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REVIEW ARTICLE

NANOTECHNOLOGY IN AGRICULTURAL DISEASES AND FOOD SAFETY

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SUMMARY

Nanotechnology can be used for combating the plant diseases either by controlled delivery of functional molecules or as diagnostic tool for disease detection. Nanotechnology, nano particles and quantum dots (QD) have emerged as pivotal tool for detection of a particular biological marker with extreme accuracy. The possibilities in future as well as some success that have been achieved so far are discussed in this review. Diagnosis of a disease in its very early stage can play important role in treatment.
Due to phenomenal advancement in nanotechnology, QDs have emerged as pivotal tool for detection of a particular biological marker with extreme accuracy. QDS being very photo-stable and optically sensitive can be used as labeling and can be easily traced with ordinary equipment. Early detection of tumor markers using quantum dots is proved to be boon for cancer diagnosis. Use of QDs has also helped in unlocking complex neurological phenomenon, such as molecular activities at synapse during neurotransmission. QDs also give important information about receptor movement if tagged with suitable antibodies. In short, optical stability and easy to handle properties have made QDs to remain at the apex of medical diagnostics.

Key words: Nanotechnology, Nanoparticles, Quantum dots, Nano-carbon, Carbon Nanotubes, Nano silver, Nano Alumino-silicate, Nano-sensors, Nano-emulsion

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1. Introduction

Whenever a new technology has emerged, it has opened many vistas to be explored. The *new* Nanotechnology with materials having unique properties than their macroscopic or bulk counter parts, has promised applications in various fields.

The essence of Nanotechnology is the ability to work at the molecular level, atom by atom, to create large structures with fundamentally new molecular organization. The aim is to exploit these properties by gaining control of structures and devices at atomic, molecular, and supramolecular levels and to learn to efficiently manufacture and use these devices.

Nanotechnology has provided new solutions to problems in plants and food science (post-harvest products) and offers new approaches to the rational selection of raw materials, or the processing of such materials to enhance the quality of plant products.

"Nanotechnology", is a brain child of late Nobel laureate Richard Feynman. Under the guidance of Nanoscience and nanotechnology, ultrasensitive bio-labels can be designed which can detect zeptomolar (10^{-21}M) quantities of proteins in samples! The heart of nanotechnology lies in the ability to compress the tools and devices to the nanometer range, and to accumulate atoms and molecules in to bulkier structures while the size remains very small.

However, it must be said here that Phytopthologists took comparatively longer time in utilizing the uniqueness of nano materials for their benefit. This review deals with the rather meager efforts in utilizing the application of Nanotechnology in controlling plant diseases. The efforts are directed towards applying the disease control molecules, slow release of pesticides and developing diagnostic tools.

In this review we provide an overview of some current efforts in the area of nanotechnology as it applies to concerns of plants and some morphologically different structures and associated manufacturing technologies that could be used to build functional food systems. Efforts are on to produce following items in future i.e. interactive, edible nano wrappers to keep the pathogens away, targeted release of chemicals, packaging, extensive nano surveillance, interactive agrochemicals as herbicides and pesticides. Even though the agro-industry is just beginning to explore its applications, nanotechnology has exhibited great potential in areas like: release systems for pesticides or fertilizers in agriculture; antibacterial or easy-to-clean surfaces in food processing machines.

There is an ever increasing consumption and demand for food. In agriculture new molecular and cellular biology tools are expected to provide disease prevention and treatment in plants e.g. **Disease diagnosis. Screening and treatment,** in farming practices involving:

- Vector and pest detection and control.
- Disease monitoring
- Smart treatment delivery systems at a Nanoscale serve as carriers and provide on board chemical detection and decision taking ability for self regulation. These smart systems deliver precise quantities of drugs or nutrients or other agrochemicals required. These intelligent systems thus monitor and minimize pesticide and antibiotic use.

2. Nano-particles controlling the plant diseases

Some of the nano particles that have entered into the arena of controlling plant diseases are nano forms of carbon, silver, silica and alumino-silicates.

Nano Carbon

Many times a simple question takes us into ocean of chemistry, that's why carbon is chosen as brick molecule for simple as well as complex architectural designs of almost all molecules by nature? What is so special with carbon? Why only carbon and not silicon or other similar elements? Why carbon is so unique? Many scientists have come forward to explain carbon's uniqueness in a unique way. But the fact is that there are many concepts yet to be understood. At such a situation Nanotechnology has astonished scientific community, because at Nano-level material shows different properties. Thus we are exposed to a huge spectrum of Nanosciences, wherein there are totally new materials, new technologies and new hope for existing problems related to agrochemicals, pesticides, herbicides-regulation and smart utilization. Brazil is trying to improve the value of its exports by developing agricultural nanotechnologies. Brazilian agriculture research corporation's (Embrapa) areas of focus are to include research for producing carbon nano-fibers to strengthen natural fibers for example those from coconuts and sisal and making nano-particles that contain pesticides and control their release. Scientists are mostly concentrating on carbon nano tubes (CNT). Carbon nanotubes are allotropes of carbon whose nanostructure is cylindrical in shape. These nanotubes have many applications, especially in the fields of nanotechnology, electronics, and architecture. Often used as thermal conductors, these nanotubes also host unique electrical properties and are surprisingly strong.

Recently scientists (Khodakovsky et al, 2009) have reported that when they planted tomato seeds in a soil that contained carbon nanotubes; these CNTs could not only penetrate into the hard coat of germinating tomato seeds but also exerted growth-enhancing effect. They envisaged that the enhanced growth was due to increased water uptake caused by penetration of CNT. This could be a boon for using CNT as vehicle to deliver desired molecules into the seeds during germination that can protect them from the diseases. Since it is growth promoting, it will not have any toxic or inhibiting or adverse effect on the plant.

Nano Silver

is the most studied and utilized nano particle for Bio-system. It has long been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. Silver nanoparticles, which have high surface area and high fraction of surface atoms, have high antimicrobial effect as compared to the bulk silver. Kim et al (2008), studied the antifungal effectiveness of colloidal nano silver (1.5 nm average diameter) solution, against rose powdery mildew caused by *Sphaerotheca pannosa Var rosae.* It is a very wide spread and common disease of both green house and outdoor grown roses. It causes leaf distortion, leaf curling, early defoliation and reduced flowering. Double-capsulized nano silver was prepared by chemical reaction of silver ion with aid of physical method, reducing agent and stabilizers. They were highly stable and very well dispersive in aqueous solution. The nano silver colloidal solution of concentration of 5000 ppm was diluted in 10 ppm of 500 kg and sprayed at large area of 3306 m^2 polluted by rose powdery mildew. Two days after the spray more than 95% of rose powdery mildew faded out and did not recur for a week. Nano silver colloid is a well dispersed and stabilized silver nano particle solution and is more adhesive on bacteria and fungus, hence are better fungicide. No wonder that maximum patents are 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) 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 is now an accepted agrochemical replacement. It eliminates unwanted microorganisms in planter soils and hydroponics systems. It is being used as foliar spray to stop fungi, moulds, rot and several other plant diseases. Moreover, silver is an excellent plant-growth stimulator. There are literally thousands of other essential uses for this odorless, nearly tasteless and colorless, totally benign, powerful, non-toxic disinfectant and healing agent.

Nano Silica-Silver composite

Silicon (Si) is known to be absorbed into plants to increase disease resistance and stress resistance (Brecht et al., 2003; Ma et al., 2001). Aqueous silicate solution, used to treat plants, is reported to exhibit excellent preventive effects on pathogenic microorganisms causing powdery mildew or downy mildew in plants, Moreover, it promotes the physiological activity and growth of plants and induces disease and stress resistance in plants (Garver et al., 1998; Kanto et al., 2004). But, since silica has no direct disinfection effects on pathogenic microorganisms in plants, it does not exhibit any effect on established diseases. Further, the effects of silica significantly vary with the physiological environment and thus, they are not registered as an agricultural chemical.

As mentioned above Silver is known as a powerful disinfecting agent. It kills unicellular microorganisms by inactivating enzymes having metabolic functions in the microorganisms by oligodynamic action (Kim et al., 1998), and is known to exhibit superb inhibitory effects on algal growth also.

Silver in an ionic state exhibits high antimicrobial activity (Kim et al., 1998; O'Neill et al., 2003; Thomas and McCubin, 2003). However, ionic silver is unstable due to its high reactivity and thus gets easily oxidized or reduced into a metal depending on the surrounding media and it does not continuously exert antimicrobial activity. Silver in the form of a metal or oxide, is stable in the environment, but because of its low antimicrobial activity it is used in relatively increased amount, which is not very desirable.

A new composition of nano-sized Silica-Silver for control of various plant diseases has been developed by Park et al (2006), which consisted of nano-silver combined with silica molecules and water soluble polymer, prepared by exposing a solution including silver salt, silicate and water soluble polymer to radioactive rays. It showed antifungal activity and controlled powdery mildews of pumpkin at 0.3 ppm in both field and greenhouse tests. The pathogens disappeared from the infected leaves 3days after spray and the plants remained healthy thereafter.

Park et al (2006) also studied the 'effective concentration' of nanosized silica-silver on suppression of growth of many fungi; and found that, *Pythium ultimum, Magnaporthe grisea, Colletotrichum gloeosporioides, Botrytis cinere* and, *Rhyzoctonia solani*, showed 100% growth inhibition at 10 ppm of the nanosized silica-silver. Whereas, *Bacillus subtilis, Azotobacter chrococuum, Rhizobium tropici, Pseudomonas syringae* and *Xanthomonas compestris pv. Vesicatoria* showed 100% growth inhibition at 100 ppm. They have also reported chemical injuries caused by a higher concentration of nanosized silica-silver on cucumber and pansy plant, when they were sprayed with a high concentration of 3200 ppm.

Nano Alumino-Silicate

Leading chemical companies are now formulating efficient pesticides at nano scale. One of such effort is use of Alumino-Silicate nanotubes with active ingredients. The advantage is that Alumino-Silicate nanotubes sprayed on plant surfaces are easily picked-up in insect hairs. Insects actively groom and consume pesticide-filled nanotubes. They are biologically more active and relatively more environmentally-safe pesticides.

Mesoporous Silica Nanoparticles

Wang et al (2002) have shown that mesoporous Silica nano particles can deliver DNA and chemicals into Plants thus, creating a powerful new tool for targeted delivery into plant cells.

Lin's research group has developed porous, silica nanoparticles systems that are spherical in shape and the particles have arrays of independent porous channels. The channels form a honeycomb-like structure that can be filled with chemicals or molecules. These nanoparticles have a unique "capping" strategy that seals the chemical inside. They have also demonstrated that the caps can be chemically activated to pop open and release the cargo inside the cells where it is delivered. This unique feature provides total control for timing the delivery. Plant cells have rigid cell wall. Hence to penetrate it they had to modify the surface of the particle with a chemical coating. It has been successfully used to introduce DNA and chemicals in to Arabidopsis, tobacco and corn plants. The other advantage is that with the mesoporous nanoparticles, one can deliver two biogenic species at the same time.

3. Nanotechnology for detecting plant diseases

A need for detecting plant disease at an early stage so that tons of food can be protected from the possible outbreak; has tempted Nanotechnologists to look for a nano solution for protecting the food and agriculture from bacteria, fungus and viral agents.

A detection technique that takes less time and that can give results within a few hours, that is simple, portable and accurate and does not require any complicated technique for operation so that even a simple farmer can use the portable system. If an autonomous nano-sensors linked into a GPS system for real-time monitoring can be distributed throughout the field to monitor soil conditions and crop, it would be of great help. The union of biotechnology and nanotechnology in sensors will create equipment of increased sensitivity, allowing an earlier response to environmental changes and diseases.

Nanotechnology to the rescue

There is an urgent need of ultrasensitive diagnostic tool which can detect the molecular defects, be it at genomic or biochemical level, rapidly. Most of the existing diagnostic tools are incapable because of the lack of interaction between the desired molecule and the one which is sensing molecules of interest. This is due to comparatively bigger size of the sensor substances used in detection and quantification. In other words, there should be one-one interaction between the sensing molecule and biomolecule of concern like seeds or other planting material.

Biological systems are charismatically orchestrated with functional nanometric devices such as enzymes, motor proteins, and Nucleic acids, fabricated by intelligent assembly of macromolecules. These magical devices function with extreme accuracy in order to drive most perceptive bio-phenomena, such as protein folding, DNA replication, spatial and temporal expression of genes, cellular proliferation, and movement of the cell assisted by extracellular matrix are some among the never ending list of vital processes. Diagnosis of molecular signature markers of a particular disease often remains a daunting task mainly because the traceable stuffs are extremely low in concentration and also because of the unavailability of sensitive diagnostic tools capable of sensing such minute amount of virus and many fungal or bacterial infections.

Why metal nanoparticles?

Nanoparticles show sharp prejudice from their bulk in many respects which becomes bonus for developing diagnostic tools. Certain nanocrystals (crystalline nanoparticles) are attractive probes of biological markers because of:

- Small size (1-100nm)
- Large surface to volume ratio (aspect ratio)
- Chemically alterable physical properties
- Change in the chemical and physical properties with respect to size and shape
- Strong affinity to target particularly proteins (in case of gold nanoparticles)
- Structural sturdiness in spite of atomic granularity
- Enhanced or delayed particles aggregation depending on the type of the surface modification, enhanced photoemission, high electrical and heat conductivity and improved surface catalytic activity (Liu, 2006; Garg et.al., 2008; McNeil, 2005; Rosi and Mirkin, 2005; Shrestha et.al., 2006)

Possibilities with Quantum dots

QDs are few nm in diameter, roughly spherical (some QDs have rod like structures), fluorescent, crystalline particles of semiconductors whose excitons are confined in all the three spatial dimensions. Their potential application in diverse fields can be attributed to the property of quantum confinement**.** In 1998, the first use of QDs for biological detection and about its photochemical properties was reported (Bruchez et.al, 1998; Chan and Nie, 1998). Diagnostics using colloidal QDs has got tremendous hoist from this milestone finding. QDs are robust and very stable light emitters and can be broadly tuned through size variations. In the past two years, wide range of protocols for bio-conjugating QDs (Tran et.al. 2002; Wang et.al, 2002; Guo et.al, 2003) have been developed in diverse areas of applications: cell labeling (WU et.al, 2003), cell tracking (Parak et.al. 2002), in vivo imaging (Dubertret et.al, 2002), DNA detection (Taylor et al, 1984; Xu et.al, 2003).

Diagnosis of a disease in its very early stage can play important role in treatment. Due to phenomenal advancement in nanotechnology, QDs have emerged as pivotal tool for detection of a particular biological marker with extreme accuracy. QDs being very photo-stable and optically sensitive can be used as labeling and can be easily traced with ordinary equipment. Early detection of diseases using quantum dots is proved to be boon.

Nanoscale Biosensors

Involving biological molecules such as sugars or proteins as target-recognition groups could be used as biosensors on foods (Charych et. al., 1996) to detect pathogens and other contaminants. In food industry, nanosensors would provide increased security of manufacturing, processing, and shipping of food products through sensors for pathogen and contaminant detection.

Benefits of using nanosensors are small, portable, rapid response and processing (i.e., real-time), specific, quantitative, reliable, accurate, reproducible, robust and stable which can overcome the deficits of present sensors.

The NANO-SENSOR for Controlled Environmental Agriculture (CEA) that provide "scouting" capabilities could tremendously improve the grower's ability to determine the best time of harvest for the crop, the health of the crop and questions of food security such as microbial or chemical contamination of the crop. Today, application of agricultural fertilizers, pesticides, antibiotics, and nutrients is typically by spray or drench application to soil or plants, or through feed or injection systems to animals. Delivery of pesticides or medicines is either provided as "preventative" treatment, or is provided once the disease causing organism has multiplied and symptoms are evident in the plant. Nanoscale devices are envisioned that would have the capability to detect and treat an infection, nutrient deficiency, or other health problem, long before symptoms were evident at the macro-scale. This type of treatment could be targeted to the area affected.

Bio-analytical Nanosensors are utilized to detect and quantify minute amounts of contaminants like viruses bacteria, toxins bio-hazardous substances etc. in agriculture and food systems. These biosensors have huge impact on Precision Farming methods. Nanotechnology has made real time monitoring possible by planting autonomous biosensors which are linked into GPS system. These Nanosensors can monitor soil conditions and crop growth over vast areas. Such sensors have already been employed in US and Australia.

Bio-Nanotechnology has designed sensors which give increased sensitivity and allow earlier response to environmental changes ones they are incorporated in equipments. Efficient detection systems which monitor and detect pathogen invasion, infection, nutrition requirement and uptake and contamination have been developed. As the Nanosensors enable us to detect and eradicate infectious diseases in plants and animals before visible symptoms appear, the heavy economic losses that occur otherwise are reduced by many folds. Smart delivery systems help in controlled delivery of nutrients, pesticides, probiotics and neutraceuticals.

Nano sensors are already being used by the store keepers to identify food items which have passed their expiry dates and by Californian vine yards to control and monitor production of finer wines. Moreover, post harvest protection of agro-products is also the concern of Nanotechnologists

Utilization of Carbon Nano Material as a sensor

Carbon is already used as an electrode for electrochemical analysis. (Sharon and Sharon 2008) In our lab at nsnRc we are working on Selection of the Carbon Nano Material for electro chemical sensor by measuring the electro-chemical properties of various synthesized CNM from different raw materials in response to different pesticides. Residual pesticides in the plants are not only harmful to human consumers, but are also not very healthy for the plants. This will involve Preparation of electro-chemical cell having CNM as an electrode in this electro-chemical sensor, to assign appropriate oxidation / reduction potential values of particular analyte.

Nano-sensor devices that use Carbon Nano Tubes or Nano-cantilevers; are small enough to trap and measure individual proteins or small molecules. A contaminant can be detected by specially engineered Nanoparticles or Nano-surfaces which trigger an electrical or chemical signal. Some Nanosensors work by initiating enzymatic reactions or by using Nano-engineered branching molecules called dendrimers as probes to bind to target chemicals and proteins. Pathogen and contaminant detection is possible with increased sensitivity and decreased response time due to Nanosensors.

Saving post harvest plant products:

Antimicrobial packaging of edible food films made with cinnamon or oregano oil, or nanoparticles of zinc, calcium other materials that kill bacteria is being tried. Green packaging using nano-fibers made from lobster shells or organic corn (both are antimicrobial and biodegradable) is also a food safety effort. Improved food packaging needs packaging materials having strength, barrier properties and stability to heat and cold. These are being achieved using nano-composite materials. Bayer Polymers have produced a nanocomposite film 'Durethan', It is a film enriched with silicate nanoparticles which reduces the entrance of oxygen and other gases, and preserves moisture, thus preventing food from spoiling. In future, incorporation of silver, magnesium oxide or zinc oxide nanoparticles (which can kill harmful microorganisms) in food or beer packages will save the contamination. Antimicrobial activity can also be imparted through addition of nano-sensors to food packages is also anticipated in the future. McHugh has suggested that these nano-sensors could be used to detect chemicals, pathogens and toxins in foods. Radio Frequency Identification (RFID) tags could be incorporated into food packages in the future. These do not require line-of-sight for reading like bar-codes and enable registration of hundreds of tags in a second. Use of nanowheels, nanofibers and nanotubes are being tried to improve the qualities of food packages.

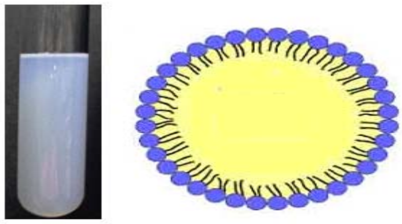
4. Nanostructures in Association-Colloidal forms for delivery of Functional Ingredients

Surfactant micelles, vesicles, bilayers, reverse micelles, and liquid crystals etc have been found to be ideal nanomaterials for nano-dispersions and nano-capsulation for delivery of functional ingredients. C*olloid* is a stable system of a substance containing small particles dispersed throughout in a liquid. Association colloids have been used for many years to deliver polar, non-polar, and amphiphilic functional ingredients (Golding and Sein, 2004; Garti et. al., 2004, 2005; Flanagan and Singh, 2006). Size of nano particles in colloids, range from 5 to 100 nm. The major disadvantage of colloids is that they can spontaneously dissociate if diluted.

Nano-emulsions

It is a mixture of two or more liquids (such as oil and water) that do not easily combine. In nano-emulsion, the diameters of the dispersed droplets are 500 nm or less. Nano-emulsions can encapsulate functional ingredients within their droplets, which can facilitate a reduction in chemical degradation (McClements and Decker, 2000).

(A) Nanoemulsion (B) Schematic representation of Nanoemulsion



Nanolamination

Technique is another viable option for protecting the food from moisture, lipids and gases. Moreover, they can improve the texture and preserve flavor as well as color of the food. Nanolaminates consist of two or more layers of nano-sized (1 – 100) thin food-grade films which are present on a wide variety of foods: fruits, vegetables, meats, chocolate, candies, baked goods, and French fries (Morillon, 2002; Cagriet. al., 2004; Cha and Chinnan, 2004; Rhim, 2004). Nanolaminates are prepared from edible polysaccharides, proteins, and lipids. Park (1999) has shown that polysaccharide- and protein-based nanolaminates are good barriers against oxygen and carbon dioxide, but poor in protecting against moisture. Whereas, lipid-based nanolaminates are good at protecting food from moisture. Trials are on to develop laminates that can protect against all the desired f actors. Coating foods with nanolaminates is done simply by spraying it on the food surface (McClements et. al., 2005).

5. Conclusion

With enhancing expertise to understand the atomic cross talk, scientists are developing new tools to formulate nanodevices capable of replacing many cellular types of machinery efficiently. Our inability to look at minute anatomical damages due to diseases and infection, such as loss of the receptor, vital proteins from cell membrane or a serious biochemical blunder in any apart of the plant will provide us a tangible to tackle molecular anomalies. This utmost need gave birth to use of principles involved in atomic interactions, nanometric devices. Nanorobotics devices marching in the body can give us abundant information for curing inimical physiological conditions such as nutrient deficiency. Thanks to the concerted efforts of scientists to bring this fiction into reality.

Nano particles for delivery of active ingredients or drug molecules will be at its helm in near future for therapy of all pathological sufferings of plants. There are myriad of nanomaterials including polymeric nanoparticles, iron oxide nanoparticles and gold nanoparticles which can be easily synthesized and exploited as pesticide or drug delivery piggybacks. The pharmacokinetic parameters of these nanoparticles may be altered according to size, shape, and surface functionalization. They can also be used to alter the kinetic profiles of drug release, leading to more sustained release of drugs with a reduced requirement for frequent dosing.

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