Type of the Paper (Review)

Nanotechnology: An Elixir to Life Sciences

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Abstract: Nanotechnology has been playing a tremendous role in all fields of life sciences including cell biology, genetics, molecular biology, botany, microbiology, zoology, evolution, ecology, and physiology. Applications of nanotechnology are more on applied side in different sectors from industry to agriculture. Agriculture sector is the backbone of developing countries for their economy. Growing world's population is putting more pressure on agriculture sector and there is a need to develop new technology to address the crises of food safety and food shortage. Today's agriculture has been entered in a new era where nanotechnology works as a technological advancement regarding entire agriculture crops, and food sector revolution, even though, it has prodigious applications in food production, food processing, food packaging, food storage and economic growth of industries. Moreover, nanotechnology is the best solution to solve problems related to better food and agriculture. Likewise, biotechnology, nanotechnology also raised high concerns upon safety on biodiversity, health and environment. Nanotechnology is providing efficient alternatives to increase the crop production by managing the insect/pests in agriculture in an eco-friendly manner. It also promotes plant efficiency to absorb nutrients. However, the concerns are very high regarding regulation, safety and approval of nanotechnology products by risk assessment authorities. The suggested review includes stupendous applications of nanotechnology in food and agriculture sector along with its prospective merits and associated risks.

Keywords: Nanotechnology, Nanoparticles, Biosynthesis, Agriculture, Food Sciences, Health Sciences, Environmental Sciences

1. Introduction

Development of agriculture is the most powerful tool that can reduce poverty and can feed an expected population of 9.7 billion people by 2050. Agricultural growth is effective in generating incomes for poor compared to other sectors. Agriculture is extremely important for economic growth, poverty and food security are at risk due to climate change that mainly affects the food insecure regions. About 25% of greenhouse gas emissions are due to agriculture, forestry and land use change. Today's food systems also affect people's health and 70% use of agricultural water would produce unsustainable levels of pollution and waste. There are certain risks associated with poor diets that leads to deaths worldwide. An estimated three billion people are victims of health crisis due to usage of wrong type of food. Several reports in 2016 suggested that hunger is a challenge worldwide [1]. Despite the use of nanotechnology in nanomedicine and nanotoxicology disciplines aimed at the betterment of human life, nanotechnology also occupies an eminent position between the newest line of technological novelties in the area of agriculture, to modernize agriculture and food production so that the demands can be fulfilled in an effective and economical way [2-4].

Likewise, biotechnology, nanotechnology also has countless applications like drug delivery, tissue culturing, food industry and biomedical engineering [5]. Precision farming techniques, nutrient delivery systems, novel functional ingredients, innovative and improved packaging, safety testing, authenticity and traceability, tracking devices, nano sensors, targeted delivery of required components, development of new products, precision processing and smart packaging are all included in stupendous applications of nanotechnology [6,7]. Now, it is accepted worldwide that nanotechnology will bring consistent benefits, and research in this proposed field is attracting large scale investments by support from academic science, leading food companies and increasing governmental financial investment and conceptual backing [8,9].

Nanomaterials can be used in different food products like titanium dioxides widely used a food additive and an antimicrobial agent in packaging of food products and food storage containers. Like titanium dioxide, silver nanoparticles are also used as antimicrobial agents in packaging of food products, storage of food containers, chopping boards and refrigerators, and they are also given as health supplement. For provision of nutrition, zinc and zinc oxide nanoparticles are used with food products. To improve analysis pattern of food, platinum and gold nano-wires are used as biosensors [10]. The suggested review article includes the monumental applications of nanotechnology in agriculture and food sector along with its prospective merits and associated risks.

2. Types of Nanoparticles

Any material having one dimension in range 1 to 100×10-9 or one to a hundred billionth of a matter having unique properties than its similar bulk material if did not cause any toxic/harms i.e. carcinogenic, immunogenic and thrombogenic to the living organism known as biocompatible nanoparticles. Now-a-days, different organic and inorganic nanoparticle have been used as carrier for targeted delivery into the living organisms e.g. genetic material, drugs, proteins, antibodies, vaccines. Both organic and inorganic materials can be biocompatible in nature some of them are listed below in **Table 1**.

3. Synthesis of nanoparticles using living systems

Nanoparticles have been produced by different method from physical to chemical. It has been found that most of these methods have certain limitations such as high cost and time-consuming and eco-toxicity. To encounter these issues, biosynthesis of nanoparticles using living systems has been found very fascinating and attractive approach. Various living systems/organisms, form unicellular to multicellular, have been used for biosynthetic production of nanoparticles such as plants, bacteria, fungi and yeast etc. Biosynthesis of nanoparticles is the need of the hour owing to the increasing demand for nanoparticles in millions of tons worldwide. Some examples of different plants, bacteria, fungi and yeast species have been given the **Table 2**.

4. Applications of Nanotechnology in Agriculture

Nanotechnology plays a decisive role in agriculture sector and its use in agriculturally based products can monitor growth of plants and can also detect diseases. Application of nanotechnology-based nanomaterials in agriculture aimed to reduce the use of conventional plant protection methods, increase yields through nutrient management or minimizing loss of nutrients by fertilization.

4.1 Seed germination and nanotechnology

Morphological and physical characteristics of crops change due to excessive salinity. For a crop subjected to salt stress, one of the most significant steps is seed germination. In a soil with high concentration of salt in the planting zone, seed germination fails [11]. Plant emergence is significantly based on seed germination, so, the time from sowing to emergence of seedling has substantial value in crop production. Increased germination can lead to strong early growth and better plant establishment. Addition of silicon can improve the salt tolerance and increases the photosynthetic activity of barely leaf cells [12].

Table 1. Types of Nanoparticles

Nanoparticles	Types	Examples	Reference
Organic Nanoparticles	Polymers	1. Poly-D- lactide-co-glycolic acid (PLGA)	[104] [105] [106]
		2. Poly lactic acid (PLA)	
		3. Poly-ε-Caprolactone (PCL)	
		4. Polyethylene glycol (PEG)	
		5. Poly ester bio-beads	
		6. Polystyrene	
		7. Gelatin	
		8. Chitosan	
		9. Inulin	
		10. Pullulan	
		11. Dendrimers	
		12. Dextran	
		13. Starch	
		14. Alginate	
	Liposomes	1. Liposome Polycation DNA nanoparticles (LPD) 2. Solid lipid nanoparticles (SLN)	[104] [106]
	Self-Assembled nanoparticles		[104] [106]
	Virus-like particles		[104] [105] [106]
	Immuno-Stimulating Complexes		[104] [106]
In-Organic Nanoparticles	Gold Nanoparticles		[104] [106]
	Silver Nanoparticles	Magnesium Silicate	[104] [106]
	Calcium-based nanoparticles	1. Calcium Phosphate 2. Hydroxyapatite	[104] [106]
	Titanium dioxide		[104] [106]
	Carbon based nanoparticles	1. Hollow carbon 2. Nano sphere 3. Carbon dots	[106] [107]
	Quantum dots		[105] [106]
	Iron core nanoparticles		

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Table 2: Biosynthesis of Nanoparticles from Plants, Bacteria, Fungi and Yeas

Organism	Nanoparticles	Plant Species	References
Plants	Gold nanoparticles	Terminalia catappa	[108]
		Banana peel	[109]
		Medicago sativa	[110]
		Azadirachta indica Azadirachta juss	[111]
		Camellia sinensis	[112]
	Silver nanoparticles	Euphorbia hirta	[113]
		Azadirachta indica	[114] [115] [116]
		Brassica juncea	[117]
		Coriandrum sativum	[118]
		Carica papaya	[119]
		Capsicum annum	[120]
		Avicennia marina	[121]
Bacteria	Silver nanoparticles	Bacillus cereus	[122]
		Escherichia coli	[123]
		Bacillus subtilis	[124]
		Bacillus sp.	[125]
		Bacillus thuringiensis	[126]
		Lactobacillus strains	[127]
		Corynebacterium	[129]
		Staphylococcus aureus	[130]
	Gold nanoparticles	Alkalothermophilic actinomycete	[131]
		Thermomonospora sp.	[132]
		Pseudomonas aeruginosa	[133]

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		Lactobacillus strain	[131]
Yeast and Fungi	Silver nanoparticles	Silver tolerant yeast strains MKY3	[129]
		Fusarium semitectum	[130]
		Fusarium oxysporum	[131]
		Aspergillus flavus	[132]
		Aspergillus niger	[133]
		Aspergillus oryzae	[134]
		Fusarium solani	[54]
		Trichodem aviride	[135]
	Gold and Silver nanoparticles	Verticillium sp. and Fusarium oxysporum	[136]

In lentil genotypes, use of Silicon-di-oxide nanoparticles, improves the seed germination under salt stress [13].

Effects of nanomaterials on development and growth of plants with special aim of using them in agriculture have been studied by different researchers. Spinach seeds containing TiO_2 nanoparticles had improved germination by 73% of dry weight, photosynthesis rate increased by three-fold, chlorophyll A formation has also increased. Size of nanomaterials was inversely proportional to spinach seeds i.e. smaller the size of particles greater will be the germination [14]. If gold nanoparticles are used for seed germination instead of TiO_2, the germination may be improved up to 90-95% and may also reduce biosafety concerns [15].

Major concern of nanoparticles in seed germination is about phytotoxicity and it depends upon type of nanomaterial i.e. bio labels were used for promoting germination like fluorescein isothiocyanate (FTIC) labeled silica nanoparticles and photostable Cadmium-Selenide (CdSe) quantum dots. Nair *et al.* (2011) stated that quantum dots affect negatively, the germination of rice but FTIC nanoparticles produced considerable results [16]. Nanoparticles has efficient physiological and biomolecular mechanism in plant that leads to better germination of seed and growth of seedling [17]. In reliance to this, the nanotechnology will improve the seed germination in highly stressed areas such as saline areas, water shortage areas like Cholistan desert, Thal desert etc. of Pakistan. Overall green synthesis of nanoparticles strongly effects on the germination of seed as shown in **Fig. 1,** which directly related to the quality of the NPs.

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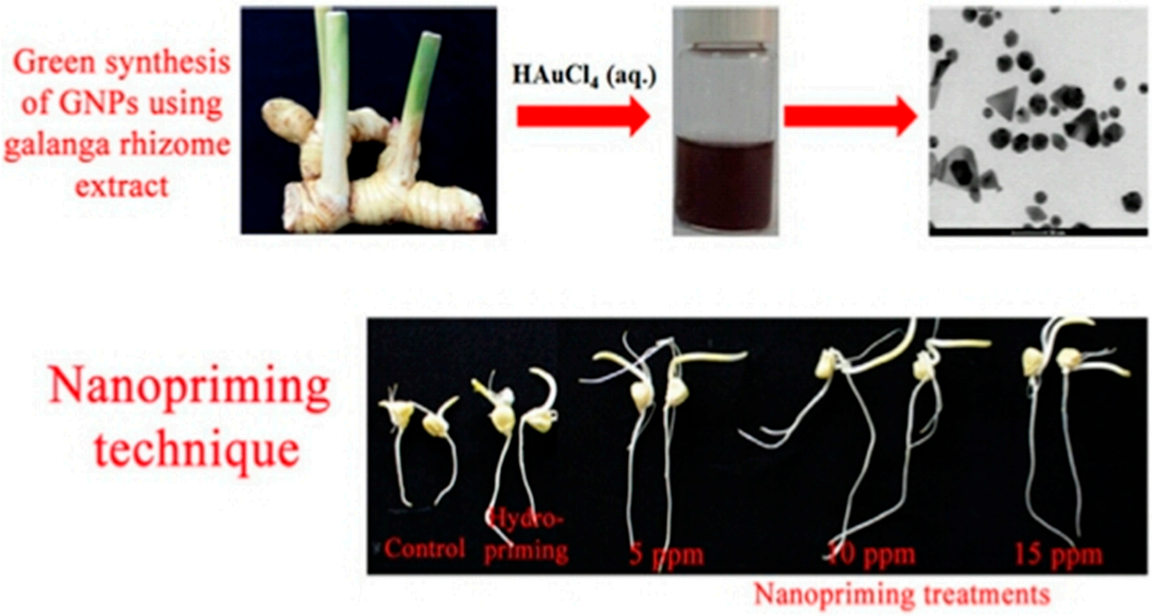

Fig. 1. Green synthesis of GNPs and its effect on seed germination. Reprinted with permission [18]. Copyright 2016 Elsevier.

4.2 Fertilizer use efficiency and crop management

Employing nanotechnology in sustainable agriculture for developmental purposes finds its applications in producing new types of fertilizers that proved promising in significantly increasing plant growth and agronomic yields to overcome the food shortage and environmental problems [19]. Fertilizers play a significant role in advancement of agriculture. Solanki *et al.* (2015) and Chien *et al.* (2011) reported that the uptake of conventional fertilizers and performance phosphate products (PPP's) do not reach to their target due to their instability in environment [20,21]. Efficient and targeted delivery of agents was possible by use of small sized nano-based smart delivery systems [22,23]. Also, they were stable in environment and provides PPP's and nutrients to crops. By the use of smart delivery systems that are nano-based, it was possible to deliver PPP's and nutrients in slow or controlled release manner [24,25].

Traditional fertilization methods are outdated and are replaced by nano-fertilizers for release of specific nutrients into the soil progressively in a controlled manner. The use of nano-fertilizers may prevent the soil from getting polluted and from excessive eutrophication [26,27]. Nanofertilizers are designed in such a way that they regulate the requirement-based release of nutrients of the crops, so they are more efficient than usual fertilizers [28]. They are being used to lessen the nitrogen loss caused by emissions, leaching, and continuous amalgamation by soil microorganisms. They allow careful release of nutrients associated with time and environmental circumstance [29].

Nanonat and ferbanat liquid fertilizer are the examples of nanotechnology-based fertilizers. Ferbanat is a new generation biostimulator that is used against soil microorganisms and stress and it contains natural elements. It contains vitamins, amino acids, micro elements, micro humates, natural biological substances and soil microfloras. Furthermore, for agricultural products, nanonat has been described as a source of minerals and vitamins to make chemical dressing by using 30–50% with biological nitrogen, calcium, potassium, magnesium, phosphorus, and the elements in it [30].

Fertilizers that have a slow-release property are the outstanding substitutes of the soluble fertilizers. The whole time during the growth of crop, plants are capable to uptake majority of nutrients with no wastage by the process of leaching. Nutrients slow release in the environment is attained by the use of a group of naturally occurring zeolites which works as a pool for those nutrients that are released in controlled manner on demand [31]. To facilitate the release of nutrients at a slow and stable rate, the particles of fertilizer are coated by nanomembranes [28]. A nano-composite comprising P, K, N, amino acids, mannose, micronutrients has been patented that boost the uptake and consumption of nutrients by grain crops [32].

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Agro-chemicals that are nanoformulated are being used throughout the primary production to enhance the efficiency of the agro-chemicals in comparison to conventional formulations as shown in **Fig. 2.** A number of pesticides that contain nanoparticles or nanotechnology based agro-chemicals are present in the market. Nanoparticles containing micronutrients can be used to increase the yield of crops [33]. Prasad et al. (2012) compared and suggested that zinc oxide nanoparticles improved stem, root growth and pod productivity than $ZnSO_4$ application [34]. Laware and Raskar, (2014) reported that ZnO-NP applied on onion crop has shown efficient growth than control [35]. The nanofertilizers can play a significant role in reclaiming soils by reducing use of chemical fertilizers that are damaging soil structure, plants, nutrient cycles, and even more damage to food chains causing heritable mutations.

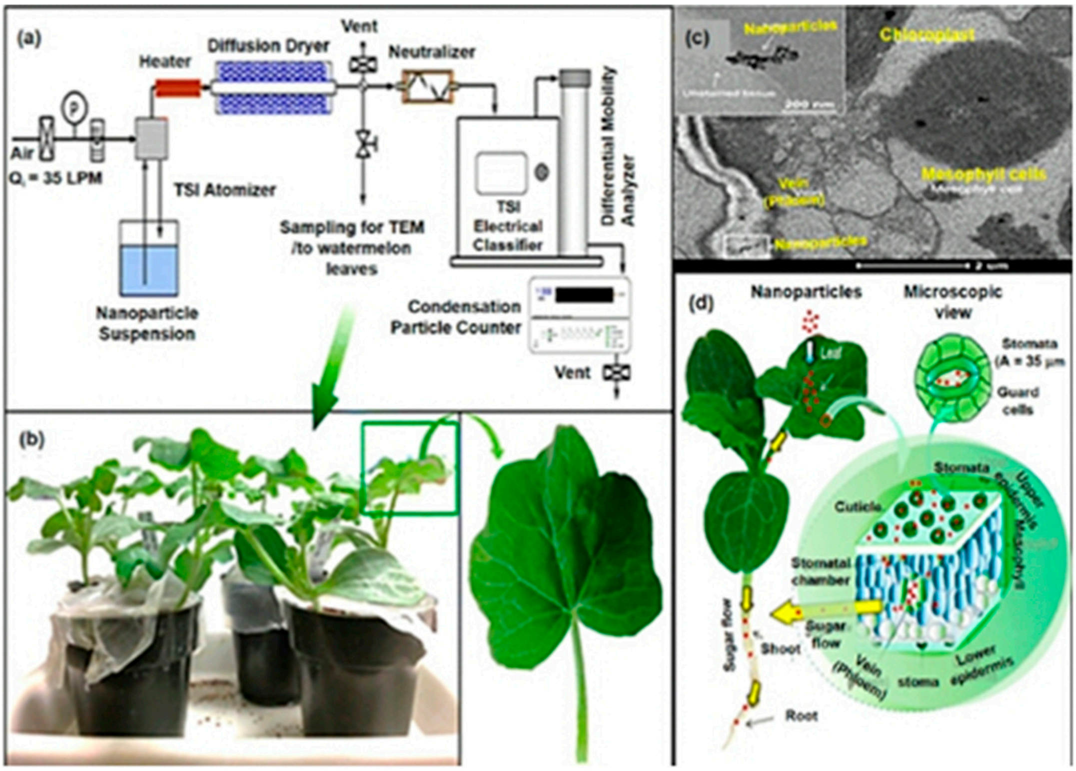

Fig.2. Aerosol-mediated nanoparticle delivery to a watermelon plant. (a) Schematic diagram of the experimental setup. (b) Watermelon plants used in this work. (inset b) Typical watermelon leaf. (c) TEM micrograph showing the presence of nanoparticles inside the leaf after applying nanoparticles for 3 days. (d) Schematic diagram of nanoparticle transport inside watermelon plants. The figure is adapted and modified with permission [36]. Copyright 2013 Springer.

4.3 Use of nanotechnology to increase water use efficiency

Cerium oxide is one of the most commonly used metallic nanoparticles. Some reports have also suggested increased yield in some crop species [37]. Water use efficiency (WUE) can be increased by using nanoparticles. Hydraulic conductivity of roots in corn seedlings was reduced dramatically by using TiO_2 nanoparticles. Singh *et al.* (2016) have found that carbon nanotubes improved the transpiration and uptake of water in plants [38]. Enhancing the key water channel proteins "Aquaporins" by using carbon nanotubes has improved the water uptake in tobacco root cell wall [39]. Cerium oxide nanoparticles improved the rubisco activity and net photosynthesis rate of soybean and also improved water use efficiency [40]. Wang et al. (2012) applied cerium oxide nanoparticles on tomato and found an increase in tomato (*Solanum lycopersicum*) yield [41]. In the same way, Wheat (*Triticum aestivum L.*) growth and yield was improved by application of CeO_2 nanoparticles [37]. Use of Gold nanoparticles showed strong adsorption capacity and increase WUE efficiently, also has reduced biosafety concerns [42]. The nanoparticles-based crops like cerium oxide,

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titanium oxide and silver have a great interest to be used in areas where there is scarcity of water like deserts, as these plant crops contain nanoparticles, use less water with great efficiency.

4.4 Monitoring of field conditions

Monitoring of field conditions in real time and growth of crop is another application of nanotechnology in which smart field sensing systems are being used to monitor temperature, light, nutritional status, moisture content, fertility of soil and presence of weeds, insects and various plant diseases [7]. Precision farming has been a goal for a long time to make the most of the crop yield and lessen the use of fertilizers, herbicides and pesticides via monitoring the variables of environment, decreasing the agricultural waste and consequently minimizing the environmental pollution using different nanosensor to increase the yield of crops as shown in **Fig 3.**

Organic farming has been a long-felt goal to enhance the crop production with a little input via monitoring environmental variables and applying targeted action. Organic farming uses remote sensors, GPS systems and computers for the measurement of highly contained environmental situations, consequently determining that if crops are growing at utmost competence or accurately spotting the location and nature of harms. With the use of centralized data to find out soil conditions and development of plant, the use of seeds, water and fertilizers can be adjusted to the lower cost of production and potentially increased crop yield that will prove to be beneficial to farmers [43]. Along with a lot of benefits which can be achieved using nanotechnology, there are biosafety concerns regarding use of nanoparticles in sensing system that needs solid evaluation of their reactivity, mobility, environmental toxicity, and stability. Some studies have been conducted to investigate unfriendly effects of nanoparticles on environment but there are no clear standards to determine their effects. The unskillful persons and lack of technical information about the method of choice has provided an appropriate clue for supporters and opponents of use of nanoparticles to present contradictory and ill-considered results. The uncertainty about use of nanoparticles posed serious concerns. So, adequate studies are necessary to identify the real risks associated with the use of nanotechnology-based sensing systems.

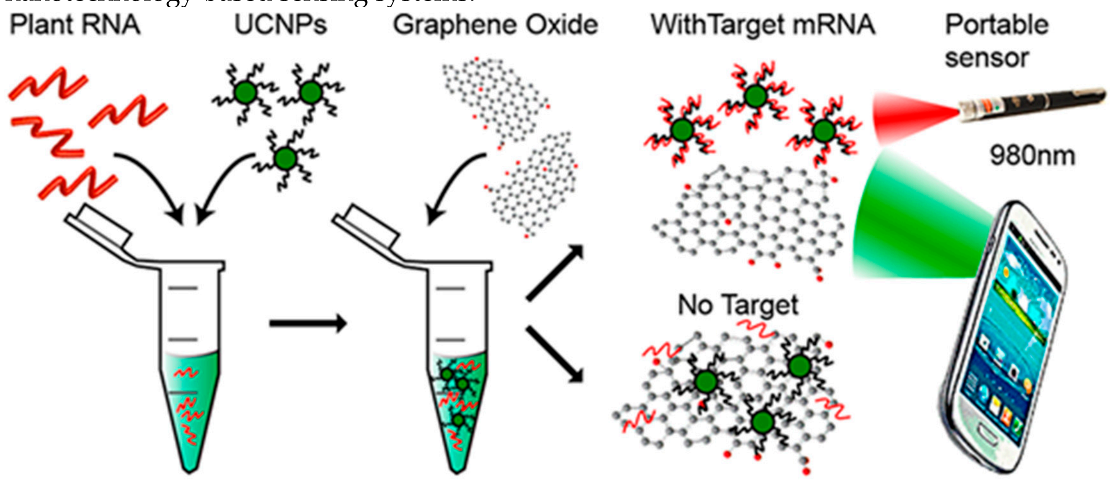

Fig.3 Schematic Illustration of the Detection of mRNA Biomarkers Related to the Nutrient Deficiency in Crops. Reprinted with permission [44]. Copyright 2018 American chemical society.

4.5 Nanoencapsulation

Nanoencapsulation is a technique which causes the release of insecticides like chemicals at a very efficient and slow rate to a specific host plant for the purpose of insect control. Nanoencapsulation with nanoparticles in the form of pesticides allows proper absorption of the chemicals into the plants [45]. Release mechanisms of nanoencapsulation include diffusion, dissolution, biodegradation and osmotic pressure with specific pH. Nanoencapsulation is currently the most promising technology for protection of host plants against insect pests. Now, most leading chemical companies are emphasizing on the formulation of nanoscale pesticides for delivery into the target host tissue through nanoencapsulation [46]. Nanoemusion can increase the availability of nutrients for crops as shown in **Fig 4**.

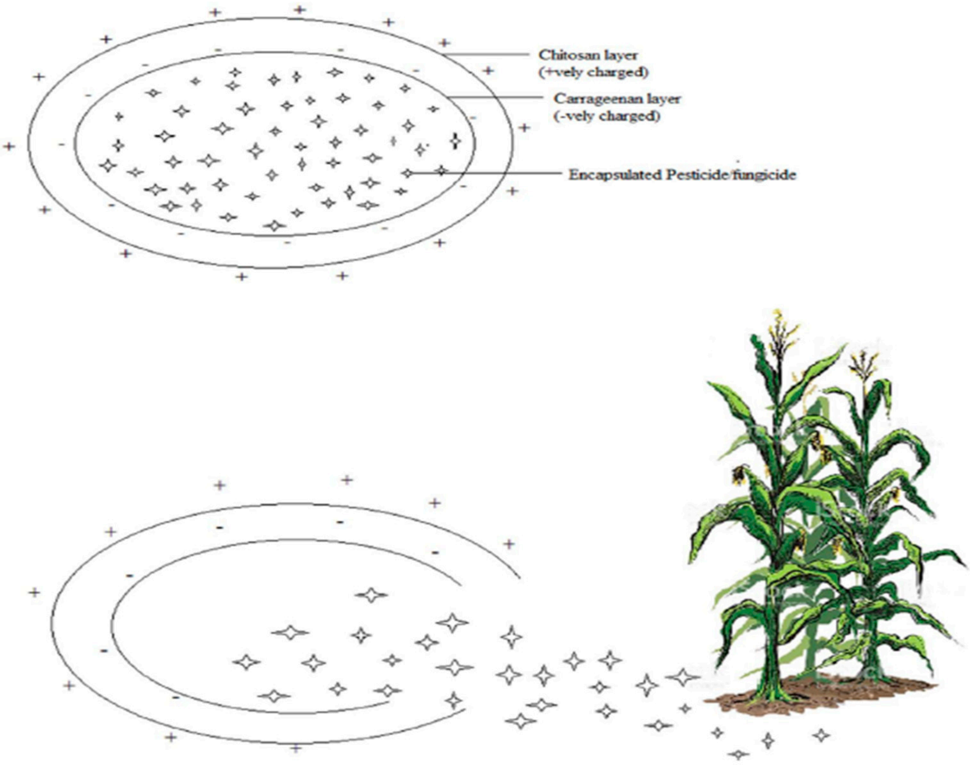

Fig. 4. Controlled release of pesticides/fungicides/nutrients from nanocoating. Reprinted with permission [47]. Copyright 2017 Elsevier.

4.6 Genome editing reagents with Nanoparticle coatings

Genome engineering has now become a very powerful genetic tool for modifying genomes to study gene function and to correct and introduce gene. In recent years, various types of reagents have been developed that can generate double strand breaks and can be repaired by non-homologous end joining or homologous recombination based double strand break repair pathway provided a suitable template is available [48].

A long-standing goal of biomedical researchers is the development of efficient and reliable ways to make precise, targeted changes to the genome of living cells. Recently, a new tool CRISPR/Cas9 has been developed from Streptococcus pyogenes which has generated considerable excitement in the history of genome editing [49]. Several attempts have been made and it took about years to manipulate the gene function which includes homologous recombination and RNAi [50]. RNAi has also become a powerful tool that enables inexpensive and high throughput cross-examination of genes, but the only limitation, it hampers the gene function temporarily and there are certain off target effects of this technology [51,52].

Viral vectors are very important for delivery of proteins. Engineering has shown that engineered gold nanoparticles can stably deliver the template DNA and Cas9 proteins *in vitro* and also in mouse model. This is an important step towards realizing non-viral CRISPR therapeutics [53]. Murthy *et al.* formulated the nanoparticle that contains a core of gold that was surrounded by CRISPR machinery components like Cas9, gRNA, template DNA and endosomal disrupting polymer for targeted editing of DNA (**Fig. 5**). They have demonstrated that cells uptake the nanoparticles along with CRISPR Cas9

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components via endocytosis, Cas9 repair the disease-causing mutation by replacing mutated gene by a process HDR [53].

Lee *et al.* (2017) reported, CRISPR vehicle composed of gold nanoparticle conjugated with donor DNA and cationic disruptive polymer can deliver Cas9 ribonucleoprotein (RNP) and donor DNA into a variety of cell types and efficiently correct the DNA mutations in mice [54]. It was also speculated that dystrophin deficient mice repair the mutated dystrophin gene when nanoparticles were injected intramuscularly and editing efficiency was found to be 5% at protein level comparing with wild type mice [55-57].

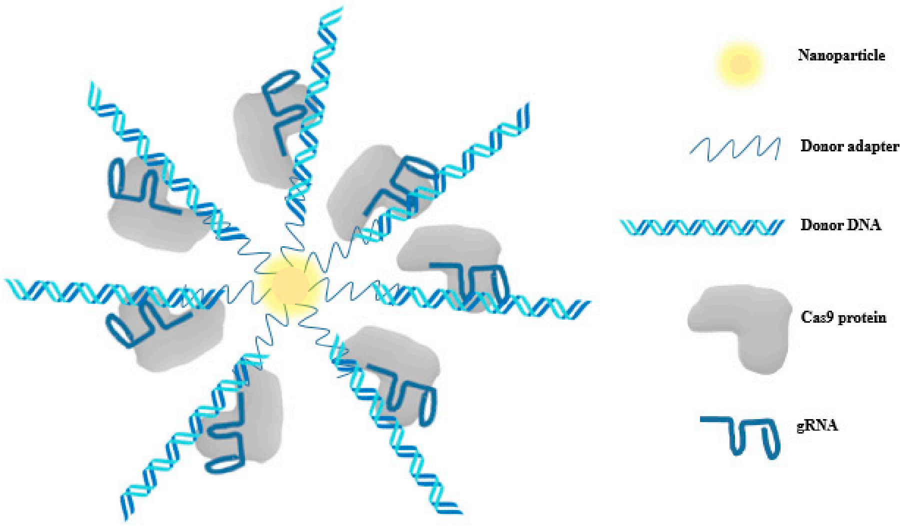

Fig. 5. Gold nanoparticle mediated delivery of CRISPR Cas9. Gold nanoparticle is surrounded with endosomal disrupting polymer, Cas9-gRNA complex and template donor DNA.

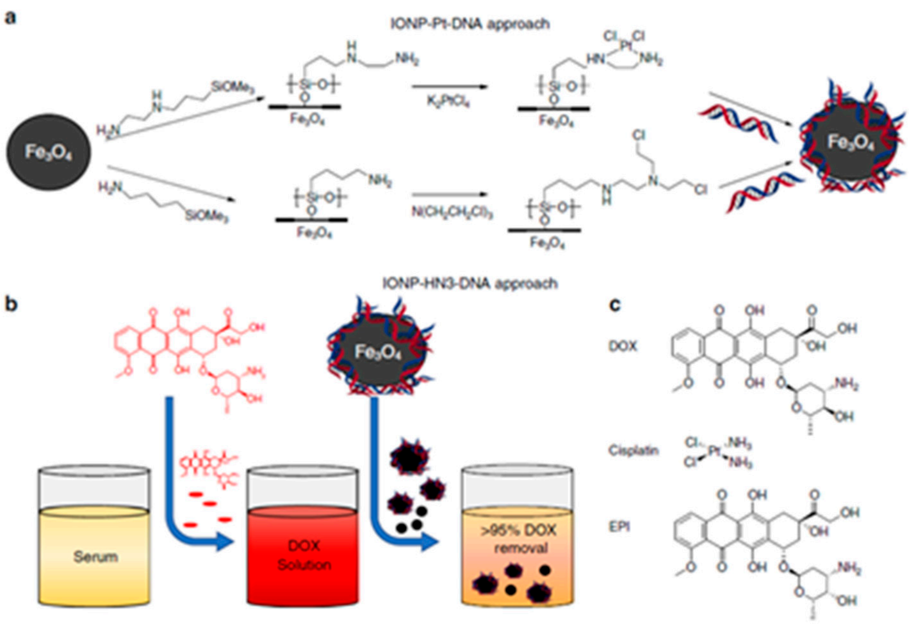

Fig. 6. Experimental approach. a Two synthetic approaches for covalently attaching genomic DNA onto iron-oxide nanoparticle (IONP) surfaces. b Drug capture concept. c Three common chemotherapy agents used in this study. Reprinted with permission [58]. Copyright 2018 Nature.

4.7 Nanopesticides

Pests have a pivotal role in decreasing the yield of crops in plants. Traditional methods to control the pests include the use of anti-pest agents in a bulk which results in increasing the cost of crop production. Large quantity of these pesticides is also a source of water and environmental pollution. The pesticides should be used in small amount so that environment could be saved and cost of crop production could be reduced [59]. Due to greater solubility, mobility and durability of nano-formulations may offer prodigious benefits. It can also provide the opportunity to reduce the active ingredient's amount to be used by the crops without their wastage. Nano-formulations based products use may release less harmful chemicals to non-target organisms. This was employed and utilized to reduce the development of resistance and protection against premature degradation of active ingredients [60-61]. Nanoparticle mediated delivery of DNA has its own applications in nanotechnology. The DNA and other desired chemicals can be delivered in tissues of plants to protect them from other insects. Liu et al. (2006) proposed about porous hollow silica nanoparticles (PHSNs) coated with validamycin can be used efficient delivery system for controlled release of water-soluble pesticides. Due to this behavior, it finds its importance in agriculture [27]. Insect, pests can also be controlled by using nano-silica and its mode of action is by physisorption. Nano-silica gets absorbed through cuticle of insects which leads to successive death of the insect in later stages [45]. A technological advanced and attractive tool for protection of environment and human health is the use of nano pesticides. The data stated in the earlier sections demonstrates that the use of conventional pesticides and nano pesticides are different from each other and hence there must be a need for redefined risk assessment approaches for nanopesticides.

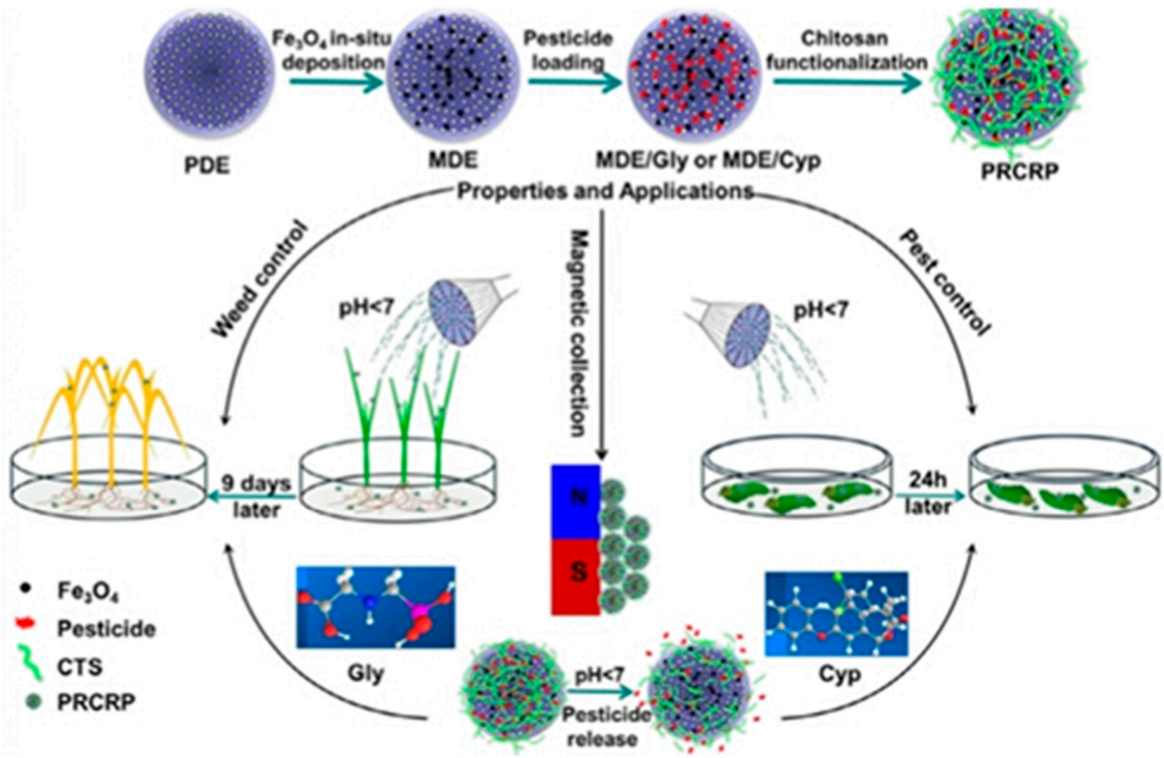

Fig. 7. Schematic diagram for the fabrication and application of PRCRP. Reprinted with permission [62]. Copyright 2017 Elsevier.

4.8 Nanobioremidiation

In many countries, among pesticides organochlorines (OC's) are restricted due to their adverse effects [63]. Food industries, waster effluents and agriculture are major producers of OC's. High concentration of OC's which cause environmental pollution by their waste discharged in water and this discharge in water is imperative due to high toxicity and potential of bioaccumulation [63]. Different types of nanomaterias are used now a day for advanced type of degradation of specific compounds. Nanoparticles like TiO_2 and Fe are considered excellent absorbents, and they are efficient in photocatalysis or degradation of OC's which open up new opportunities to explore other nanoparticles as well. These types of methodologies are economic, fast and very efficient [63]. Bioremediation by the use of nanoparticles is very important for slowly degradable compounds like pesticides. The harmful compounds present in pesticides may be degraded or converted into less harmful compounds. Otherwise, there is a concern if these harmful compounds enter the food chain can cause serious problems. Hence, nanotechnology can also be used for environmental protection of compounds [64]. Uranium remediation, groundwater remediation, hydrocarbon remediation, solid waste remediation, waste water remediation and heavy metal remediation are the major applications of nanotechnology. The important nanomaterials used innano bioremediation are nano sized dendrimers, nanoiron and its derivatives, carbon nanotubes, engineered nanoparticles single, enzyme nanoparticles etc. [65]. Nanoparticles are being used for the cleaning of soil and water. Nanoparticles such as aluminum oxide, nanoscale iron particles and lanthanum particles are used for the cleaning of soil and purification of water [66]. Nanotechnology based techniques for water treatment are supported by membrane filters that are originated from magnetic nanoparticles, nanoporous ceramics and carbon nanotubes instead of using UV light and chemicals are widespread in conventional water treatment [67]. Magnetic separations and the use of magnetic nanoparticles is now achievable at very low gradients of magnetic field. Nanocrystals, such as monodisperse magnetite (Fe_3O_4) like nanocrystals have an irreversible and strong relation with arsenic though keeping their magnetic properties. A simple magnet could be used to eliminate arsenic and nanocrystals from water. This type of treatment could be exploited for filtration process of irrigation water [68].

Zeolites are the silicates of crystalline aluminum that assist in retention and infiltration of water in soil because of their extremely porous properties and the capillary suction it applies. For non-wetting sands, it is an exceptional modification because of its performance as a natural wetting agent and also supports distribution of water through soils. In sandy soils, retention of water is significantly improved by this and porosity is also increased in clay soils. Improvement in water retention capability of soils results in an increased production of crops in areas that are affected by drought. Zeolite will additionally improve the ability of soil to hold nutrients and produce improved yield production by consequent application [43]. Due to its powerful potential, it is expected that their application will increase at a great leap in the near future, and it will play a critical role in sustainable development.

4.9 Limiting growth and development of microorganisms in plants

Botrytis cinerea is a significant post-harvest pathogen which is a source of gray mold disease. It destroys a large quantity of fruit and vegetables of great economic importance during their growth season and post-harvest storage. During storage, it is crucial to overcome this disease because of its development at low temperatures like -0.5 °C and quickly spreads among vegetables and fruit [72]. It has been observed that the growth of *Botrytis cinerea* could directly be inhibited by Chitosan of varying molecular weight in in-vivo and in-vitro assays. Resistance of tomato fruit against gray mold disease is enhanced by Chitosan and is capable of being used as an alternative for the synthetic fungicides in vegetable and fruits up to some extent as a natural compound. If nano-ZnO, nano-$CaCO_3$ and nano-silicon is used along with Chitosan film, then the decay incidence, red index, respiration rate and weight loss of coated vegetables and fruit could be decreased [73]. An analysis technique named as reflective interferometry is produced, by use of nanotechnology that offers a

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label free, rapid and biomolecules optical detection in compound mixtures. This new technique provides quality assurance of food via detecting *Escherichia coli* in a food sample by light scattering detection in mitochondrial cell. The working principle of this sensor is a protein of a distinguished and known bacterium placed on a chip of silicon can attach with any other *E. coli* that exists in food sample. This attachment will lead to a nanosized light scattering which could be detected via digital image analysis [74].

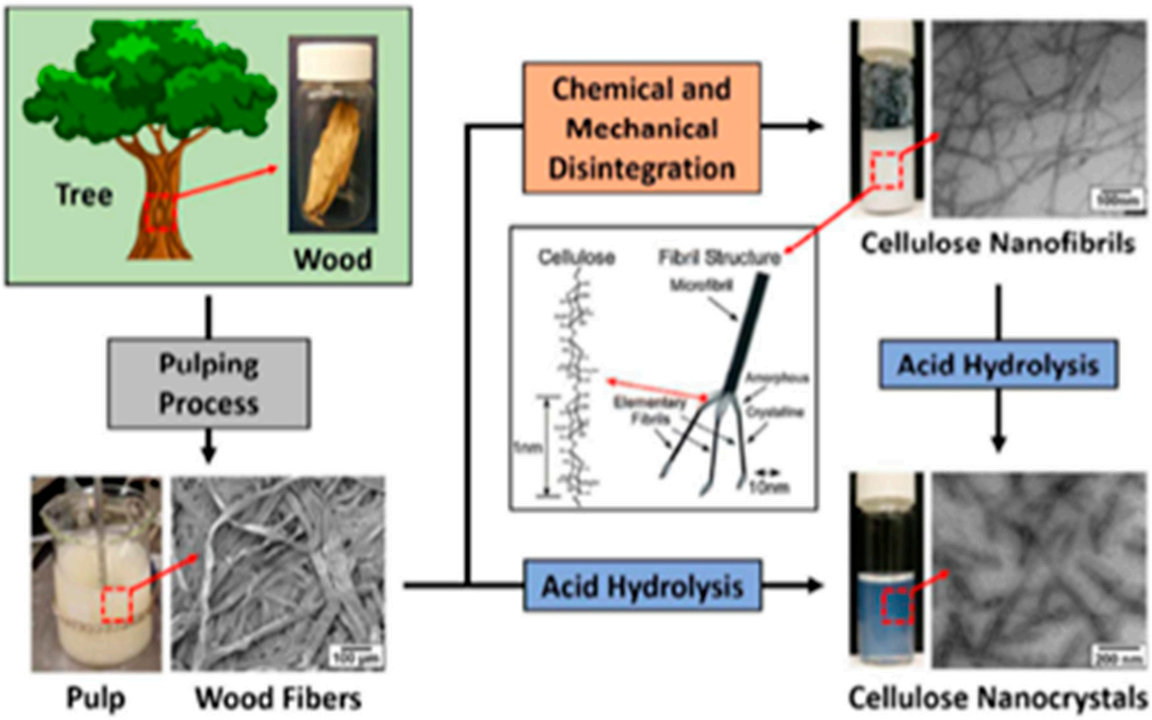

Fig. 8. Schematic diagram showing the process of extracting CNs from trees along with micrographs of the wood fibers during the various stages of the extraction process. (Sections of this schematic diagram are reproduced with permission [69] © 2011 IOP Publishing, [70] © 2011 The Royal Society of Chemistry, [71] © 2007 American Chemical Society)

5. Applications of Nanotechnology in Food Sector

Similarly, nanotechnology has possible applications in agriculture as well as in food industry. Technological improvements lead us to develop new tools for detection of pathogens, delivery systems for treatment of diseases, food packaging and targeted delivery of bioactive compounds. Moreover, application of these new tools related to nanotechnology in food systems has provides us with new methods of environmental safety and to enhance the nutritional value of food products [76]. Applications of nanotechnology in agriculture and food sector is given in the **Figure 2**.
Native β-lactoglobulin is a food protein that is about 3.6 nm in length and it goes through denaturation biofactors like pressure, pH and heat and the resembling denatured components join to form larger structures, like aggregates and fibrils. These large structures can be joined together to make large networks of gel e.g. yogurt [77]. Casein micelles are being used as nanovehicles that entrap, protect and deliver sensitive hydrophobic nutraceuticals present inside many food products. Food system is modernized by nanotechnology and it is influencing the food science in a positive manner as it is generating innovative texture, process ability, taste and stability of food. Nano derived assemblies are being used as a tool to deliver bioactive nutrients for better bioavailability [78].

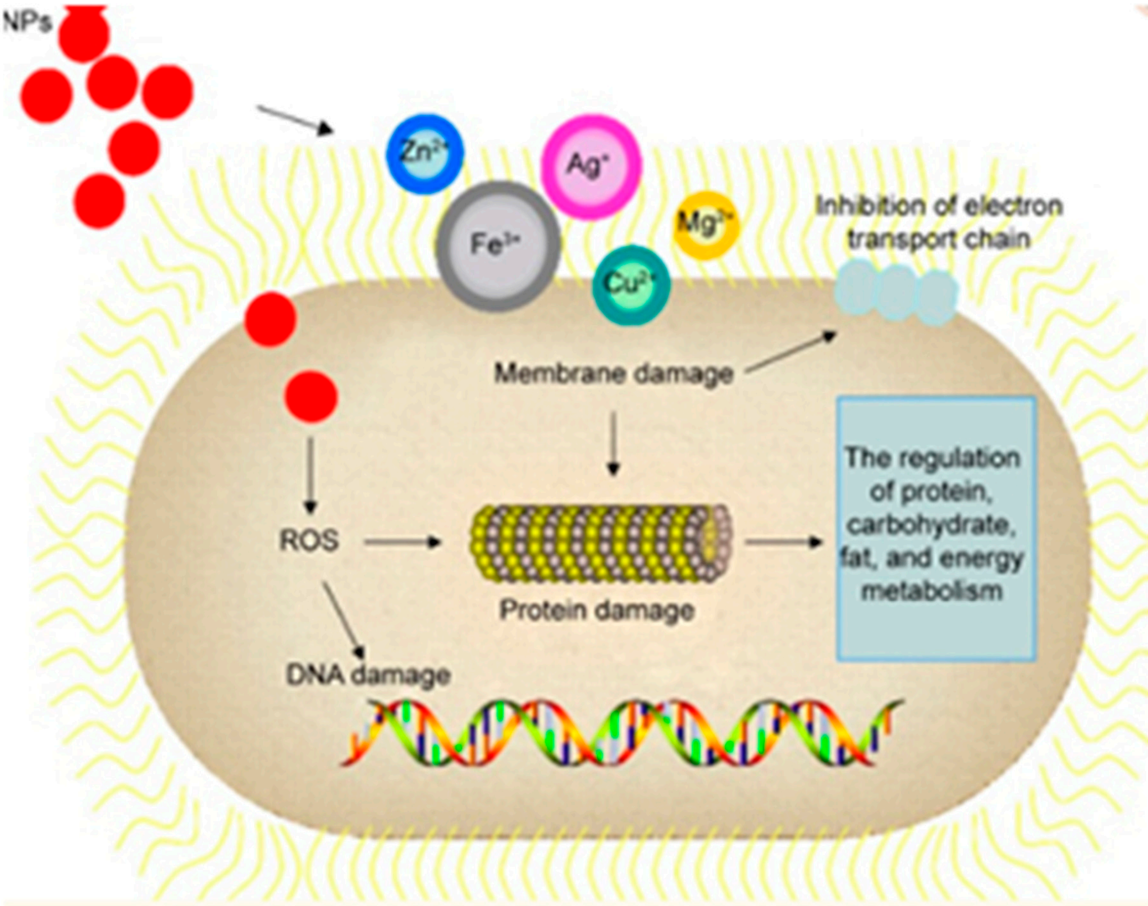

Fig. 9. Mechanisms of NP action in bacteria cells. Reprinted with permission [75]. Copyright 2017 Dove press

5.1 Nanotechnology and Food Processing

Nanotechnology is serving as a platform for the development of interactive and functional foods that respond according the requirements of body as they have the potential to deliver nutrients more effectively. Various nanotechnology-based approaches are being used to produce "on demand" foods that will remain dormant inside the body and nutrients will be delivered to cells when required. The main element in processing of food is adding up nanoparticles to already present foods to enhance the nutrient absorption. Microcapsules are designed in such a way that they break open only after they have reached the stomach, so the repulsive taste of fish oil incorporated inside them could be avoided [80].

The Oil Fresh Corporation of United States has developed a nanoceramic product that has reduced the use of oil in fast food shops and restaurants by half because it has a large surface area [81]. Nanotechnology based spray is available in which Nanodroplets ranging in size up to 87 nm are used to increase the uptake of supplements like vitamin B12 [82]. Nanoporous media, magnetic nanoparticles, carbon nanotubes and nanofibers are being used for immobilization of enzymes [83]. For hydrolysis of soybean oil immobilization of lipase on magnetic nanoparticles and nanofibers has been reported [84,85].

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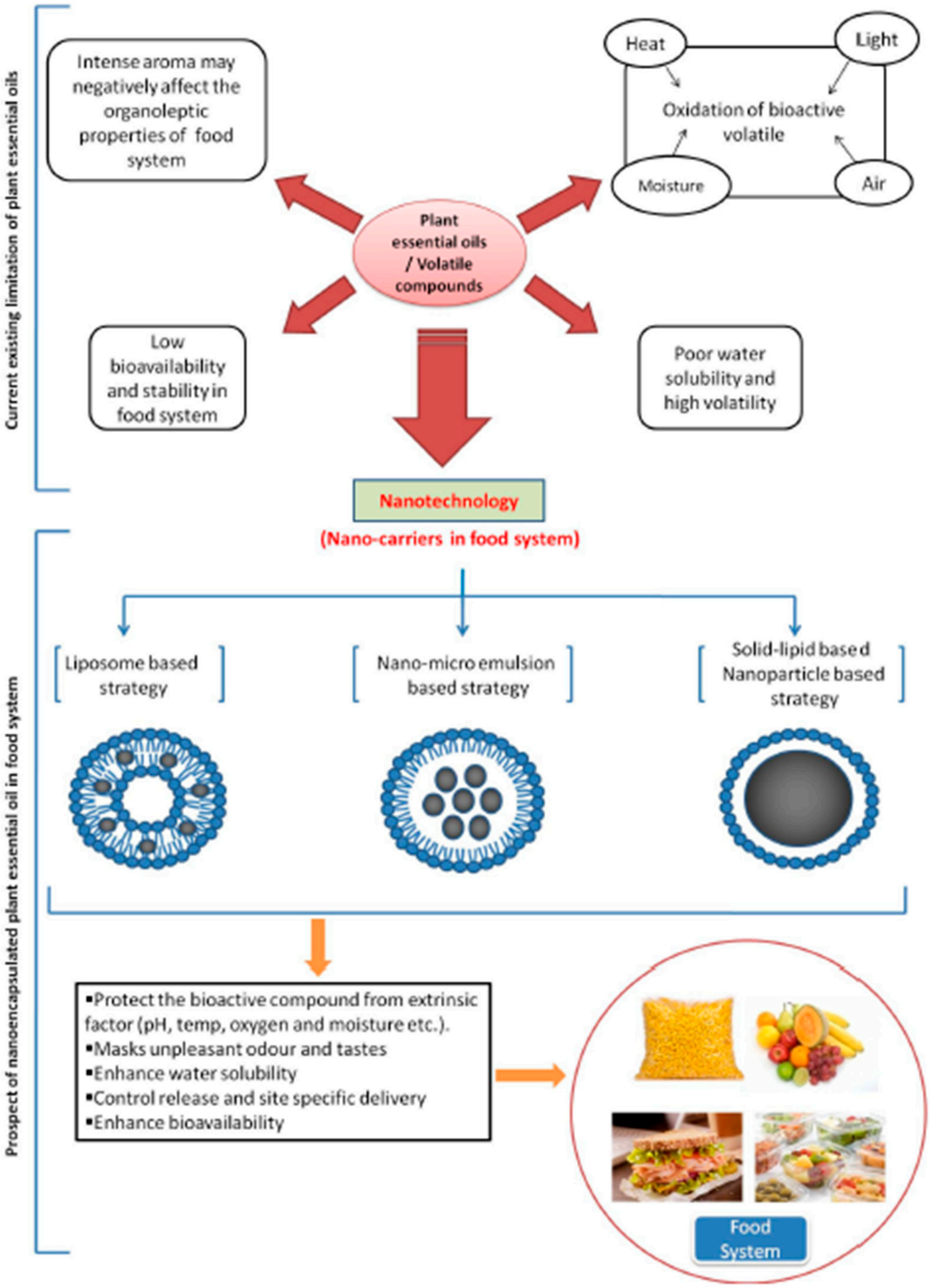

Fig. 10. Limitation of plant essential oils as food preservative sand prospect of nanotechnology for the development of carrier agents to enhance the practical applicability of essential oils in food system. Reprinted with permission [79]. Copyright 2018 Eleviser.

Active ingredients that are nano encapsulated such as fatty acids and vitamins are commercially available to be used in food processing and preservation of meat, beverages and cheese etc. Like in case of industrial cured meat and sausage production, several additives are needed to accelerate the production, improve taste and stabilize color. A German company named Aquanova, has launched a nanotechnology-based product of size 30 nm to encapsulate fatty acids and vitamins like C & E to be used as preservatives and marketed with a brand name of "NovaSol". NovaSol offers faster processing of meat, cheaper ingredients and higher stability of color claimed by German industry “Fleischwirtschaft" [10].

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The stupendous and profound applications of nanotechnology in food sector play its role in increasing the nutritional value, smart sensing and there must be the need for safer techniques and risks management.

5.2 Smart food packaging via nanotechnology

Customers demand that the food should be fresh for a longtime, and the materials for packaging should be safe, healthy and easy to handle. Developing and determining the effective material for packaging is a main issue in food science. Using a huge amount of silicate nanoparticles, a hybrid system has been developed that is more airtight plastic packaging to keep food fresh for a longer time than plastics [86].
Using natural biopolymers like protein and starch, bio-degradable nanocomposites has been developed that act as better food packaging material because they offer improved organoleptic properties such as odor, flavor and appearance [87]. Silicate nanoparticles and nano silver, magnesium and zinc-oxide are used to enhance the ability of packaging materials to act as barriers. When nanoparticles are used in the packaging material of food then direct contact with food is feasible only by nanoparticle migration. Nanoparticle migration from metals like clay nanocomposite films used in smart packaging of materials for gas barrier attributes to vegetable samples [88].
The perfect solution for plastic beer bottles is the use of nanocomposites, as the use of plastic for this purpose resulted in flavor problems and spoilage. Microgravure is a process launched by a Japanese company Nano Material Inc., important in coating plastic films with nanocomposite barrier material and it can perform as a better substitute to alumina and silica coated packaging films for foods. These nanocomposites made possible the use of biodegradable and edible films. It helps to decrease the packaging waste related to the processed food and also supports preservation of food by increasing their shelf life [89].
Significant factors like gases, moisture and accumulation of lipids cause rotting of food. For the protection of food from such agents nano-lamination is done. Food is nano-laminated by coating them with nano-laminates or the food surface is simply sprayed by nano-laminating agents. Besides preserving the food, the texture, color and flavor of food can also be improved [90]. Harmless nano-laminates and thin protective films are produced from edible proteins, lipids and polysaccharides [91]. They have been proved to be a good barrier against carbon dioxide and oxygen. Lipid based nano-laminates are more effective for protection against moisture. Another development in this area is development of nanoparticles coatingsto protect fruits from shrinkage and weight loss [92].
Nanocomposites which can be degraded biologically are of great importance to environment. By using smart packaging, one may choose the product of choice that has increased shelf life which is indicated by nature of food. Consequently, smart sensors are very fruitful for the consumers providing better quality food identification and for producers that allows fast distribution and further authentication of food products.

5.3 Nanoencapsulation of probiotics

Nanoencapsulation is used for the development of designer probiotics that are bacterial preparations desired to be delivered to specific parts of the gastro-intestinal tract to interact with particular receptors. These Nano encapsulated probiotics behave as de novo vaccines, having the ability to modulate the immune responses [46]. Biopolymer assemblies that are stabilized by different types of non-covalent forces are showing considerable progress [46].
Drug administration by means of oral route is considered the most convenient means of delivery. The smart carrier systems have the ability to control or time release the bioactive compounds that leads to better dosage pattern and minimizing side effects. Nanocarriers or nano-encapsulation is advantageous and ruled over the conventional methods of drug delivery. Drug can be protected from degradation due to enzymes and their bioavailability can be increased by using nanocarriers.

Nanoencapsulation of certain prebiotics has opened new methods of oral drug delivery as they have natural ability in gastro intestinal (GI) tract to protect the encapsulated material.
A nanoparticle that resembles starch can stop lipids oxidation and consequently improves the o/w emulsions stability. Curcumin is a natural pigment that is responsible for yellow coloration of turmeric and its health benefits could be improved by encapsulation in Nano emulsions [93]. Nanoemulsions can also improve the oral bioavailability and stability of curcumin and epigallocatechingallate [93]. A stearin rich fraction of milk is used alone or with α-tocopherol to prepare o/w Na^{2+}-caseinate stabilized Nano emulsions. Due to the reduced size, nanoemulsions have replaced the conventional emulsions. Adsorption rate is increased due to the large surface area provided by reduced size of nanoemulsions and this is the basic principle in making the nano-emulsions more efficient.

Fig. 11. Synthesis of encapsulated urease protein to detect Urea. Reprinted with permission [94]. Copyright 2017 Elsevier

5.4 Nanotechnology and food safety

Food products can be kept fresh for a longer period of time by using nano-sensors, and they are used in food production and food packaging [31]. Nanotechnology has significantly increased the sensitivity of nano-sensors that even they are able to detect spoilage or changes in product quality with respect to time. To ensure food safety, scientists of Good Food Project have developed a portable nano-sensor which has the tendency to detect pathogens and toxins in food in real time [95].
Nano-sensors that can monitor grain quality have now been developed which detect the source and the type of spoilage and respond to various analytes and volatiles in food storage environment. Thousands of nanoparticles can be placed on a single sensor to correctly detect the harmful fungus and insects in stored grain. Nanosensors are of small size, lightweight and require low power and can be used into the crevices of grain, where the pests often hide in stored grain [96]. An electronic tongue for presence of spoilage in food packaging is developed which detect the gases released by spoiled food can be detected by an array of nanosensors strip which change the color giving a clear visible signal of whether the food is fresh or not [97]. So, the food industry has been revolutionized by nanotechnology as it by provides strong and high resistance packaging materials, more effective antimicrobial agents and efficient sensors to detect trace contaminants, microbes and gasses in packaged foods.

6. Nanotechnology improving animal production and health care

Livestock has a close relation with agriculture and plays an important role in human nutrition. Nanotechnology has a vital role in improving nutritional efficiency of feed, product quality and management of diseases and provides state of threat remedies for the challenges in these areas [98]. Nanotechnology has improved the profile of nutrients and also their efficiency in developing countries. The Best way to elevate protein synthesis is the supplementation of nutrients and utilization of minor nutrients in animals. Cellulosic enzymes can help in utilizing energy from the plant products. Numerous delivery systems are available like liposomes, biopolymeric nanoparticles, micelles, nano-emulsions, protein carbohydrate complexes, dendrimers, solid nano lipid particles which are efficient in nutrient supplementation. The above discussed systems are better suited to the environmental stresses and have high absorption, better solubility in food and feed and controlled release kinetics [78].

Nanotechnology has promising applications in anima1l production as it provides enhanced nutrition and effectiveness of animal feeds by fortifying it with nanosupplements, detoxifying nanomaterials, antimicrobial additives and Nano biosensors to diagnose animal disease. For example, an animal feed additive containing a natural biopolymer is obtained from yeast cell wall that binds mycotoxins to provide protection against mycotoxicosis in animals. A nano additive derived from nanoclay that binds aflatoxin is also used in animal feed [7]. By modifying animal feed, animal production could be improved and product quality and value is also improved that helps in food production resourced from animals like milk fatty acids, vaccinic acid, cis-9, trans-11 conjugated linoleic acid with suitable health recommendations. Atherogenesis and cancer in humans can be prevented by these types of products.

Delivery of nutrients on basis of nanotechnology helps to control the biosynthesis and concentration of conjugated linolenic acid (CLA) and vaccinic acid (VA) in milk fat of lactating ruminants efficiently. It also helps to examine the biological benefits of functional foods with high level of CLA/VA [99].

There are substantial losses due to various diseases in animal production. Many infectious diseases have emerged since past four decades and according to WHO, 75% of them are zoonotic. The two important tools for integrated animal disease management are detection and intervention to reduce/eradicate the disease. There are various techniques that have been offered by nanotechnology which are highly specific and sensitive in detection and diagnostics are time saving, robust, and are convenient to use and economical. Simple and quick remedies of certain problems are offered by use of nanotechnology. Nowadays, new drugs and vaccines are developed through nanotechnology which are cheaper and very efficient than conventional methods of drug manufacture [100].

Nanotechnology is also making progress in dairy industry as a protective coating named as “Nansulate”. It is launched by Industrial Nanotech company to provide protection against high temperature and corrosion to dairy processing equipment. An insulating oxide material is used by the company, and they claim that material is not good for the conduction of thermal energy in the world. Thermal energy conduction via this material is delayed by the minute contact area among the little particles connected in a 3-D network to make up the conduction path [101]. No doubt, intensive research is needed for nanotechnology as it has environmental concerns ahead, but there are prodigious benefits offered by nanotechnology in disease diagnosis, drug delivery and treatment.

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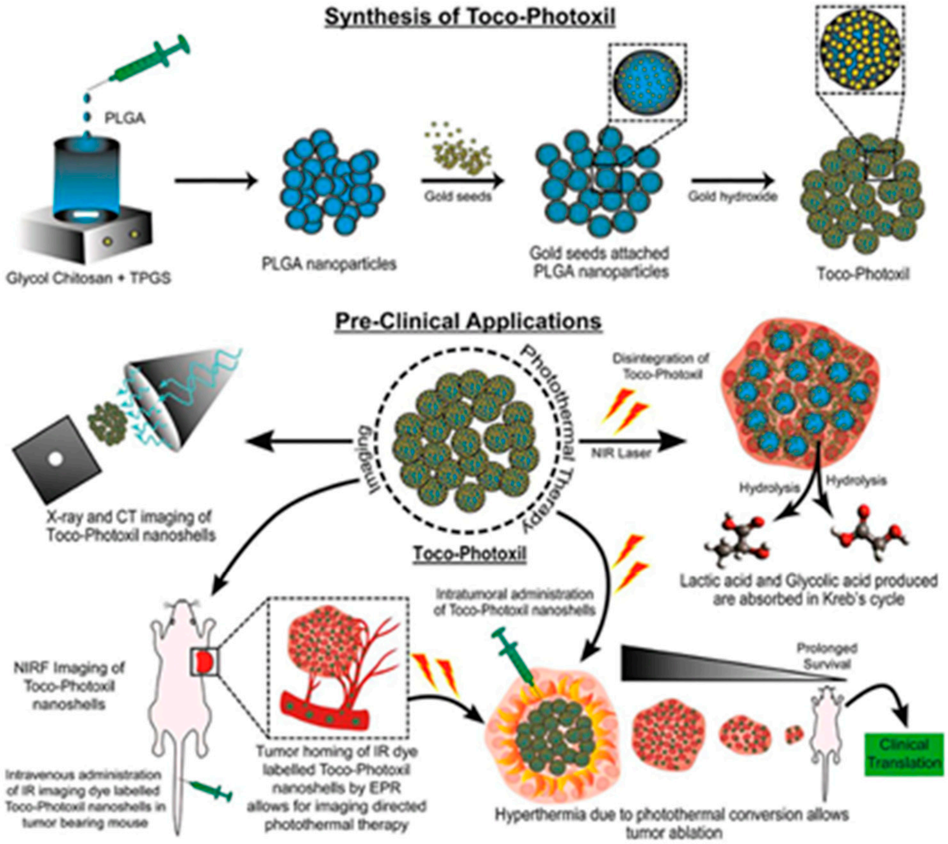

Fig.12. Illustration showing the synthesis and preclinical applications of Toco-Photoxil for imaging guided photo thermal therapy. Reprinted with permission [102]. Copyright 2018 Nature.

Nanotechnology: Pros and Cons

The use of nanoparticles depends upon many factors which should be considered before doing research/experiments *i.e.* size of particles, surface chemistry of the particles (positive, negative and neutral), pore size of the particle (mesoporous, semi porous, porous and solid), shape of the particles and the nature of the material (organic and inorganic). All these factors determine the high efficient delivery of antigen/DNA/drug/vaccine/protein/antibodies etc. to the target site [103]. The nanoparticles i.e. polymers, gelatin, chitosan, inulin, pullulan, dendrimers, dextran, starch, alginate, liposomes, self-assembled particles, VLPS and ISCOMs are highly biocompatible in nature as compare to the gold, silver, carbon, silica, calcium and titanium dioxide nanoparticles [104]. Nowadays nanoparticle has been used in most of the researches due to number of different benefits; 1) More availability of antigen/gene/protein/drug to the target site, 2)due to small size large surface area exposed and ultimately more reactivity and availability of the antigen to the target site, 3) Large amount of antigen/protein/ drug/ gene can be loaded to the nanoparticles, 4)more circulation time in blood stream, 5) less dose of antigen/ protein/ DNA required, 6) Easily pass through the membrane, 7) less toxicity, 8) metallic nanoparticles not only use for drug delivery but also useful for imaging of tumor cells, 9) sustain and control release to the target site, 10) we can use nanoparticles in all the administration routes [105].

As, it a thumb rule, anything which have some benefits also have some limitations, similarly, all the above mentioned in-organic nanoparticles are also biocompatible in nature but up to a certain limit if their concentration/ dose increase these may cause cellular toxicity [105]. 1. Whenever we inject nanoparticle into the cells, the cellular catalytic protein which keep balance in cellular activities will make corona formation especially by the opsonin protein and ultimately cause degradation of the nanoparticles by the phenomenon known as opsonization. So, the chance of targeted delivery will be reducing down. 2. There is another issue if we will use cationic nanoparticle as such these will bind to the surface of cell membrane and ultimately cause inflammation [105]. 3. Aggregation phenomenon may occur due to small size and large surface area it will cause difficulty in handling the samples in liquid form. 4. Due to their small size these will accumulate into the site where these will never pass or any part of living organism which allow only smaller particles than nanoparticles [105].

Conclusion

Nanotechnology excelling in almost every field of life thus it could be considered as the future of any nation. It has applications in food industry, pollution control, genetic engineering, water treatment, agriculture, medical sciences and many other grounds to help mankind by improving quality of life. World agriculture is already facing serious issues and increasing population and food demand has further intensified its problems. Modern agricultural practices have been improved by the use of nanotechnology by making them susceptible, technical and safer. Agriculture products are performing efficiently due to nanotechnology usage in packaging and storage conditions. Additionally, nanotechnology has been making the food products more nutritious and fresher with enhanced shelf life. Delivery of fertilizers has also been improved similarly as of pesticides, herbicides and plant growth regulators just because of nanotechnology. There are health safety concerns due to the possibility of nanomaterials accumulation in local food products. There are concerns raised to the use of nanopesticides on the biosafety and their impact on environment and possibly on humans, especially to those workers which are chronically exposed to nanotechnology. Nanoparticles has the tendency to penetrate human organ and organelles. So, their exposure concentrations, exposure time, sites of penetration, accumulation, immune response and retention in body substantial effects should be assessed carefully. Estimation level of nanoparticle release into the environment and their exposure levels must be evaluated for further discussion. There is a little bit known about toxicology of nanoparticles due to lack of validated test methods.

As there are many unpredicted risks of using this technology that may come through its productive potential thus, we should be very careful about its use. Standardized test procedures against nanoparticles are obligatory for evaluation of the risk assessment on human exposure and their effects on living cells. It is very important to create a fully trained workforce for research and development in this area. To create a fully trained workforce, first step is making the public well aware about the importance of nanotechnology and its potential benefits. The future of nanotechnology is still uncertain because of adverse effects of nanoparticles on the environment and human health. There is a need to reduce the limitations that nanotechnology is facing in the course of its development for provision of desirable and healthier future. This could be achieved by the collaboration between international organizations of developing and developed countries.

Chemical synthesis of nanomaterials is considered toxic for nature or environment. To overcome this, from plant systems, nanomaterials are being synthesized and it is termed as green nanotechnology. Green nanotechnology is energy efficient and safe process and nanomaterials synthesized by it are eco-friendly sustainable. Hence, there is more shift in a faster rate towards the green nanotechnology. Still, there are concerns at its peak, what are the consequences of green nanotechnology on environment in near future. The potential risks associated with green nanotechnology must be considered and mitigated with this emerging field of agriculture.

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