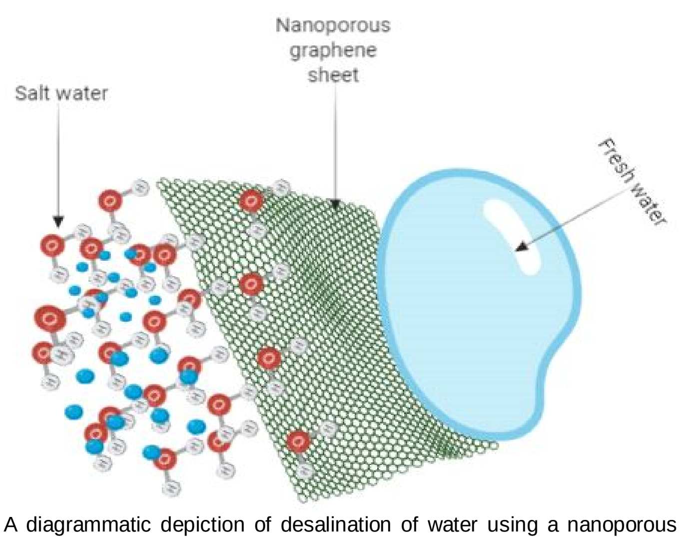

Fig 4: A diagrammatic depiction of desalination of water using a nanoporous graphene sheet.

carefully evaluated for its toxicity as higher concentrations might affect the process of nitrification (Masrahi, *et al*., 2014). AuNPs in minute quantities promoted shoot to length ratio in *Lactuca sativa* seeds with no reports of toxicity (Pestovsky and Martínez-Antonio, 2017). In *Bacopa monnieri*, biologically synthesized Ag nanoparticles were found to induce carbohydrate and protein synthesis and decrease the total phenol content (Krishnaraj, *et al*., 2012). Biologically synthesized Zn nanoparticles effectively enhanced shoot (10.8%), chlorophyll content (18.4%) and grain yield (29.5%) in pearl millet (Tarafdar, *et al*., 2014). Nanoanatase-treated parsley seeds enhanced germination, root and shoot length, chlorophyll content, *etc*. (Mousavi, *et al*., 2011). Carbon nanomaterials are most commonly used owing to their excellent mechanical, thermal, chemical and electrical properties. They have shown a phenomenal enhancement in the germination and growth of *Solanum lycopersicum* seeds. CNTs were able to penetrate the thick seed coats of tomato, thereby activating the water uptake mechanism leading to higher biomass production and faster germination rates (Villagarcia, *et al*., 2012). The plants treated with nanomaterials have shown a significant increase in their biomass and could also grow in various conditions of humidity.

Nanofoods

Nanofoods are foods that are made by applying nanotechnological tools during their cultivation, production, processing, or packaging (Walz, *et al*., 2017). But, nanofoods are not produced by nanomachines. The potential of nanotechnology in nutraceutical and functional foods is realized recently by applying the new concepts in engineering for the targeted delivery of a micronutrient or any bioactive compound (Walz, *et al*., 2017). They have better release efficiency, better encapsulation, good retention time, increased surface area, direct nanoparticle uptake and enhanced nutritional quality than traditional encapsulating material. Nano-emulsions, liposomes, micelles, biopolymer complexes have made nanofood encapsulation possible (Nile, *et al*., 2020). Various nanoparticles' effects like improved germination rate, root and shoot length, the biomass of seedlings on crops like wheat, ryegrass, Tomato, radish, lettuce, spinach, pumpkin, corn and cucumber, corn, *etc*. have been studied. Iron oxide nanoparticles have improved agronomical traits, including the grain yield of soybean (Shanti *et al*., 2011). Carbon-based nanoparticle fullerol has improved the yield of a medicinally rich vegetable crop, bitter melon, fullerol treatment resulted in an increased biomass yield of 54%, a fruit number of 59% and a fruit weight of 70%, which improved up to 128% of fruit yield. The content of two anti-cancerous phytomedicines, cucurbitacin-B and lycopene, were also enhanced (Kole, *et al*., 2013).

Soon, nanotechnology will be the base for novel food, food flavors, food texture, *etc*. Nanofoods have been used in processed or textured food to enhance their taste. Spoilage of perishable food items has become a significant issue due to the lack of appropriate processing, preservation and packaging techniques. This urgently calls for smart packing methodologies like nanotechnology for minimizing post-harvest losses (Vishwakarma, *et al*., 2018). To increase mango's shelf life, researchers at Tamil Nadu Agricultural University developed nanomatrices that could be infused with the packaging material. Antimicrobial nanofilms were developed by Kodak to prolong the freshness of food as they could absorb oxygen (Asadi and Mousavi, 2006). The shelf-life of Jujube, asparagus, *etc*., was increased when packaged in an AgNP-infused film (Li, *et al*., 2009). T_iO_2, CNTs, nisin and chitosan have been used for the same purpose (An, *et al*., 2008). GuardIN Fresh, a nanotechnology-based antimicrobial system, is made to keep a check on ethylene-based ripening of fruits and vegetables (Vishwakarma, *et al*., 2018). Nanocarrier systems have been used to deliver nutrients and supplements in the form of liposomes or biopolymer-based nanoencapsulated substances. They have been widely used in food packaging, such as plastic polymers containing or coated with nanomaterials for improved mechanical or functional properties. Inorganic nanosized additives have been developed for food, healthy food, animal feed; surface-functionalized nanomaterials have been used in food packaging and animal feed and nanocoatings for food contact surfaces for barrier or antimicrobial properties (Vasile, 2018).

Packaging can be coupled with nanosensors to evaluate the internal and external condition of the food package or container, *i.e.*, food color, gases given off by the spoiled food, microbial load and chemical compounds, including toxins (Nachay, 2007). Hence, Agrifood is the way to improve the quality, sustainability and freshness of consumables.

Challenges associated with nanotechnology on agriculture

Nanotechnology is proved highly beneficial in agriculture, but it has certain limitations. Before applying any technology to the fields, risk assessment is indispensable (Mwaanga 2018). Adverse effects reported in plants include slow growth, increased oxidative stress, chromosomal abnormalities, decreased photosynthetic rate, disturbances in water transport, decrease in the concentration of growth hormones such as Indole Acetic Acid, metabolic disorders or necrosis, or increased susceptibility to natural toxins (Morales-Díaz, *et al*., 2017). The soil naturally contains some nanoscaled materials resulting from natural or industrial processes, treatment or disposal of wastewater, or the entry of biosolids into the soil. The use of carbon-based and metal-based engineered nanomaterials (ENMs) leads to the undesired accumulation of nanomaterials and disturbs the ecosystem balance (McKee and Filser, 2016).

In traditional fertilizers, elements are present in the form of ground minerals. So, the elements such as Iron and Zinc generally don't pose a health concern for the consumer. However, nanomaterials and nanoparticles' effects in this manner on plants in both the short and long term remain questionable (Remédios, *et al*., 2012). Like conventional fertilizers, nanofertilizers can also cause eutrophication of water bodies. Moreover, because of the slow release of fertilizers, the contamination is difficult to resolve. Nanofertilizers need to be released in the correct form and slowly to minimize the losses by leaching, gasification and competition with other organisms (DeRosa, *et al*., 2010) (*Ditta et al., 2016*). Nanoparticles can get absorbed by the microorganisms in the soil, roots in the plant from where they get accumulated in the plant's upperparts. When the higher trophic levels consume these microorganisms, plants, or their waste products, protozoa, arthropods, annelids, mollusks, insects (Hawthorne, *et al*., 2014) birds, mammals, their ill- effects are visible in their future generations (América Berenice Morales-Díaz, *et al*., 2017). The nanoparticles and the nanomaterials released into soil and water can affect both biodiversity and the relative abundance of certain species of microorganisms found in the soil (Colman, *et al*., 2013), the rhizosphere and the interior of plant tissues. Some of the practical issues to be addressed for the successful usage of nanotechnology in agriculture are the nanomaterial's adverse effects on the exposed workers, the nanomaterials' reactivity with the environment, phytotoxicity of nanomaterials, biosafety and toxicological aspects of the nanoformulations and damage to non-target species.

Regardless of Agri-nanotechnology advancements, many gaps need to be filled to further resolve the existing issues in nanotechnology applications in the agriculture sector. These include process design for easy scale-up at the industrial level, knowledge regarding the regulatory aspects of the usage of nanotechnology (Amenta, *et al*., 2015), up-gradation of methods to better understand the dynamics and life cycle of the newly developed nanomaterials (Kookana, *et al*., 2014), studies regarding the resistance to nanosystems by target organisms, involving all stakeholders, governmental and non-governmental agencies and consumer associations to extend support to nanotechnology products.

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