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Original Research Article

Significance of Nanotechnology and Molecular Farming in Agriculture

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Abstract

Climate change, urbanization, soil degradation, unsustainable use of natural resources, accumulation of pesticides and fertilizers are the hot issues for today's agriculture. Traditional agriculture practices are unable to cop up these challenges. Nanotechnology and molecular farming are the emerging strategies in the area of agricultural research. These strategies have opened up a wide array of opportunities in agriculture and related food production systems. It has great potential to improve the quality of life through its applications in agriculture and the food production systems. Insect pests management through the formulations of nanomaterials-based pesticides and insecticides, enhancement of agricultural productivity using bio-conjugated nanoparticles for slow release of nutrients and water, nanoparticle-mediated gene transfer in plants for the development of insect pest-resistant varieties and use of nanomaterials for preparation of different kind of biosensors are required for precision farming. This article emphasize the role of nanotechnology and molecular farming in various applications for improving the crop production, controlling pathogenic infections and their by increasing the yield of crop. This paper reviews some of the potential applications of nanotechnology in the field of agriculture and recommends many strategies for the advancement of scientific and technological knowledge currently being examined.

Keywords*: Agriculture, Nanotechnology, Molecular Farming, Crop Production, Precision Farming*

1. Introduction

The productivity and sustainability of agriculture is facing serious challenges. Climate change, urbanization, sustainable use of natural resources and soil degradation and accumulation of pesticides and fertilizers are the hot issues for today's agriculture. Conventional agricultural techniques have certain concerns such as overdependence on supplementary irrigation; susceptibility to weather conditions; poor input and energy conversion which can be reduce by nanotechnology (Mukhopadhyay and Sharma 2013). Modern agriculture practices have certainly revolutionized the food supply worldwide but these techniques have several limitations. Worldwide, 16% agriculture land produces 40% of crop production (Gleick, 2014 and Postel *et al*, 1996). Water table is decreasing with alarming speed as more and more ground water is pumped out for irrigation (IFPRI, 2014 and Rodell *et al*, 2009). Excessive use of fertilizers and pesticides in improper way which increases nutrients and toxins concentration in groundwater and surface waters, that affect the health and water purification costs, and decreasing fishery and recreational prospects. Therefore, continuous irrigation and drainage strategies have increased the weathering of soil minerals and soil acidity. Therefore, disrupted ecosystem cycles affect the life cycle of human beings.

Unscientific and excessive use of chemical fertilizers and pesticides has increased disturbed the basic integrity of the ecosystem. Degraded ecosystems have become a serious threat to human health and civilization. The benchmark for ecosystem degradation is linked to its failure to retain carbon and prevent escape of various forms of nitrogen from the soil to water bodies and the atmosphere. A huge amount of biomass was added to soils during the Green Revolution era through a many-fold increase in yields of root mass from crops. Similarly, several attempts have been made to increase the organic matter in soils by adding crop residues. However, these efforts could neither retain carbon for long nor check pollution from nitrogen. The situation is aggravated with the rise in soil temperature across ecosystems. Many soils throughout the world, especially those brought under the Green Revolution during the second half of the last century, are contaminated with harmful trace metals and pesticide residues. It is not practically possible to clean up these lands through bioremediation (including phytoremediation) without relocating farmers and withdrawing their livelihood (Karn *et al*, 2009) At the same time, opportunities exist to reengineer plants (Eapen *et al*, 2005) for which nanobiotechnology could be promising.

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2. Molecular Farming

Molecular farming is the production of recombinant proteins in plants with the intention to use protein itself as a product, in purified form, crude extracts, or in planta, without alteration in phenotype, performance, or metabolism. Production of an antibody and human serum albumin in plant cell suspension cultures and transgenic plants are some of the initial examples of molecular farming (Alivisatos *et al*, 1996 and Han *et al*, 2001). This led to the rapid exploration of many different plant species as hosts for the production of recombinant pharmaceutical proteins, also called as molecular pharming (Hirsch *et al*, 2003, Li *et al*, 1996). Many proof-of-principle studies have been published in the subsequent years, but the commercial advancement came when a defined regulatory framework was accepted for plant-derived biologics, culminating in 2012 with the approval of taliglucerase alfa, a recombinant form of human glucocerebrosidase developed by Protalix Biotherapeutics for the treatment of the lysosomal storage disorder Gaucher's disease (Mazzola, 2003).

Veterinary products produced in plants are also gaining attention, reflecting imminent regulatory changes enforcing the reduction of antibiotic use in food and dairy animals (Scott, 2005, Seeman, 2004 and Tolles *et al*, 2003). Advantages of pharmaceutical products obtained by molecular farming are cheap, larger scalability and not toxic.

Present studies describe the impact of molecular farming on agriculture practices coupled with nanotechnology. Also, focused on the commercialization of non-pharmaceutical products of molecular farming products such as proteins, and growth factors, which are used as chemical reagents in research, ornamental constituents, sensors, and biocatalysts which convert plant biomass into sugars and also assists the process of bioremediation. Presently, various commercially available non-pharmaceutical products of molecular farming are produced in the small to medium range, depending upon the culture conditions not on field cultivation, as they need extra regulatory scrutiny. However, molecular farming along with nanotechnology could only be successful, if early diagnosis of plant diseases and increased productivity of crop is achieved.

3. Nanotechnology?

Nanotechnology is a branch of diverse sciences including life sciences, material science and information technology, which design, synthesize and characterize the materials and devices, whose smallest functional entity in at least one dimension, is on the nanometer scale (Silva, 2004). It involves the engineering with diverse science that design the nano tools/devices having size in the range of nano scale i.e.1-100nm (Huang *et al*, 2007).

It is a promising field of science that resolves problems and issues which are impossible to solve solely by an engineer or a biologist. Combination of nano-science and technology involve the studying the fabrication and working of a material in a nano scale that help in manipulation and invention of new tools, materials and structures at the molecular level, by rearranging their atom sequence into functional structures having size in nano scale (Ray *et al*, 2009). A comparison of size of cell/macromolecule has been shown in Table 1.

Table 1: Classification of size cells and macromolecules

SN	Type of cell/macromolecule	Size (nm)
1	Leukocycte	10,000
2	Bacteria	1,000-10,000
3	Virus	75-100
4	Protein	5-50
5	Deoxyribonucleic acid (DNA)	~2
6	Atom	~0.1

A leukocycte has the size of 10,000nm, a bacteria 1,000-10,000nm, virus 75-100nm, protein 5-50nm, deoxyribonucleic acid (DNA) ~2nm (width), and an atom ~0.1nm [Hirsch *et al*, 2003][4] All organisms, from microbes to humans, are powered by highly evolved molecular and cellular machines that operate at the nano level. (Predicala, 2009; Prasanna, 2007). Arrangement of atoms and molecules converge the technology with biology, physics, chemistry and computer at nano level is called 'nanobiotechnology' (Mazzola, 2003, Ricca, E. and Cutting, 2003 and Salata, 2004). Nanobiotechnology is an interdisciplinary field of research, which is based on the combined efforts of chemists, physicists, biologists, medical doctors and engineers. We are in starting phase to understand the nanoscale methods, which are used in nature to create self-replicating, self-monitoring, self-controlling and self-repairing tools, materials and structures (Scott, 2005 and Seeman, 2004). Table 2 shows the agri products ending with food processing and packaging with supplements.

Table 2 Recent advances in nanotechnology in agriculture (Source: Mukhopadhyay, 2014)

SN	Product	Application	Institution
1	Nanocides	Pesticides encapsulated in nanoparticles for controlled release BASF, Ludwigshafen, Germany Nanoemulsions for greater efficiency	BASF, Ludwigshafen, Germany
2	Buckyball fertilizer	Ammonia from buckyballs	Syngenta, Greensboro, NC, USA
3	Nanoparticles	Adhesion-specific nanoparticles for removal of *Campylobacter jejuni* from poultry	Kyoto University, Kyoto, Japan
4	Food packaging	Airtight plastic packaging with silicate nanoparticles	Clemson University, Clemson, SC, USA
5	Use of agricultural waste	Nanofibers from cotton waste for better strength of clothing	Bayer AG, Leverkusen, Germany
6	Nanosensors	Spoilage of packed food, Pathogen detection	Cornell University, Ithaca, NY, USA. Nestle Kraft, Chicago, USA
7	Precision farming	Nanosensors coupled to a global positioning system tracking unit for real-time examining of soil conditions and crop growth	US Department of Agriculture, Washington, DC, USA
8	Livestock and fisheries	Nanoveterinary medicine (nanoparticles, buckyballs, dendrimers, nanocapsules for drug delivery, nanovaccines; smart herds, cleaning fish ponds (Nanocheck [Nano-Ditech Corp., Cranbury, NJ, USA]), and feed (iron nanoparticles)).	Cornell University NanoVic, Dingley, Australia

Table 3 Examples of marketable developments of non-pharmaceutical proteins produced in plants

Product	Company	Application	Plant species	Country	Advantage	Source
Trypsin avidin endo-1,4β-Dglucanase	ProdiGene/Sigma Aldrich	Technical reagents	Maize seeds	United States	Cost, animal -free	http://www.sigmaaldrich.com
Cellobiohydrolase	Infinite Enzymes/Sigma-Aldrich	Technical reagents	Maize seeds	United States	Cost, integrated production	http://www.sigmaaldrich.com
Growth factors, cytokines, thioredoxin, TIMP-2	Agrenvec	Research reagents	Tobacco leaves, transient	Spain	Cost, Animal free	http://www.agrenvec.com
Growth factors, Cytokines	ORF Genetics	Research reagents	Barley seeds	Iceland	Cost, animal-free	http://www.orfgenetics.com
Epithelial growth factor	Sif Cosmetics	Cosmetics	Barley seeds	Iceland	Cost, animal-free	http://www.sifcosmetics.cm
Albumin, lactoferrin, lysozyme, transferrin, insulin	Ventria Bioscience/ InVitria	Research reagents	Rice seeds	United States	Cost, animal-free	http://www.invitria.com
Aprotinin	Kentucky Bio-Processing	Research reagents	Tobacco leaves, transient	United States	Cost	http://www.kbpllc.com
Collagen	CollPlant	Research Reagents, tissue culture, health applications	Transgenic tobacco	Israel	Cost, animal free	http://www.collplant.com
Trypsin, enterokinase, growth factors, cytokines	Natural Bio-Materials	Research reagents, cosmetic ingredients	Rice cell Suspension	South Korea	Cost, animal-free	http://www.nbms.co.kr
Antibody	Center for Genetic Engineering and Biotechnology	Purification of a hepatitis B vaccine	Transgenic tobacco	Cuba	Cost	http://gndp.cigb.edu.cu
α-Amylase	Syngenta	Bioethanol production	Maize seeds	United States	Cost, integrated production	http://www.syngenta.com
Phytase	Origin Agritech	Feed Maize seeds	Maize seeds	China	Increased mineral availability, integrated production	http://www.originseed.com.com
Growth factors	NexGen	Tissue culture reagent	Tobacco leaves, transient	South Korea	Cost, animal-free	http://www.nexgen.com

4. Molecular farming and nanotechnology integration for agriculture systems

Molecular biology is the basic foundation of nanotechnology. Researchers are reducing the size and are trying to make possible life from the nano-scale for some time. In 1976, Genentech Company has developed an automated process for synthesing DNA and constructed a fully functioning artificial genes and artificial DNA which gave a new direction in the field of genetic engineering in medicine and agriculture. Biological molecules and cell phenomenona have offered unique properties that assist nanotechnologists to achieve their goals which are not possible by other method. For instance, construction of scaffolds of silicon for nanostructures is not necessary as DNA's ladder structure offers nanotechnologists a natural framework for assembling nanostructures; and its highly specific bonding properties bring atoms together in a predictable pattern to form a nanostructure (Tolles and Rath, 2003; Tomànek and Enbody 2000).

DNA is an important component of nanomachines for the construction of nanostructures. DNA store genetic information and act as the basis of the next generation of computers. As microprocessors and microcircuits have confined in the form of noprocessors and nanocircuits by mounting DNA molecules onto silicon chips which replace microchips with electron flow-channels etched in silicon. Such biochips are DNA-based processors that use DNA's unique information storage capacity. Biochips use the properties of DNA to resolve computational obstacles, by simplifying the mathematical calculations. Other biological molecules are assisting in our continual quest to store and transmit more information in smaller places. For instance, research communities are using light-absorbing molecules, such as those found in our retinas, to increase the storage capacity of CDs a thousand-fold (Whitesides, 2003). Nanobiotechnology, is a promising field of opportunity that fuse nano/microfabrication and biosystems to the benefit of both. It congregates all possible applications of genomics including mammalian, plant and microbial and provides the basic tools for collecting sequence information and designing innovative devices to solve queries of the genomic information and the application of this knowledge in diverse fields, particularly medicine and agriculture (Yasuda, 2004). Table 3 shows the marketable developments of non-pharmaceutical proteins produced in plants.

5. Approaches of nanotechnology

5.1. Nanofabricated gel-free systems and high throughput DNA sequencing

As a central process, DNA sequencing needs to be improved in terms of its throughput and accuracy. Nanofabrication technology will be critical toward this goal both in terms of improving existing methods as well as delivering novel approaches for sequence detection. The scaling down in size of the current sequencing technology allows the process to be more parallel and multiplex. Research in nanobiotechnology is advancing toward the ability to sequence DNA in nanofabricated gel-free systems, which would allow significantly more rapid DNA sequencing. Coupled with powerful approaches such as association genetic analysis, DNA sequencing data of the crop germplasm, including the cultivated crop gene pool and the wild relatives can potentially provide highly useful information about molecular markers related with agronomically and economically important traits. Thus, nanobiotechnology can enhance the rate of progress in molecular marker-assisted breeding for crop improvement (Ganesh, 2017).

5.2. Micro-chips and expression profiling

Microchips for DNA or protein sequencing (bio-chips) are the very first tools of nanobiotechnology. These technologies had connected the traditional microelectronic industry to recently advanced biotechnology. Example of nano-biotechnology is microfluidic bio-chips, also known as lab-on-a-chip devices which is based on alteration in microscopic living cells immersed in fluids. These embedded cells are used for biochemical processing including sampling, mixing, amplification, separation detection and analysis. These biochips and microfluidic chips techniques have many applications ranging from high throughput screening, cell analysis and drug discovery to transportable devices for minimal-invasive therapy, precision surgery as well as drug delivery(Ganesh, 2017).

Microarray-based hybridization technique involves the measurement of expression of thousands of genes including gene regulation and function simultaneously; this is known as 'expression profiling' and this method acting as a central tool in biological research. Sequence determination and patterns of gene expression by these techniques gives considerable higher accuracy than present conventional technologies. Thousands of DNA or protein molecules are immobilized on glass slides to create DNA chips and protein chips, respectively. Recently, in microarray technology beads are used in place of glass slides. These nanofabrication techniques used, to pattern surface chemistry for a variety of biosensor and biomedical applications. The main application areas of these techniques are: (i) Determination of new genomic sequences. (ii) Scanning of genes for polymorphisms that might have an impact on phenotype. (iii) Comprehensive survey of the pattern of gene(s) expression in organisms, when they are exposed to biotic/abiotic stress. The fundamental principle underlying the microarray technology has motivated the researchers to create many types of microarrays to answer scientific questions and discover new products.

5.3. DNA microarrays

DNA microarrays has offered several uses (i) detection of mutations in disease-related genes; (ii) monitoring of gene activity; (iii) identification of genes which are vital to crop productivity; and (iv) in bioremediation for improving screening of microbes. Gene sequence and mapping data mean little until we determine what those genes do—which is where protein arrays come in (Ganesh, 2017).

5.4. Protein microarrays

DNA arrays collect the information of DNA, which has no significance unless it is not used at protein level. The configuration and functions of proteins are more complex than DNA. Proteins are less stable than DNA. Each cell has exhibited diverse enzymes which are specific for doing particular function. Protein content in each cell varies with individual, age, present and past environmental conditions. Protein microarrays have been are used to: (i) discover protein biomarkers that indicate disease stages; (ii) assess potential efficacy and toxicity of pesticides (natural and synthetics); (iii) measure differential protein production across cell types and developmental stages, and in both healthy and diseased states; (iv) study the relationship between protein structure and function; and (v) evaluate binding interactions between proteins and other molecules (Ganesh, 2017).

6. Applications of nanotechnology in agriculture

The aim of nanotechnology in agriculture is to increase the crop productivity by improving the quantity of absorbable nutrients in the soil. For enhancing efficiency of agriculture, nanotechnology depends on two parameters (i) ions must be present in plant-absorbable forms in the soil system, and as nutrient transport in soil-plant systems requires ion exchange (e.g. NH_4^+, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, Zn^{2+}), adsorption-desorption (e.g. phosphorus nutrients) and solubility-precipitation (e.g. iron) reactions, nanomaterials must facilitate processes that would ensure the availability of nutrients to plants according to speed and requirement of plants (Mukhopadhyay, 2014). Clay minerals can be used as receptacles. Suspension and hydrogel forms of nanomaterials containing plant nutrients can be used that help in their easy storage and easy transport and without any damage during release of nutrients. Similarly, nanoparticles of zerovalent iron nanoparticles and iron rust have been used for cleaning of contaminated soil with pesticides, heavy metals, and radionuclides. Like, calcium carbonates nanoparticles, Iron nanoparticles have offered unique soil binding properties, which help in formation of soil microaggregates and macroaggregates (Liu and Lal, 2012).

Applications of nanotechnology offer many opportunities in the area of molecular farming by improving genome of plants, (Eapen and D'Souza, 2005 and Kuzma, 2007) delivery of genes and drug molecules to target sites at the cellular level in plants and animals,(Maysinger, 2007) and nanoarray-based technologies for gene expression in plants overcome the stress and development of sensors (Ahmed *et al*, 2013) and protocols for its application in precision farming (Day, 2005) management of natural resources, early diagnosis of pathogens and contaminants in food products, smart delivery systems for agrochemicals like fertilizers and pesticides, and integration of smart systems for food processing, packaging, and monitoring of agricultural and food system security (Chau *et al*, 2007 and Moraru *et al*, 2003) Nanofertilizers are the alternatives to traditional fertilizers, storage of nutrients in soils and hence, risk of eutrophication and contamination of drinking water eliminated.(DeRosa *et al*, 2010 and Bhalla and Mukhopadhyay, 2010). Also, it has been observed that contaminated soil can be cleaned by nanoremediation which is rapid and cost effective. Various applications of nanotechnology in agriculture have been shown in Figure 1.

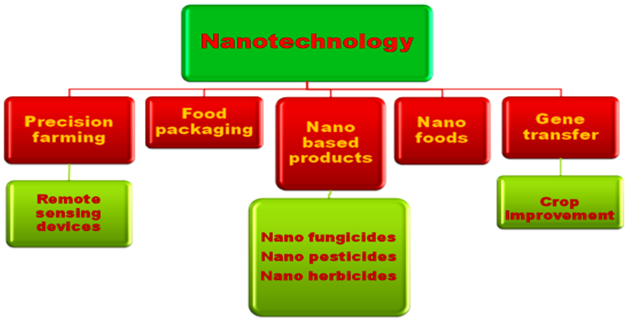

Figure 1 Applications of nanotechnology in agriculture

6.1 Nanofabrication in agriculture

Nanofabrication is the design and manufacture of devices that measure dimensions in nanometers. It is a vibrant field; so many new classes of materials with innovative fabrication technology are expected to appear in the future. Current engineered nanomaterials are grouped into four classes, 19 i.e. carbon-based materials, metal-based materials, dendrimers, and composites. It is difficult to generalize the processes of nanofabrication with accuracy, because they are fabricated by methods specific to the requirements of the materials themselves, and in many cases are protected by intellectual property rights. Recent advances made in nanotechnology in agriculture sector have been shown in Table 4(Source: Mukhopadhyay, 2014).

Delivery of plant materials like fertilizers, pesticides, growth hormones and use of highly advanced biosensor for precision farming have been made possible by using nanoparticles or nanochips. Nano-encapsulation traditionally used fertilizers, pesticides and herbicides facilitate the gradual and sustain release of precise amount of nutrients and agrochemicals to the plants (Duhan *et al*, 2017). Various types of nanomaterials have been used for plant protection, nutrition and management of farm practices as they exhibit small size, high surface to volume ratio and exceptional optical properties. Metal oxides, ceramics, magnetic materials, semiconductor, quantum dots, lipids, polymers, dendrimers and emulsions (Puoci *et al*, 2008). Improvement in micro fabrication and nanotechnology can plan a significant role in detection of viruses, enhancing the detection limit, easy to operate and economic viral diagnosis (Cheng *et al*, 2009).

Table 4 Agricultural products made through nanotechnology

SN	Agriculture	Food processing	Food packaging	Supplements
1	Single molecule detection to determine enzyme/substrate interactions	Nanocapsules to improve bioavailability of neutraceuticals in standard ingredients such as cooking oils	Antibodies attached to fluorescent nanoparticles to detect chemicals or food borne pathogens	Nanosize powders to increase absorption of nutrients
2	Nanocapsules for delivery of pesticides, fertilizers and other agrichemicals more efficiently	Nano-encapsulated flavor enhancers	Biodegradable nanosensors for temperature, moisture and time monitoring	Cellulose nanocrystal composites as drug carrier
3	Delivery of growth hormones in a controlled fashion	Nanotubes and nanoparticles as gelation and viscosifying agents	Nanoclays and nanofilms as barrier materials to inhibit contamination and reduced oxygen absorption	Nanoencapsulation of neutraceuticals for better absorption, enhanced stability or delivery at precise site
4	Nanosensors for monitoring soil conditions and crop growth	Nanocapsule infusion of plant based steroids to substitute a meat's cholesterol	Electrochemical nanosensors to detect ethylene	
5	Nanochips for identity preservation and tracking	Nanoparticles to selectively bind and remove chemicals or pathogens from food	Antimicrobial and antifungal surface coatings of nanoparticles (silver, magnesium, zinc)	Vitamin sprays dispersing active molecules into nanodroplets for improved absorption

6.1.1. Biosynthesis of nanoparticles and their application in agriculture

Various chemical methods for synthesis of particles are available which require toxic chemicals which are harmful for living organisms. Hence, there is requirement to use eco-friendly practices. Different types of biological sources such as bacteria, fungi, higher plants and viruses have been used for the synthesis of NPs (Kuppusamy *et al*, 2016).

6.1.2. Nano-sized carriers

Smart nano carriers have been developed for the proficient intake of fertilizers, herbicides, pesticides and plant growth regulators. These smart nano carriers confined in the vicinity of roots of the crop plants growing in the soil rich in organic material. Therefore, this approach has increased the consistency and minimizes the efforts for reduced deformations persuaded by atmospheric factors (Ditta and Arshad, 2015; Srinivas, 2016).

6.1.2 (i). Nano-herbicides

Nanoherbicides have been used for removing unwanted plants called as weeds from crops devoid no harmful effect in the environment. Encapsulation of herbicide in polymeric nanoparticles reduces toxicity and they are eco-friendly in nature. Inappropriate application of herbicides for prolonged duration has damaged the subsequent crops. Constant exploitation of identical herbicide is responsible for weeds resistance against identicle weedicide. Efficiency of nano zerovalent iron (nano ZVI) has been observed to dechlorinate herbicide atrazine (2-chloro-4ethylamino-6-isopropylamino-1, 3, 5-triazine) from atrazine-contaminated water and soil. Specific nanoparticles coated with herbicide have been used for their delivery in roots of weeds, consequently, these molecules enter inside the roots sphere of the weed plants and from where they are translocated to cells and interrupt physiological processes such as glycolysis and leads to death of plants (Duhan *et al*, 2017).

Various types of nanoherbicides have been synthesized and their effects on the plants have been observed such as effect of poly(e-caprolactone) atrazine on *Brassica sp*., nanoemulsion of chitosan and sodiumtriphosphate paraquat, clomazone/Alginate/ chitosan NPs on *Allium cepa*, effect of complex of paraquat, formulation of AgNPs chitosan matrix on *Eichhornia crassipes* and Nanoemulsion of Glyphosate isopropylamine (GIPA) on *Elwsine indica*.

6.1.2.(ii). Nano-fertilizers

Growth and survival of plants require a number of essential constituents. Some of these include nitrogen, phosphate, potassium, calcium, magnesium, sulfur, iron, manganese and zinc. Typically, these constituents are found in soil; although they are not in sufficient concentration required for growth of plant. Some of companies have provided fertilizers which contain almost all the components necessary for growth of plant (Cicek and Nadaroglu, 2015). Nanotechnology in the field of sustainable agriculture has offered several applications by providing novel varieties of fertilizers which has amplified the agriculture yield which meet out the food requirements by augmenting the productivity of agriculture (Iavicoli *et al*, 2017).

Applications of nanotechnology have reduced the ill consequences of fertilizers; fertilizer carriers by using smart fertilizers. These fertilizers help in the controlled release of such smart fertilizers which is an efficient and inexpensive strategy. Smart nano fertilizers have increased the surface to volume ratio and hence plant uptake of nano fertilizers by plants due effective adhesiveness. Hence, minimizes the rate of recurrence and quantity of fertilizer use. Thus, reduces the necessity of fertilizer as well as labor costs. In comparison to traditional fertilizers, the nano-fertilizers have improved the crop productivity. Applications of nano-fertilizers have also minimized the loss due to elution and evaporation in the soil and improve the nutrients retaining capacity in the soil (Ditta and Arshad, 2015).

Wu *et al*. (2008) have explained the significance of chitosan-coated NPK composed fertilizer help in controlled-release and water-retention abilities that is mediated by an interior coating of chitosan, and an outer coating was poly (acrylic acid-co acrylamide) (P(AA-co-AM)). It has been found that the product has facilitated the control release of the nutrients and the nutrients released not surpass 75% on the 30th day. In addition, chitosan is an effective recyclable product, while the *P*(AA-co-AM) is also degradable in soil, therefore, matrix polymers as well as their degraded products are not hazardous.

Study of Liu *et al*. (2009) have compared the nano-calcium carbonate with humic acid, and organic fertilizer in groundnut, and demonstrated that at low concentrations the nano-calcium carbonate has offered various fascinating properties such as large leaves and leaf area, increased count of leaves, dry weight, the soluble sugar and peanut protein content. Corradini *et al*. (2010) have stated that nanoparticles of chitosan exhibit antibacterial activity by for slow dispersing of fertilizers (Kashyap *et al*. 2015). The application of nanoporous zeolites have also examined for high efficiency as well as gradual release of fertilizers (Chinnamuthu and Boopathi 2009). It has been observed that SiO2 nanoparticles have elevated the germination of tomato (*Lycopersicum esculentum*) seeds (Manzer and Mohamed 2014, Ramalingam *et al.* 2015).

Kottego *et al.* (2011) have demonstrated that modification of hydroxyapatite nanoparticles using urea and encapsulation of these nanoparticles with *Gliricidia sepium's* wood spaces under pressure, and correlated with commercially available fertilizers. Initially these nano-fertilizers have showed an outburst and their by slow release. The impact of TiO_2 nanoparticles have also been studied by growing corn plants and stated that TiO_2 has showed better results on the growth of corn plants. Titanium nanoparticles have also amplified the light absorption ability and photo-energy transfer. Nanoparticles of SiO_2 and TiO_2 have augmented the nitrate reductase activity in soybean plants, and better plant absorption capacity (Lu *et al*. 2002).

6.2.1. Nano-particles controlling the plant diseases

Various types of nanoparticles have been used in the field of agriculture in controlling plant diseases such as nano forms of carbon, silver, silica and alumino-silicates. Goswami *et al*. (2010) have reported the use of various nanoparticles viz. silver nanoparticles (SNP), aluminium oxide (ANP), zinc oxide and titanium dioxide in protection of rice weevil and grasserie disease in silkworm caused by *Sitophilus oryzae* and baculovirus BmNPV (B. mori nuclear polyhedrosis virus), respectively (Goswami *et al*. 2010). In their study they performed bioassay, in which they performed a bioassay and prepared solid and liquid formulations of the above-mentioned nanoparticles and applied these formulations on rice and kept in a plastic box with 20 adults of S. oryzae and observed the effects for 7 days. It was found that hydrophilic SNP showed effective results on the first day while on next day; more than 90 % mortality was obtained with SNP and ANP. After 1 week of exposure, 95 and 86 % mortality were reported with hydrophilic and hydrophobic SNP and approximately 70 % of the insects were killed when the rice was treated with lipophilic SNP. However, 100 % mortality was observed when rice were treated with ANP. likewise, in another bioassay was performed for grasserie disease in silkworm (B. mori), a considerable decline in viral load was reported when leaves of B. mori were treated with ethanolic suspension of hydrophobic alumino-silicate nanoparticles (Goswami *et al*. 2010).

6.2.2. Nano Carbon

Carbon is selected as brick molecule for simple as well materials to improve the quality of plant products. Carbon nanotubes are allotropes of carbon whose nano configuration is in cylindrical shape having many applications, particularly in the areas of nanotechnology, electronics, and architecture and commonly used as thermal conductors. These carbon nanotubes possess unique electrical properties. When these CNT introduced into planted tomato seeds in a soil these CNTs could not only penetrate into the hard coat of germinating tomato seeds but also exerted growth enhancing effect(Khodakovsky *et al*, 2009). They observed better growth which was due to increased water uptake caused by penetration of CNT. Therefore, these CNTs acting as vehicles for delivery of desired molecules into seeds during their germination which keep protect them from diseases. CNTs are growth promoting, not toxic or inhibiting or adverse effect on the plant.

6.2.3. Nano Silver

Silver nanoparticles have exhibited powerful inhibitory and bactericidal effects as well as a broad scale of antimicrobial activities. Various properties of these silver nanoparticles are high surface area, large fraction of surface atoms and unique antimicrobial activity as compared to the bulk silver. Colloidal nano silver solution has showed antifungal activity against powdery mildew caused by *Sphaerotheca pannosa Var rosae* (Kim *et al*, 2008). Powdery mildew is most commonly spread diseases to both green house and outdoor grown roses that result in leaf distortion, leaf curling, premature defoliation and reduced flowering. Moreover, double capsulized nano silver has been prepared by chemical reaction of silver ion with the help of physical method, reducing agent and stabilizers which are very stable and well dispersive in aqueous solution. Nano silver colloid is a well dispersed and stabilized silver nano particle solution and more adhesive due to which more and more bacteria and fungus are entrapped, hence acting as a better fungicide. Marvelously, maximum number of patents has been filed for 'Nano silver for preservation and treatment of diseases in agriculture field.' This popularity of nano silver has caused concern about regulating and classifying the nano silver as pesticide (Anderson 2009). In May 2008, the International Center for Technology Assessment (ICTA) has submitted a petition to EPA requesting that it regulate nano-silver used in products as a pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Silver has an accepted agrochemical replacement which eliminates unwanted microorganisms in fertile soils and hydroponics systems. It has been used as foliar spray to prevent fungi, moulds, rot and numerous other plant diseases. In addition, silver has exhibited an excellent plant-growth stimulator. There are thousands of other vital uses for this odorless, nearly tasteless and colorless, totally benign, potent, safe antiseptic and healing agent.

6.2.4. Nano Silica-Silver composite

Silicon (Si) has been reported that when it is absorbed by the plant, it makes plant tolerant for increased diseases and stress (Brecht *et al*., 2003; Ma *et al*., 2001). Aqueous silicate solution has been used for the treatment of the plant for e.g. protective effects have been shown against powdery mildew or downy mildew. Beside this, it also increases the growth, physiological activities and resistance to diseases and stress in plants (Garver *et al*., 1998; Kanto *et al*., 2004). Silver is a potent disinfecting agent and destroy unicellular microorganisms by inactivating the enzymes involved in metabolic reactions of microorganism by oligodynamic reaction (Kim *et al*., 1998) and also suppress the growth of algae. Kim *et al*, 1998, Neill *et al*, 2003 and Mc Cubin, 2003 have reported that Silver exhibited antimicrobial activity. Ionic state of silver is unstable because of its high reactivity it is converted into oxidized/reduced forms depending on the conditions of physiological environment and therefore, does not exhibit constant antimicrobial activity. Ionic or oxides states of silver are stable in the environment but poor antimicrobial activity. Hence, Park *et al* (2006) have prepared a hybrid of nano sized Silica-Silver for prevention of various diseases in plants. This nano sized hybrid was combined with silica molecules and water soluble polymer prepared by a solution containing silver salt, silicate and water soluble polymer to radioactive rays. Silica-silver hybrid at 0.3ppm has showed excellent antifungal activity and controlled powdery mildews of pumpkin in field as well as greenhouse tests. Park *et al* (2006) have also investigated the 'effective concentration' of nano sized silica-silver to control the growth of various fungi; and found that at 10 ppm there was 100% inhibitiory effect on the growth of various fungi such as *Pythium ultimum*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides*, *Botrytis cinere* and, *Rhyzoctonia solani*, showed 100% growth inhibition at 10 ppm of the nanosized silica-silver. Nano sized silica-silver has also exhibited the 100% antibacterial activity at concentration 100 ppm against *Bacillus subtilis*, *Azotobacter chrococuum*, *Rhizobium tropici*, *Pseudomonas syringae* and *Xanthomonas compestris* pv. *Vesicatoria*. Higher concentration (3200 ppm) of these nano sized silica-silver has caused chemical injuries on cucumber and pansy plant (Park *et al*, 2006).

6.2.5. Nano Alumino-Silicate

Top chemical companies have prepared the pesticides used in agriculture in nano scale by using Alumino-Silicate nanotubes with active constituients. The merit of these Alumino-Silicate nanotubes is that when these nanotubes are sprayed on the plant parts they can be easily sticked in the insect hairs and consume pesticides filled nanotubes. These Alumino-Silicate nanotubes are highly active and eco-friendly pesticides.

6.2.6. Mesoporous Silica Nanoparticles

Nano-silica is a nanomaterial prepared from silica and has many applications in medicine and drug development as catalyst and used as nano-pesticide. Barik *et al*. (2008) have reported the use of nano-silica as nano-pesticide. Wang *et al* (2002) have reported that mesoporous Silica nano particles can deliver DNA and chemicals into plants thus, acting as an influential novel tool for site-specific delivery into plant cells. Porous, silica nanoparticles systems that are spherical in shape have been developed which are porous, silica nanoparticles systems, spherical in shape and arrays of independent porous channels that form honeycomb-like structure that can be filled with chemicals or molecules (Lin). Nano silica has been used as an insect pest as insects use large number of lipids found in the cuticle of the plants to prevent water from dessication but nano-silica absorbed into the cuticular lipids by physiosorption and leads to death of insects by physical means when applied on leaves and stem surface. Surface charged modified hydrophobic nano-silica (~3–5 nm) can be use to control a range of agricultural insect pests and animal ectoparasites of veterinary importance (Ulrichs *et al*. 2005).

7. Nanotechnology for mutation breeding

Nanotechnology has also shown its ability in modifying the genetic constitution of the crop plants thereby helping in further improvement of crop plants. Mutations –both natural and induced– have long since played an important role in crop improvement. Instead of using certain chemical compounds like EMS, MMS and physical mutagen like X-ray, gamma ray etc. for conventional induced mutation studies, nanotechnology has showed a new dimension in mutation research. In Thailand, Chiang Mai University's Nuclear Physics Laboratory has come up with a new white-grained rice variety from a traditional purple coloured rice variety called 'Khao Kam' through the usage of nanotechnology.

The word "Kam" means deep purple, and the rice variety is known for its purple stem, leaves and grains. Using nanotechnology, the scientists changed the colour of the leaves and stems of Khao Kam from purple to green and the grain becomes whitish. The research involves drilling a nano-sized hole through the wall and membrane of a rice cell in order to insert a nitrogen atom. The hole is drilled using a particle beam (a stream of fast -moving particles, not unlike a lightning bolt) and the nitrogen atom is shot through the hole to stimulate rearrangement of the rice's DNA. This newly derived organism through the change at the atomic level is designated as 'Atomically Modified Organisms (AMOs). Recent advances made in agriculture by using nanotechnology have been shown in Table 6.

Table 6 Nano-agrochemicals and nano-materials under development (Source:Chinnamuthu and Boopathi, 2009)

Category	Type of product	Product name	Nano content	Purpose
Nano-agrochemicals	Super" combined fertilizer and pesticides	Pakistan-US Science and Technology Cooperative Program	Nano-clay capsule contains growth stimulants and biocontrol agents	Slow release of active ingredients, Reducing application rates
	Herbicide	Tamil Nadu Agricultural University (India) and Technologico de Monterry (Mexico)	Nano-formulated	Designed to attack the seed coat of weeds, destroy soil seed banks and prevent weed germination
	Pesticides, including herbicides	Australian Common wealth Scientific and Industrial Research Organization	Nano-encapsulated active ingredients	Very small size of nanocapsules increases their potency and may enable targeted release of active ingredients
Nano-materials	Nutritional suppliments	Nanoceuticals 'mycrohydrin' powder, RBC Life sciences	Molecular cages 1-5 nm diameter made from silica mineral hydride comple	Nano-sized mycrohydrin has increased potency and bioavailability. Exposure to moisture releases H- ions and acts as a powerful antioxidant
	Nutritional drink	Oat Chocolate Nutritional Drink Mix, Toddler Health	300nm particles of iron (SunActive Fe)	Nano-sized iron particles have increased reactivity and bioavailability.
	Food packaging	Adhesive for McDonald's burger containers, Ecosynthetix	50-150nm starch nanospheres	These nanoparticles have 400 times the surface area of natural starch particles. When used as an adhesive they require less water and thus less time and energy to dry.
	Food additive	Aquasol preservative, AquaNova	Nanoscale micelle (capsule) of lipophilic or water insoluble substances	Surrounding active ingredients within soluble nanocapsules increases absorption within the body (including individual cell)
	Plant growth treatment	Primo Maxx, Syngenta	100nm particle size emulsion	Nano-sized particles increases the potency of active ingredients, potentially reducing the quantity to be applied

8. Plant Disease Diagnostics

Disease caused by various pathogens is one of the major factors for decreasing crop productivity. The problem with the disease management lies with the detection of the exact stage of prevention. Most of the times pesticides are applied as a defensive manner leading to the residual toxicity and environmental hazards and while on the other hand application of pesticides after the appearance of disease lead to loss of crop. Among the several diseases, the viral diseases are the most difficult to control, as one has to stop the spread of the disease by the vectors. But, once it starts showing its symptoms, pesticide application would not be of much use. Therefore, detection of exact stage such as stage of viral DNA replication or the production of initial viral protein is the key to the success of control of diseases particularly viral diseases. Nano-based viral diagnostics, including multiplexed diagnostic kit development, have taken momentum in order to detect the exact strain of virus and stage of application of some therapeutic to stop the disease. Detection and utilization of biomarkers that accurately indicate disease stages is also a new area of research. Measuring differential protein production in both healthy and diseased states leads to the identification of the development of several proteins during the infection cycle. These nano-based diagnostic kits not only increase the speed of detection but also increase the power of the detection. (Prasanna, 2007). Figure 2 shows how crop improvement is one of the potential applications from Nano Products.

8.1 Management of insect-pests using nanotechnology: as modern approaches

As the conventional/traditional methods have certain limitations therefore, there is a need to develop a new approach for the management of insects and pests. Nanotechnology plays an important role in the field of agriculture by improving the productivity of crops by controlling insects and pest. In past few approaches were concerned for the management of insect pest. Hence, there is a need to explore the applications of nanotechnology in the agricultural field. In earlier reports suggested that metal nanoparticles had been used which were effective against plant pathogen and these were used for the formulation of pesticides, insecticides and insect repellant (Barik *et al*, 2008). Nanotechnology has many significant applications in molecular farming by transferring DNA mediated by nanoparticles. Nanoparticles have also been used to transfer useful chemicals into plant parts to protect the host plant against insects. Efficient delivery of water soluble pesticides, porous hollow silica nanoparticles loaded with validamycin used for the protection of host plant against various insecticides in a controlled manner in plants which require immediate and long term release of soluble pesticides (Liu *et al*, 2008). Nano-emulsion (Figure 2) of oil in water has been used for the preparation of pesticides and these nano-emulsions can be used as pesticides against various insects which affect the productivity of crop/ in the agriculture (Wang *et al*, 2007). Beside, this oils in water nano-emulsion other nano-pesticides such as essential oil-loaded solid lipid nanoparticles have also been used in the agriculture (Liu *et al*, 2006).

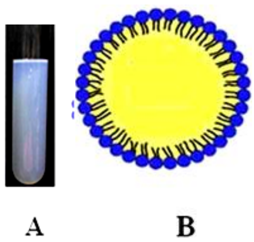

Figure 2:- (A) Nano encapsulation (B) Schematic representation of nano-emulsion

Bhattacharyya *et al*. (2010) reported that in future nanotechnology can revolutionize agriculture including pest management. Nanotechnology can also accelerate 'green revolution' in next two decades. Nano-encapsulation has been currently being used as the most important and promising strategy for protection of host plants against insect pests. Nano-encapsulation involves the encapsulation of insecticide in different kind of nanoparticles so that insecticide is released slowly but released to a particular host plant for insect pest control (Figure 3).

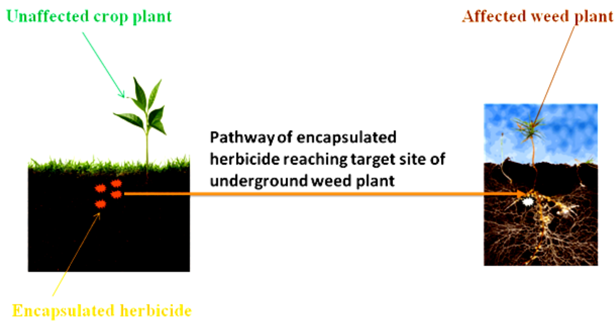

Figure: 3 Nanoparticle targeting the specific receptors of weed plants

Nano-encapsulation with nanoparticles in form of pesticide allows for proper absorption of the chemical into the plants unlike the case of larger particles (Scrinis and Lyons 2007). Teodoro *et al*. (2010) have investigated the insecticidal activity of nanostructured alumina against two insect pests viz. *S. oryzae* L. and *Rhyzopertha dominica* (F.), which are key insect pests in stored food supplies throughout the world. They reported mortality after 3 days of continuous exposure to nanostructured alumina-treated wheat. Therefore, as compared to commercially available insecticides, inorganic nanostructured alumina provide a cheap and reliable alternative for control of insect pests, and attracted researchers interest to expand the frontiers for nanoparticle-based technologies in pest management.

9. Advantages of nanomaterials over corresponding bulk materials

Nanomaterials have unique properties such as increased surface area, cation exchange capacity, ion adsorption and complexation. The main difference between nanomaterials and solid materials is that a large number of atoms are exposed to the surface of the nanomaterials (Maurice and Hochella, 2008). Nanoparticles also differ from the microzides in terms of different surface compositions, different types and densities of sites, and different reactivity with respect to processes such as adsorption and redox reactions (Hochella *et al*, 2008 and Waychunas *et al*, 2005) which can be used for the preparation of nanoparticles used for agriculture purpose.

10 Miscellaneous applications of nanotechnology in the field of agriculture

Nanotechnology has potential to reform the various area of agriculture and food industry by designing various advance devices for the treatment of diseases, fast detection, improving the nutrients absorbing capacity of plants. This can be achieved by developing smart sensors and smart delivery of nano-pesticides at the affected site which can fight with crop infecting viruses and other crop pathogens (Rickman *et al*. 1999). Hence, future research should be focused on nano-based catalysts which will augment the efficiency of pesticides and herbicides and require very low quantity. Nanotechnology has a good impact to make eco-friendly environment by using renewable energy resources, filters or catalysts to reduce pollution by absorbing or degrading various pollutants and thus, helps in protection of environment (Tungittiplakorn *et al*. 2005). Bhattacharyya *et al*. (2011) have reported the relevance of nanotechnology in diverse area such as nano-food, nano-food packaging and nano-farming and highlighted the use of nanoparticles and their effects on maintaining the ecological stability. Nanoparticles of polyethylene glycol loaded with garlic essential oil and exhibited insecticidal activity against adult *Tribolium castaneum* found in the stored products. It was found that about 80 % control efficacy against adult *T. castaneum* which was due to the slow and persistent release of the active components from the nanoparticles Yang *et al*. (2009).

Conclusion

Nanotechnology has the potential to revolutionize the existing conventional techniques used in various areas including agriculture. Nanotechnology has many applications in biotechnology genetics, plant breeding, disease control, fertilizer technology, precision agriculture, and related fields. Nanotechnology has offered concrete explanation against several agriculture-related issues such as insect pest management using traditional methods, adverse effects of chemical pesticides and development of improved crop varieties. Nanomaterials used for different applications like efficient management of insect pests and formulations of potential insecticides and pesticides. Gene delivery mediated by use of nanoparticles would be helpful for the making novel insect resistant varieties. Hence, nanotechnology provides green and eco-friendly substitutes for insect pest management without impairment of nature.

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