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Nanotechnology: Scope and Application in Plant Disease Management

Mujeebur Rahman Khan and Tanveer Fatima Rizvi

Department of Plant Protection, Aligarh Muslim University, Aligarh 202002, India

Abstract: Nanotechnology is one of the most fascinating and rapidly advancing sciences and possess potential to revolutionize many disciplines of science, technology, medicine and agriculture. Conversion of macromaterials in to nano size particles (1-100 nm) gives birth to new characteristics and the material behaves differently. Nanoparticles can be produced by different methods, chemical and biological, the former is commercially used. Nanomaterials can be potentially used in the crop protection, especially in the plant disease management. Nanoparticles may act upon pathogens in a way similar to chemical pesticides or the nanomaterials can be used as carrier of active ingredients of pesticides, host defence inducing chemicals, etc. to the target pathogens. Because of ultra small size, nanoparticles may hit/target virus particles and may open a new field of virus control in plants. The disease diagnosis, pathogen detection and residual analysis may become much more precise and quick with the use of nanosensors. The present paper critically analyzes the relevance, scope and application of nanotechnology in plant disease management in future crop production.

Key words: Nanoparticles, biosynthesis, nanopesticide, disease management

INTRODUCTION

The term nanotechnology was first coined by Taniguchi (1974) to the science that largely deals with synthesis and application of nano size particles (1-100 nm or 1.0×10^{-9} m) of any material (Fig. 1). When a material is reduced to nano size, it acts differently and expresses some new properties completely lacking in its macro scale form. The nanoparticles (NPs) have a high surface to volume ratio that increases their reactivity and possible biochemical activity (Dubchak *et al.*, 2010). For example, when 1 g gold is converted into nano scale, the particles may cover an area of 100 km². Gold nanoparticles (2.5 nm) melt at much lower temperatures (~300°C) than a gold slab (1064°C) (Buffat and Borel, 1976). Absorption of solar radiation is much higher in materials composed of nanoparticles than its thin film. The gold nanoparticles show toxic effect on bacteria, *Salmonella typhimurium*, in which the macro gold did not exhibit (Wang *et al.*, 2011). Similarly, the silver nanoparticles have anti-bacterial and anti-fungal properties while silver in macro form does not do this (Sofi *et al.*, 2012). Nanoparticles are of different shapes and many times smaller than bacterial cells (Fig. 1). The nanoparticle can not be imaged by the optical microscopes. The particle size is even smaller than a virus particle like influenza virus (80-120 nm diameter) and tomato mosaic virus (300 nm length and 10-18 nm diameter). The nanoparticles appear as point of scattering light under high resolution. The nanoparticles move

under Brownian motion and their speed varies strongly with the particle size. Nanoparticles may be spherical, polyhedral rod shaped, etc. (Fig. 2).

Nanotechnology has a wide scope of application in medicine, industry and agriculture and can revolutionize the entire society, if exploited properly. In agriculture, nanotechnology has potential scope for use in the natural resource exploitation and conservation, and production and protection of the crops and livestock. Nanomaterials may also provide solutions to technological and environmental challenges in the areas of solar energy conversion and water-treatment. Significance of nanotechnology has been realized internationally and several countries have set up especial committees/groups to support and monitor nano technological advancements and to harness its benefits for the mankind. The Indian Government has setup a Nano Research Project during the Eleventh Five Year Plan.

Nanotechnology has two major aspects, the first aspect is synthesis of nano-size materials and second, the use or application of nano materials for the desired objectives. The synthesis deals with the conversion of the matter of macro size into the particles of nano size, 1-100 nm. The synthesis of nanoparticles is not a simple process and requires specific skill and facilities depending on the method of synthesis. After the synthesis, characterisation of nanoparticles is another important step which ascertains attainment of the required particle size of the material and their relative uniformity.

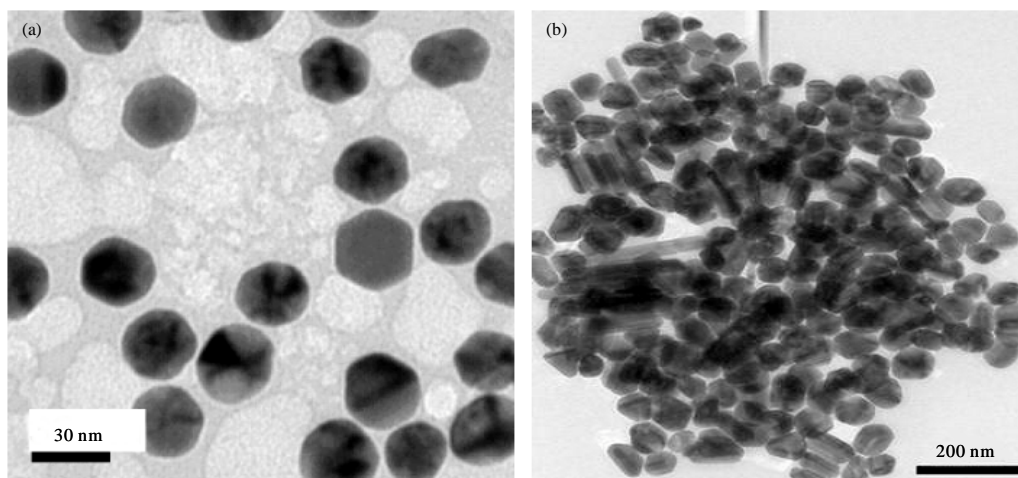


Fig. 1(a-b): Transmission electron micrograph of a collection of (a) Gold (Thompson, 2010) and (b) Silver nanoparticles (<http://pubs.rsc.org/en/content/articlehtml/2010/md/c0md00069h>)

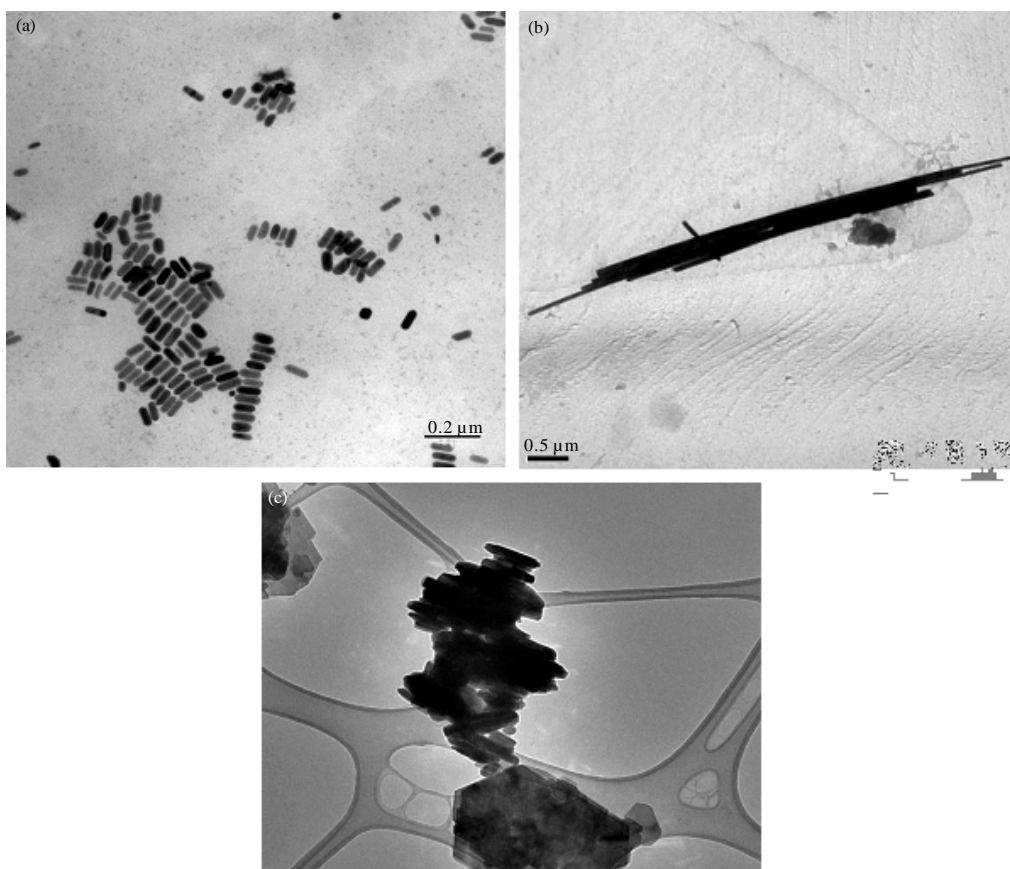


Fig. 2(a-c): Transmission electron micrograph image of typical (a) Nanorods, (b) Nanowires and (c) Nanoclays (EMRP, 2010)

Here, we summarize different methods of synthesis of nanoparticles to present an overall picture of nanotechnology followed by scope and application of nanomaterials in the plant disease management.

SYNTHESIS OF NANOPARTICLES

The nanoparticles are synthesised by chemical, physical and biological methods. The property of the nanoparticles and efficacy of synthesis vary with procedure of synthesis. The chemical methods have been found to synthesize the nanoparticles more efficiently than other methods.

Chemical synthesis of nanoparticles: The commercial synthesis of nanoparticles is largely done by chemical methods. There are different chemical methods to synthesize the nanoparticles, however, choice of the methods may vary with the material. Some of the important chemical methods are reduction method, colloidal method, sonochemical method etc., which are described here briefly.

Chemical reduction method: In 1857, Michael Faraday for the first time reported a systematic study of the synthesis of colloidal gold using chemical reduction route. The chemical reduction of copper salts is the easiest, simplest and the most commonly used method to synthesize copper nanoparticles. In fact, the production of nano sized metal copper particles with good control of morphologies and sizes can be achieved with the reduction method (Song *et al.*, 2004). In the chemical reduction methods, a copper salt is reduced by a reducing agent such as sodium borohydride (Aslam *et al.*, 2002), ascorbate (Wang *et al.*, 2006), polyol (Park *et al.*, 2007), isopropyl alcohol with Cetyl Trimethyl Ammonium Bromide (CTAB) (Athawale *et al.*, 2005) as well as glucose. Chemical reduction of copper salts using ascorbic acid (Vitamin C) is a new and green approach in which ascorbic acid is used both as the reduction and capping agent (Umer *et al.*, 2012).

Microemulsion/colloidal method: Hoar and Schulman (1943) observed that an appropriate amount of water, oil, surfactant and an alcohol or amine-based co-surfactant produced clear and homogeneous solutions called microemulsion. Microemulsion is a good technique for the synthesis of nanoparticles in which two immiscible fluids such as water in oil (W/O) or oil in water (O/W) or water in supercritical carbon dioxide (W/Sc. CO₂) become a thermodynamically stable dispersion with the aid of a surfactant. A typical emulsion is a single phase of three

components, water, oil and a surfactant (Chen *et al.*, 2006). Normally, oil and water are immiscible but with the addition of a surfactant, the oil and water become miscible because the surfactant is able to bridge the interfacial tension between the two fluids (Kitchens and Roberts, 2004). Microemulsion consists of surfactant aggregates that are in the ranges of 1-100 nm. The micro emulsion is said to be oil in water (O/W), if water is the bulk fluid and oil is in less quantity with small amounts of surfactant. Similarly, the system is said to be water in oil (W/O), if oil is the bulk fluid and water is present in less quantity. The product of oil in water and surfactant (O/W) is called micelles which is an aggregate form to reduce free energy (Umer *et al.*, 2012). Hydrophobic surfactants in nanoscale oil and micelles point towards the centre of aggregate, whereas the hydrophobic head groups toward water, the bulk solvent. The water in oil microemulsion carries oil or organic solvent as bulk. The system is thermodynamically stable and called reverse micelles (Dadgostar, 2008). Purely metallic nanoparticles (Cu, Ag, Co, Al), oxides (TiO₂, SiO₂), metal sulphides (CdS, ZnS) and various other nanomaterials are prepared using this technique (Cason *et al.*, 2001). The pioneering study by Pileni and Lisiecki (1993) demonstrated synthesis of copper and silver nanoparticles using reverse micelle system. The use of salts and the presence of anions within reverse micelles have been shown to alter the physical properties of the water environment and surfactant layer resulting in variations in the size and shape of the reverse micelles and metallic particles synthesized (Hassan *et al.*, 2002; Lisiecki *et al.*, 2000).

Sonochemical method: In the sonochemical process, powerful ultrasound radiations of 10-20 kHz are applied to molecules to enhance the chemical reaction (Suslick *et al.*, 1991). Acoustic cavitation is a physical phenomenon which is responsible for sonochemical reaction. This method, initially proposed for the synthesis of iron nanoparticles (Suslick *et al.*, 1996), is now a days, used to synthesize different metals and metal oxides (Pol *et al.*, 2003). The major advantages of the sonochemical method are its simplicity, operating conditions (ambient conditions) and easy control of the size of nanoparticles by using precursors with different concentrations in the solution (Vijayakumar *et al.*, 2000). Ultrasound power affects the occurring chemical changes due to the cavitation phenomena involving the formation, growth and collapse of bubbles in liquid (Vijayakumar *et al.*, 2000). The sonolysis technique involves passing sound waves of fixed frequency through a slurry or solution of carefully selected metal complex precursors. In a solvent with

vapour pressure of a certain threshold, the alternating waves of expansion and compression cause cavities to form, grow and implode (Pol *et al.*, 2003). Sonochemical reactions of volatile organometallics have been exploited as a general approach to synthesize various nanophase materials by changing the reaction medium. There are different theories on the mechanism of breakup of the chemical bond under 20 kHz ultrasonic radiations. According to one of these theories, bubble collapse occurs at very high temperatures (5000-25,000 K) during the sonochemical process (Gedanken, 2004). Upon collapse of the bubble which occurs in less than a nanosecond, the system undergoes a very high cooling rate (K sec^{-1}). The organization and crystallization of nanoparticles is hindered by this high cooling rate. The reaction occurs in a 200 nm ring surrounding the collapsing bubble, if the precursor is a nonvolatile compound. The temperature of the bulk is lower compared to the ring and temperature of collapsing bubble is higher than the temperature of the ring.

Microwave method: In this method, microwave radiations are introduced in the reaction solution. The microwave-assisted synthesis of copper nanoparticles has become popular due to its simplicity, ease of operation, rapid volumetric heating and kinetics, short reaction period and increasing yield of the products compared to the conventional heating methods (Komarneni, 2003). Microwaves are a form of electromagnetic energy with frequencies in the range of 300 MHz-300 GHz. The commonly used frequency is 2.456 GHz. Blosi *et al.* (2011) reported that microwave-assisted polyol synthesis of crystalline particles with radius ranging from 90-260 nm (diameter 45-130 nm). Very small size nanoparticles can be synthesized in cases where microwave is applied to the reaction solution. The main reasons for using microwave are the fast and homogeneous reaction conditions during the nanoparticle synthesis. Zhu *et al.* (2004) found a fast method for the production of copper nanoparticles by using copper sulphate as a precursor and sodium hypophosphite as the reducing agent in ethyl glycol under microwave irradiation. The size of copper nanoparticles prepared by this method was 10 nm.

Electrochemical method: In the electrochemical method, electricity is used as the driving or controlling force to produce nanoparticles of a metal. Electrochemical synthesis is achieved by passing an electric current between two electrodes separated by an electrolyte. As a

result, the synthesis of nanoparticles takes place at the electrode-electrolyte interface. For example, to synthesize copper nanoparticles, the electrolytic solution of copper salt and sulphuric acid are usually used. The main advantages of electrochemical techniques are avoidance of vacuum systems as used in the physical techniques, low costs, simple operation, high flexibility, easy availability of equipment and instruments, less contamination (pure product) and eco-friendly process. Raja *et al.* (2008) used copper sulphate and sulphuric acid as the electrolytic solution and supplied 4 V and 5 A for 30 min, the process yielded copper nanoparticles of 40-60 nm size.

Solvothermal decomposition: In the solvothermal processes, the chemical reaction takes place in a sealed vessel such as bomb or autoclave, where solvents are brought to temperatures well above their boiling points. When water is used as solvent, it is called a hydrothermal process. The hydrothermal process involves a heterogeneous chemical reaction in a closed system in the presence of aqueous or nonaqueous solvent above the room temperature and at a pressure >1 atm (Byrappa and Yoshimura, 2001). At 647.15°K and 221 bar, water is said to be a supercritical fluid. Supercritical water/fluid act both as a liquid and gas. A supercritical fluid decreases the surface tension at the interference of solid and dissolves the chemical compounds which are very difficult to be dissolved at NTP. Mostly, these techniques are employed to take advantage of the enhanced solubility and reactivity of metal.

There are many advantages of using supercritical conditions such as, simplicity, very low grain size, presence of a single phase and synthesis of high purity nano crystals with high crystallinity and eco-friendly nature (Byrappa, 2005). The use of supercritical conditions is becoming very popular for the synthesis of copper, silver, gold, platinum, germanium and silicon nanoparticles because of the short residence time, rapid reaction rates and particle growth of the process. Supercritical water conditions for the production of nanoparticles have been reported by many scientists (Reverchon and Adami, 2006; Chen *et al.*, 2010). Yang *et al.* (2001) used solvothermal reduction process for the preparation of nanoparticles. The reaction was initiated under ambient conditions before the mixture was transferred to an autoclave and heated to 8020°C. By varying the solvent and reaction temperatures, they were able to vary the sizes and morphologies of the products from loment-like to octahedral to spherical nanoparticles. Chen *et al.* (2010) used Sodium Dodecyl Benzenesulfonate (SDBS) as surfactant which also acted

as stabilizer during hydrothermal reduction of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$. Shape and structure of copper nanoparticles greatly depend upon the reaction temperatures and quantity of SDBS (Amendola *et al.*, 2006).

Production of magnetic nanoparticles: The most common approaches used to produce magnetic nanoparticles are physical vapour deposition, mechanical attrition and chemical routes from solution. In both, the vapour phase and solution routes, the particles are assembled from individual atoms to form nanoparticles. Alternatively, mechanical attrition involves the fracturing of larger coarse-grained materials to form nanostructures.

Biosynthesis of nanoparticles: Soil microorganisms and plants (extracts) are important bioagents and possess great potential and scope in the synthesis of nanoparticles. Development of an efficient and eco-friendly processes of synthesis of nanoparticles is an important aspect of bionanotechnology (Khan *et al.*, 2009). One of the important option to achieve this objective is to use 'natural bioresources' such as microorganisms and plant based materials to produce nano size particles of the matter.

Microbial synthesis of nanoparticle: Soil microorganisms constitute one of the vast and strong natural factories to harness beneficial effects through biotechnology (Khan, 2007) and nanotechnology (Gurunathan *et al.*, 2009). Numerous microorganisms have been found to produce nanoparticles in the substrate (Khan and Anwer, 2011; El-Rafie *et al.*, 2012). Several researchers have shown that macro matter can be converted into nanomaterial with the use of microorganisms. Gurunathan *et al.* (2009) reported synthesis of silver nanoparticles (Ag NPs) through reduction of Ag^+ ions by the culture supernatant of *E. coli*. The synthesized silver nanoparticles were purified by using sucrose density gradient centrifugation. The purified sample was further characterized by UV-vis spectra, fluorescence spectroscopy and TEM. The purified solution yielded the maximum absorbance peak at 420 nm and the TEM characterization showed a uniform distribution of nanoparticles of an average size of 50 nm.

Manonmani and Juliet (2011) worked on extracellular biosynthesis of "silver nanoparticles" using *Escherichia coli* and characterization of nanoparticles by UV visible spectroscopy, FTIR and SEM of nanoparticles against different human pathogenic bacteria in food. This technique is used to combine function of Surface Enhanced Resonance Raman Scattering (SERRS) and silver nanoparticles to produce antigen-antibody

interaction onto biological products. El-Rafie *et al.* (2012) used fungus *Fusarium solani* for biosynthesis of silver nanoparticles. They also examined the factors affecting the biomass concentration, pH of the reaction medium, AgNO_3 concentration and the ratio of AgNO_3 to biomass concentration on the production of Ag NPs. Optimum conditions for biosynthesis of AgNPs could be attained using biomass of *F. solani* (10 g/100 mL), AgNO_3 (0.078 g/100 mL), pH, 12, temperature, 25°C and duration, 24 h that yielded stabilized concentration of 2000 ppm Ag NPs with a mean diameter range of 8-15 nm.

The biosynthesis of Ag and Au nanoparticles (NPs) was investigated by Mourato *et al.* (2011) using an extremophilic yeast strain isolated from acid mine drainage in Portugal. A successful route for the metal NPs synthesis was obtained using the yeast biomass that yielded Ag NPs (20 nm) and Au NPs (30-100 nm). Using the bacterium *Bacillus licheniformis*, the biosynthesis of silver nanoparticles of 50 nm size has been achieved (Kalimuthu *et al.*, 2008). Similarly, extracellular biosynthesis of silver nanoparticles was achieved by *Aspergillus niger* (Gade *et al.*, 2008). *Geobacter sulfurreducens* reduced Ag(I) as insoluble AgCl or Ag(+) ions, via a mechanism involving c-type cytochromes, precipitating extracellular nanoscale silver (Law *et al.*, 2008). Abd *et al.* (2013) isolated silver nanoparticle from culture of gram positive bacteria *Morganella morganii*.

A good quantity of gold nanoparticles was obtained with the use of extremophilic bacteria, *Thermomonospora* sp. (Ahmad *et al.*, 2003a, b). Konishi *et al.* (2004) have reported synthesis of gold nanoparticle with the help of mesophilic bacterium *Shewanella*, using H_2 as an electron donor. The bacterium *Rhodospseudomonas capsulate* produced gold nanoparticles of 10-20 nm size (He *et al.*, 2007). Bacterial cell supernatants of *Pseudomonas aeruginosa* have been found to reduce the gold ions for extracellular biosynthesis of gold nanoparticles (Husseiny *et al.*, 2007). Similarly, cell-free extracts of *Micrococcus lactilyticus* reduced the uranium (vi) to uranium (iv) (Woolfolk and Whiteley, 1962). Also, *Alteromonas putrefaciens* grown in the presence of hydrogen as electron donor and U (vi) as electron acceptor reduced U (vi) to U (iv) (Lovley *et al.*, 1989). Lovley *et al.* (1991) demonstrated that *G. metallireducens* GS-15, grown anaerobically in the presence of acetate and U (vi) as electron donor and electron acceptor, respectively, reduced soluble U (vi) to insoluble U (iv), oxidising acetate to CO_2 .

Stenotrophomonas maltophilia SELTE02, a strain isolated from rhizospheric soil of selenium hyperaccumulator legume *Astragalus bisulcatus*, has

showed promising transformation of selenite to elemental selenium and accumulation of selenium granules in either the cell cytoplasm or extracellular space (Di Gregorio *et al.*, 2005). The facultative anaerobic bacterium, *E. cloacea* (Losi and Frankenberger, 1997), purple nonsulfur bacterium, *Rhodospirillum rubrum* in oxic and anoxic conditions and *Desulfovibrio desulfuricans* (Tomei *et al.*, 1995) are reported to bioreduce selenite to selenium both inside and outside the cell. *E. coli* has also been found to deposit elemental selenium both in the periplasmic space and cytoplasm (Silverberg *et al.*, 1976). *P. stutzeri* is also known to aerobically reduce selenite to elemental selenium (Lortie *et al.*, 1992). Recently, Yadav *et al.* (2008) have showed that *P. aeruginosa*, isolated from rhizospheric seleniferous soil, biosynthesized nanostructured selenium by biotransforming selenium oxyanions both intracellularly and extracellularly to spherical amorphous allotropic elemental red selenium.

Similarly, the extracellular culture filtrate of *Lactobacillus* sp. has been shown to produce titanium nanoparticles at room temperature in the form of spherical aggregates ranging in size from 40-60 nm (Prasad *et al.*, 2007). The gram-negative cyanobacterium, *P. boryanum* has been reported to produce extracellular Pt (ii) organics and metallic platinum nanoparticles of 30-300 nm size (Konishi *et al.*, 2007). The sulphate reducing bacterium, *Desulfovibrio desulfuricans*, anaerobically bioreduced and biocrystallised palladium (2+) ions to palladium nanoparticles on the cell surface in the presence of formate as an exogenous electron donor within minutes at neutral pH (Yong *et al.*, 2002). De Windt *et al.* (2005) have demonstrated that an iron reducing bacterium, *S. oneidensis*, reduced Pd (ii) to Pd (0) nanoparticles in the presence of lactate as electron donor on the cell wall and within the periplasmic space. An actinomycetes, *Thermomonospora* sp., has been reported to synthesize extracellular monodispersed, spherical gold nanoparticles of an average size of 8 nm (Ahmad *et al.*, 2003a, b). Furthermore, an alkalotolerant actinomycete *Rhodococcus* sp. accumulated intracellularly gold nanoparticles of 5-15 nm size.

Some common cyanobacteria, *Anabaena*, *Calothrix* and *Leptolyngbya* have also been found to produce intracellular Au, Ag, Pd and Pt nanoparticles. The NPs were naturally released in the culture medium and stabilized by algal polysaccharides for their easy recovery (Brayner *et al.*, 2007). The yeast, *Candida glabrata*, has been used for the intracellular production of monodispersed spherical and peptide-bound CdS quantum dots measuring 2 nm by forming a metal-thiolate complex with phytochelatin

(Dameron *et al.*, 1989). *Schizosaccharomyces pombe* also produced wurtzite-typed hexagonal lattice structured CdS nanoparticles of 1-1.5 nm size (Kowshik *et al.*, 2002). Another yeast, *Pichia jadinii*, intracellularly produced spherical, triangular and hexagonal gold nanoparticles of 100 nm size (Gericke and Pinches, 2006). *S. cerevisiae* has been found to produce gold nanoparticles in the size range of 2-10 nm (Jha *et al.*, 2009).

Biosynthesis of nanoparticles using fungi such as *F. oxysporum* (Bansal *et al.*, 2004), *Collitotrichum* sp. (Shankar *et al.*, 2003), *Trichothecium* sp., *Trichoderma asperellum*, *T. viride* (Fayaz *et al.*, 2010), *Phaenerochaete chrysosporium* (Vigneshwaran *et al.*, 2006), *F. solani* (Ingle *et al.*, 2009), *F. semitectum* (Basavaraja *et al.*, 2008), *Aspergillus fumigatus* (Bhainsa and D'Souza, 2006), *Coriolus versicolor* (Sanghi and Verma, 2009), *Aspergillus niger* (Gade *et al.*, 2008), *Phoma glomerata* (Birla *et al.*, 2009), *Penicillium brevicompactum* (Shaligram *et al.*, 2009), *Cladosporium cladosporioidis* (Balaji *et al.*, 2009), *Penicillium fellutanum* (Kathiresan *et al.*, 2009) and *Volvariella volvacea* (Philip, 2009) has been extensively studied.

PHYTOSYNTHESIS OF NANOPARTICLES

Plant extracts are well known for different uses such as, plant growth promotion (Derbalah *et al.*, 2011), pathogen suppression and disease control (Ikeura *et al.*, 2011). Interestingly, plant extracts can also serve as one of the most cost effective and eco-friendly material for synthesis of nanoparticles. Extracts of a number of plant species have been found to produce nanoparticles. Plant extracts from *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica* and *Citrus sinensis* were used for the synthesis of silver nanoparticles (Ag NPs) from silver nitrate solution. The atomic force microscope showed the formation of silver nanoparticle with an average size of 28, 26.5, 65, 22.3 and 28.4 nm corresponding to *O. tenuiflorum*, *S. cumini*, *C. sinensis*, *S. tricobatum* and *C. asiatica*, respectively. The leaf extract of *Ocimum tenuiflorum* acted as reducing as well as stabilizing agent and produced silver nanoparticles in the range 25-40 nm (Patil *et al.*, 2012). The leaf extract of *Piper longum* as reducing as well as capping agent yielded silver nanoparticles from 1 mM AgNO₃ solution. Nanoparticles were characterized by UV-vis absorption spectroscopy, FTIR and SEM. SEM analysis showed the spherical nanoparticles with 17.6-41 nm in size. Vijayakumar *et al.* (2013) used the extracts of *Artemisia nilagirica* (Asteraceae) to produce Ag NPs of 70-90 nm. Silver nitrate was used as the metal precursor

and hydrazine hydrate as a reducing agent. Scanning Electron Microscopy (SEM) and energy-dispersive spectroscopy (EDX) were used to characterize the nanoparticles obtained from *A. nilagirica*. Silver nanoparticles have been synthesized using leaf extract of *Acalypha indica* (Krishnaraj *et al.*, 2012) and root extracts of *Morinda citrifolia* (Suman *et al.*, 2013) as a reducing agent. Leaf extract of Chinese tea, (*Camellia sinensis*) is also reported to produce silver nanoparticles (Loo *et al.*, 2012).

NANOPARTICLES IN PLANT DISEASE MANAGEMENT

Different types of organic and inorganic salts have been used for controlling disease for many years (Talibi *et al.*, 2011). Organic acids and salts were used to control citrus blue mold by Latifa *et al.* (2011). Besides, certain organic amendments are also used to control plant diseases (El-Sherif *et al.*, 2007; Abd El-Hai *et al.*, 2010; Kundu and Saha, 2014). Use of nanoparticles in plant disease management is a novel and fancy approach that may prove very effective in future with the progress of application aspect of nanotechnology. The nanotechnology has potential prospects of use in plant disease management in different ways. The most simple and obvious way is direct application of nanoparticles in the soil on seeds or foliage to protect plants from pathogen invasion. In this way, the NPs may suppress the pathogens in a way comparable to chemical pesticides. When nanoparticles are to be applied directly in soil, their effects on non-target organisms especially the mineral fixing/solubilizing microorganisms will be of great significance. Secondly, the nanomaterials, carbon tubes, cups, etc., can also be used as a carrier of some fancy chemicals such as pheromones, SAR inducing chemicals, polyamine synthesis inhibitors or even concentrated active ingredients of pesticides for their controlled release especially under flooded conditions (Khan *et al.*, 2014a). Hence, to reckon the scope and application of nanoparticles in plant disease management, the effects can be discussed through two major point of views i.e direct effect of NPs on pathogens and use of nanomaterials in formulating the pesticides i.e., nanopesticides. In view of ultra-small size of the particle and their very high degree of reactivity/sensitivity, the NPs may also prove very effective in the diagnosis of plant pathogens/diseases and pesticide residue analysis.

Effect of nanoparticles on the pathogens/microorganisms: Since, physio chemical properties of nanoforms from its macroform vary greatly, it becomes

important to examine the effect of NPs on microorganisms to harness the benefit of this technology in the plant protection especially against phytopathogens. Nanoparticles because of ultra-small size, even smaller than a virus particle and high reactivity, may affect the activity of microorganisms. The silver has been generally found non injurious to microorganisms. However, silver NPs inhibited the colonization of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*. The highest antimicrobial activity of silver nanoparticles (30 nm) synthesized by *Solanum tuberosum* and *Ocimum tenuiflorum* leaf extracts was found against *S. aureus* and *E. coli*, respectively. The information so far available on this aspect has shown that the nanoparticles have definite effect on the colonization of bacteria and fungi. However, these effects are suppressive as well as stimulatory and hence cannot be generalized.

Effect of nanoparticles on bacteria: Antibacterial activity of zinc nanoparticles against *P. aeruginosa* has been reported by Jayaseelan *et al.* (2012). The maximum zone of inhibition in the colonization of the bacteria (22±1.8 mm) was recorded at 25 ng mL⁻¹ ZnO NPs. The study showed that the ZnO NPs proved to be a novel antimicrobial material. The antibacterial activity of the synthesized Ag NPs/PVP (hybrid materials based on polyvinylpyrrolidone with silver nanoparticles) against three different groups of bacteria-*Staphylococcus aureus* (gram positive bacteria), *E. coli* (gram-negative bacteria), *P. aeruginosa* (nonferment gram-negative bacteria), as well as against spores of *Bacillus subtilis* has been studied (Bryaskova *et al.*, 2011). The antibacterial activities of CuO NPs has also been reported against *S. aureus*, *Bacillus subtilis*, *P. aeruginosa* and *E. coli* (Azam *et al.*, 2012). Guzman *et al.* (2009) found that silver nanoparticles showed high antimicrobial and bactericidal activity against gram positive bacteria such as *E. coli*, *P. aeruginosa* and *S. aureus* which are highly methicillin resistant strains. The antibacterial activity of nanoparticles was found to be dependent of NPs concentration, physiology, metabolism, intracellular selective permeability of membranes and the type of microbial cell.

Effect of nanoparticles on plant pathogenic fungi: Plant pathogens viz., bacteria, fungi, viruses and nematodes are important limiting factors in the production of food material (Khan *et al.*, 2011, 2012; Khan, 2012). Numerous methods are used to control pathogens but none of them offer perfect control of

the disease (Khan and Jairajpuri, 2010a, b, 2012). Hence, a great scope exists for the exploitation of nanotechnology for the management of plant pathogens. The nanoparticles have also been found suppressive to fungi. Singh *et al.* (2013) reported that among nanoforms of 15 micronutrients, CuSO_4 and $\text{Na}_2\text{B}_4\text{O}_7$ were found most effective in controlling rust disease of field peas. Microelements such as manganese and zinc also suppressed the damping off and charcoal rot diseases in sunflower (Abd El-Hai *et al.*, 2009). The Ag NPs/PVP were tested for fungicidal activity against different yeasts and molds such as *Candida albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata* and *Aspergillus brasiliensis*. The hybrid materials showed strong antifungal effects against the tested microbes (Bryaskova *et al.*, 2011). Fungicidal effect of zinc oxide nanoparticles (ZnO NPs) against two postharvest pathogenic fungi, *Botrytis cinerea* and *Penicillium expansum*, have been reported. Traditional microbiological plating, Scanning Electron Microscopy (SEM) and Raman spectroscopy were used to study antifungal activities of ZnO NPs and to characterize the changes in morphology and cellular compositions of fungal hyphae. The ZnO NPs (70 ± 15 nm) at the concentrations greater than 3 mmol L^{-1} significantly inhibited the growth of *B. cinerea* and *P. expansum*, the later fungus was found more sensitive to the treatments. The NP treatments caused deformation in the hyphae of *B. cinerea* and prevented the development of conidiophores and conidia in *P. expansum* which eventually led to the death of fungal hyphae. Krishnaraj *et al.* (2012) examined the effect of silver nanoparticles on plant pathogenic fungi, *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *B. cinerea* and *Curvularia lunata* and found that 15 mg L^{-1} concentration of NPs greatly inhibited the activity of all the tested pathogens. The zinc nanoparticles (25 mg mL^{-1}) suppressed the colonization of *A. flavus* (Jayaseelan *et al.*, 2012).

NANOPESTICIDES

Presence of active ingredient of a formulation in the root zone of a plant at the initial stage of crop growth helps in the protection of the plant from pathogen invasion and bringing down its population below economic threshold level (Khan *et al.*, 2014a, b). Since, all the propagules/spores of a pathogen do not approach/invade the host at one time, rather they attack intermittently, hence persistence or slow/ gradual release of active ingredient in the root zone shall enhance the effectiveness of the formulation (Khan *et al.*, 2011).

Moreover, timely and slow release of the chemical shall reduce the net amount of pesticide required for the disease control. This would subsequently minimize the adverse effects of the pesticide on the man and environment (Khan and Jairajpuri, 2012). The controlled release of the active ingredient can be achieved through a nanotechnological approach by using nanomaterials as carrier for the chemicals. Such formulations may greatly decrease the amount of pesticide input and associated environmental hazards. Nanopesticides shall reduce the rate of application because the quantity of the chemical actually being effective is at least 10-15 times smaller than that applied with classical formulations, hence a much smaller than the normal amount could be required to achieve satisfactory control of the disease. Due to better kinetic stability, smaller size, low viscosity and optical transparency, nanoemulsions can serve as a better pesticide delivery systems (Xu *et al.*, 2010). The micro or nano emulsion, as a carrier for pesticide delivery, can improve the solubility and bioavailability of the active ingredients of the chemical.

Nanopesticides contain very small particles of pesticide's active ingredients or other small engineered structures with useful pesticidal properties (Bergeson, 2010b). Nanopesticides can increase the dispersion and wettability of agricultural formulations (reduction in organic solvent runoff) and unwanted pesticide movement (Bergeson, 2010a). Nanomaterials and biocomposites exhibit useful properties such as stiffness, permeability, crystallinity, thermal stability, solubility and biodegradability (Bouwmeester *et al.*, 2009; Bordes *et al.*, 2009) needed to formulate the nanopesticides. The nanopesticide formulations also offer large specific surface area and hence, increased affinity to the target (Yan *et al.*, 2005). Nanoemulsions, nanoencapsulates, nanocontainers and nanocages (Fig. 3) are some of the nanopesticide delivery techniques that may prove effective in plant protection programmes (Lyons and Scrinis, 2009; Bouwmeester *et al.*, 2009; Bergeson, 2010b). Corradini *et al.* (2010) explored the possibility of utilizing chitosan nanoparticles, a highly degradable antibacterial material for slow release of NPK fertilizer. Liu *et al.* (2006) developed kaolin clay-based nano layers to be used as cementing and coating material for control release of fertilizers. More of such studies have been summarized by Ghormade *et al.* (2011). Primarily, nano-clay materials offer interactive surfaces with high aspect ratio for encapsulating "agrochemicals such as fertilizers, plant growth promoters and pesticides" (Ghormade *et al.*, 2011).

Generally, there are three kinds of Controlled Release Systems (CRS), viz., zero-order release, first-order release

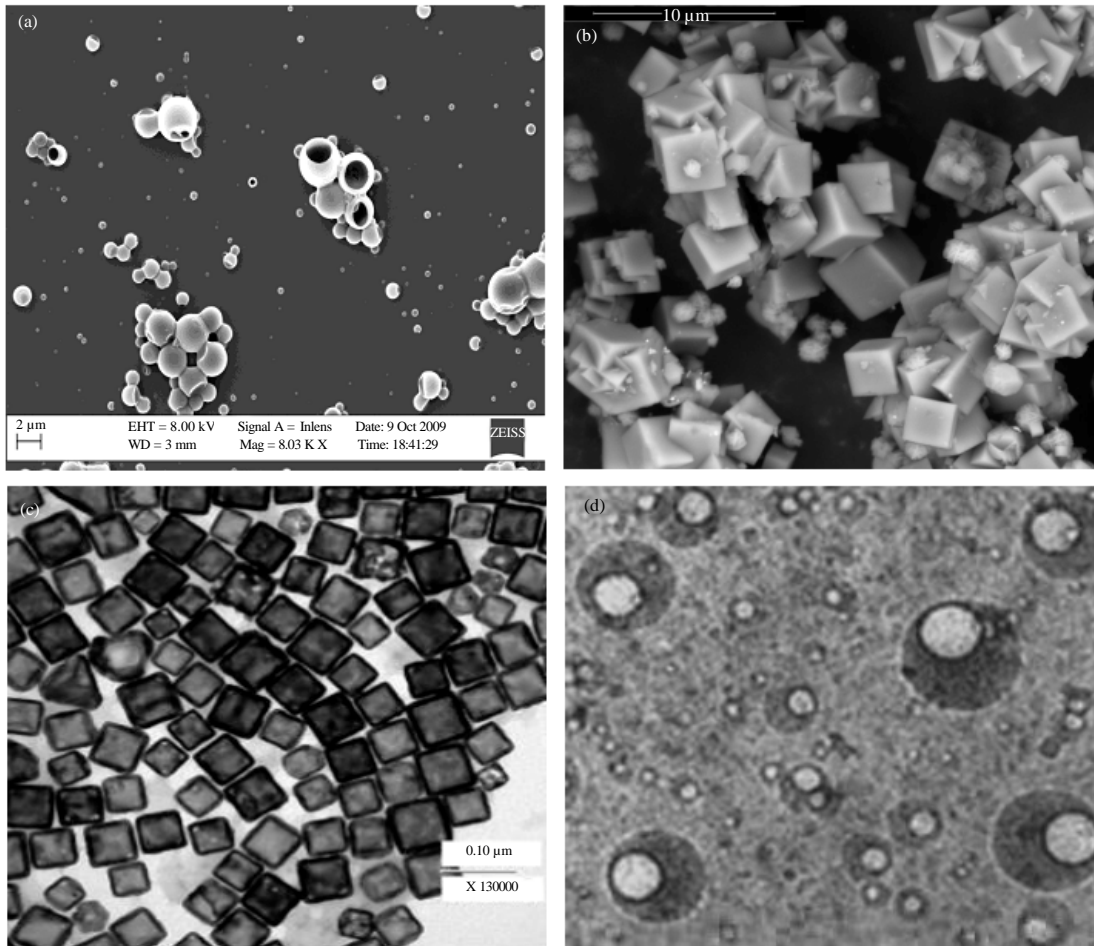


Fig. 3(a-d): Scanning Electron micrographs of (a) Nanocapsules (Masuda, 2010), (b) Nanocontainers (<http://www.itc.uji.es/notDestacada/PublishingImages/Nanocontainers.jpg>), (c) Nanocages ([http:// bit.bme.jhu.edu/showimg/nanocage.jpg](http://bit.bme.jhu.edu/showimg/nanocage.jpg)) and (d) Nanoemulsions (https://encrypted-tbn3.gstatic.com/images?q=tbn:And9GcSpWbJHE3y CQMIK2fsgAtu_ VSfBRRiG77nchzulLNLOyjc2VDO0w)

and square-root-time release which may be selected in view of the environmental condition and pest/pathogen biology. Biodegradable microbial polymers like polyhydroxyl alkanates have proved effective for controlled release of pesticides (Pepperman *et al.*, 1991). Temperature sensitive polymers e.g., intelimer can regulate the release of pesticide with the progress of season depending on the temperature fluctuations, thereby protecting the active ingredient from unwanted leaching and degradation (Cameron *et al.*, 1992). Some important controlled release devices are microcapsules, microspheres, coated granules and granular matrices that may be used in formulating the nanopesticides. A microcapsule is a reservoir system where active ingredient of the pesticide is contained within the core which is surrounded by a membrane. Micro-encapsulation has a

potential scope in formulating nanopesticides. A microsphere is a monolithic system consisting of spherical or irregular shape particles (20 nm-2000 μm size) in which the active agent is dissolved or dispersed in a polymer matrix (Sotthivirat *et al.*, 2007). Various naturally occurring polymers (polysaccharides and proteins), synthetic polymers (polystyrene, polyacrylamide, polyamides, polyesters etc. (Shukla *et al.*, 1992) and inorganic materials (silica, zeolites, inorganic oxides, glass beads, ceramic etc.) (Chuan *et al.*, 2013) can be explored to test their suitability in formulating nanopesticides helpful in crop protection.

Basically, the nano-formulations should degrade faster in the soil and slowly in plants with residue levels below the regulatory criteria in foodstuffs. Yan *et al.*

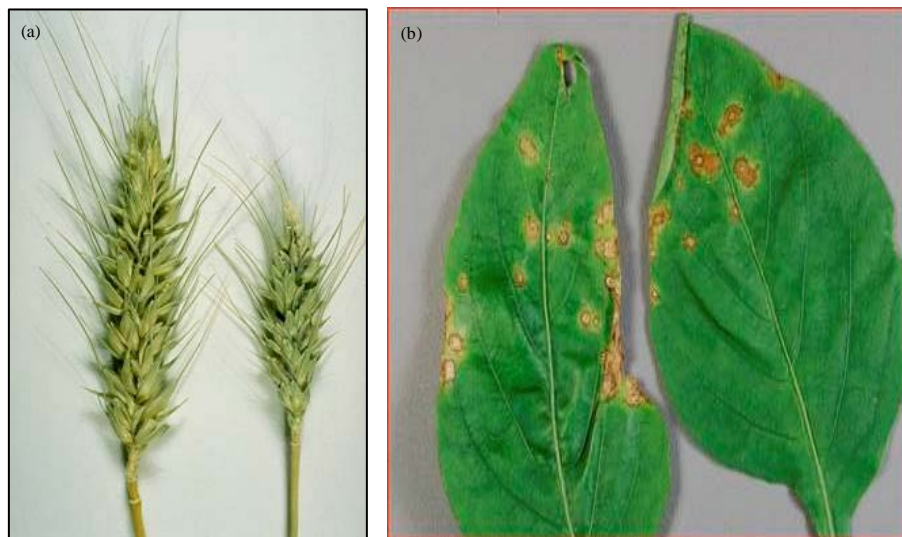


Fig. 4(a-b): Symptoms of (a) Karnal bunt (https://encrypted-tbn2.gstatic.com/images?q=tbn:ANd9GcQiOpHDQZUE_3ytLOfUAjM9I8VDN0TE0AHSfmDZ43OrwG621uyiw) and (b) Bacterial leaf spot (<http://extension.udel.edu/kentagextension/tag/bacterial-spot/>)

(2005) reported the development of Sodium Dodecyl Sulphate (SDS) modified photocatalytic TiO_2/Ag nanomaterial conjugated with dimethomorph (DMM) which met the above criterion. Khot *et al.* (2012) used common pesticides to prepare the nanopesticide. Modified formulation with 96 nm average granularity, increased dispersivity and decomposition of the pesticide in soil have much greater effectiveness in the seedling of cabbage and cucumber. The modification of the nanomaterials using SDS greatly increased the absorption of DMM. Guan *et al.* (2010) formulated encapsulated nano-imidacloprid with above properties that could be used for pests control during vegetable production. The SDS modified Ag/TiO_2 imidacloprid nano formulation was developed using a microencapsulation technique that used chitosan and alginate. It was tested on soybean plants that were transplanted to soil pH of 6.2. The formulation residues in soil and the plants degraded faster during the first eight days and were minimal to undetectable after 20 days. The SDS in the above applications was used to increase the photo-degradation of the nanoparticles in soil.

Alternatively, Mohamed and Khairou (2011) developed highly photo-degradable Ag/TiO_2 particles (57 nm) which were synthesized using polyoxy ethylene lauryl ether (POL). They tested Ag/TiO_2 nanoparticles synthesized using both POL and SDS for the degradation of 2, 4-D herbicide under visible and UV radiation. Results confirmed that the POL synthesized nanoparticles photo-degraded faster during the same

exposure period. All the above results cannot be considered as safeguarded due to lack of any national/international nano-regularity institutional framework/standards related to the use of nanomaterials in agriculture (Bowman and Hodge, 2007).

Nanoparticles in the detection of phytopathogens and pesticide residue

Detection of phytopathogens: A prior and accurate detection of pathogens would help in timely application of pesticides/fertilizers in the field to protect the crops from diseases (Bergeson, 2010a). Nanoparticles can be used as biomarkers or as a rapid diagnostic tool for detection of phytopathogens (Boonham *et al.*, 2008), viruses (Yao *et al.*, 2009) and fungi (Chartuprayoon *et al.*, 2010). Nanoparticles based sensors might offer improved detection limits in detecting viral pathogens in plants (Baac *et al.*, 2006). Nanoparticles can either be directly modified for use in pathogen detection or for a diagnostic tool to detect compounds indicative to a diseased condition. Nano-chips are types of microarrays that contain fluorescent oligo capture probes through which the hybridization can be detected (Lopez *et al.*, 2009). The nanochips are known for their high sensitivity and specificity in detecting single nucleotide change in bacteria and viruses (Lopez *et al.*, 2009). Yao *et al.* (2009) utilized a fluorescence silica nanoparticles in combination with antibodies to detect *Xanthomonas axonopodis* pv. *Vesicatoria* that causes bacterial spot disease in plants (Fig. 4). Singh *et al.* (2010) used nano-gold based

immunosensors that could detect Karnal bunt disease in wheat (*Tilletia indica*) using Surface Plasmon Resonance (SPR). Application of SPR sensor in the seed certification and plant quarantines may prove highly effective and accurate in the detection of infection in plants. Research on pathogen detecting nanosensors for their field application would be highly valuable for rapid diagnosis and disease management.

Plants respond to different stress conditions through physiological changes (Khan and Khan, 2011). One such response is the induction of systemic defence which is thought to be regulated by jasmonic acid, methyl jasmonate and salicylic acid (Khan and Haque, 2013). Wang *et al.* (2010) harnessed this indirect stimulus to develop a sensitive electrochemical sensor, using modified gold electrode with copper nanoparticles, to monitor the levels of salicylic acid in oil seeds to detect the pathogenic fungus, *Sclerotinia sclerotiorum*. They successfully and accurately measured salicylic acid using this sensor. Research on similar sensors and sensing techniques needs to be expanded for detecting pathogens, their by products or monitor physiological changes in plants.

Pesticide residue detection: There are around 1050 chemicals reported by Food and Drug Administration (FDA) as pesticide residues (FDA, 2005). Nanomaterials based nanosensors can be used to detect such pesticide residues as an alternate to traditional gas or liquid chromatography-mass spectroscopy (GC/LC-MS) techniques. The traditional techniques are accurate and reliable but involve time consuming steps. Nanosensors for pesticide residue detection offers high sensitivity, low concentration detection limits, super selectivity, fast responses and small sizes (Liu *et al.*, 2008). The nanosensors aimed to detect the pesticide residues of methyl parathion (Kang *et al.*, 2010), parathion (Wang and Li, 2008), fenitrothion (Kumaravel and Chandrasekaran, 2011), pirimicarb (Sun and Fung, 2006), dichlorvos and paraoxon (Vamvakaki and Chaniotakis, 2007) have proved to give high degree of accuracy and handyness. Additionally, Van Dyk and Pletschke (2011) have also emphasised the higher sensitivity of enzyme based NP biosensors in the detection of residues of organochlorines, organophosphates and carbamates. In some of these biosensors, nano C, Au, hybrid Ti (titanium), Au-Pt (platinum) and nanostructured lead dioxide PbO₂, TiO₂ and Ti were used to immobilize the enzymes on sensor substrate and to increase the sensor sensitivity.

Toxicity or biosafety of nanoparticles: Toxicity or biosafety of nanoparticles and nanopesticides is a major concern of application of nanotechnology in the plant

disease management. With nanopesticide applications, the uncertainty exists on the long-term effects of the formulation on the human health and environmental quality. There is an urgent need to assess the possible uptake of nanopesticides that can occur when agricultural workers inhale it during applications. Shi *et al.* (2010) studied the toxicity of chlorfenapyr (nanopesticide) on mice. It was reported that the chlorfenapyr nano formulation from 4.84-19.36 mg kg⁻¹ was less toxic to mice than the common formulation. Thus, such nano formulation pesticides may decrease adverse environmental and human effect as compared to classical pesticides. In order to ensure health and environmental safety, further such studies on the biosafety of the nanopesticides are needed.

Formulation stability is also an important aspect of the biosafety of nanomaterials. Liu *et al.* (2008) successfully formulated a stable nanopesticide (bifenthrin) using polymer stabilizers such as poly (acrylic acid)-b-poly (butylacrylate) (PAA-b-PBA), Poly Vinyl Pyrrolidone (PVP) and polyvinyl alcohol (PVOH). A flash nano-precipitation technique was used to prepare 60e200 nm bifenthrin particles. While using such techniques commercially, stability of the polymers over an extended period of time needs to be considered. Anjali *et al.* (2010) reported formulation of artificial polymer-free nano permethrin as an effective larvicide that was stabilized by plant extracted natural surfactants.

Environmental and human risk of nanoparticles: The ultra-small sizes that make the nanoparticles of immense usefulness, unfortunately the same characteristic is a basic cause of several adverse effects which may represent following hazards to environment, animals, human beings and plants:

- Nanopesticides, nanofertilizers and other nanoformulations, when air borne may deposit on leaves and floral parts of plants. They may plug stomata and create a fine physical and toxic barrier layer on stigma preventing pollen germination and tube penetration into stigma. The NPs may also enter the vascular tissue and impair translocation of water, minerals and photosynthates
- The nanoparticles may get inhaled by the human beings and animals deep into lungs resulting into various ill effects and disorders. The particles may enter the bloodstream and accumulate in lungs and kidneys, Bowman's capsule of nephron etc
- The entry of NPs in to lungs and blood stream, may cause pro-inflammatory effects depending on the nature of NP, leading to inflammation, protein fibrillation, induction of genotoxicity, etc

- Use of nanopesticides may create new kinds of contamination in soil and water bodies due to enhanced transport, longer persistence and higher reactivity of the particles

CONCLUSION

Perusal of the literature and critical analysis of the relevant information have revealed that nanotechnology has potential prospects of use and application in the detection, diagnosis and management of plant diseases. The limited studies so far conducted on this aspect are sufficient enough to warrant potential future use of nanotechnology in plant disease management. The researches have shown that direct application of nanoparticles significantly suppressed the plant pathogenic fungi and bacteria tested. Nanomaterials, nanotubes and microcapsules can efficiently carry higher concentration of active ingredients of pesticides, host resistance inducing chemicals, polyamine synthesis inhibitors, etc and would regulate the release of chemicals from the nanocarriers as per requirement. Enzyme based biosensors coated with nanoparticles of Au, Ag, Co, Ti, etc., may greatly help in the precise and quick diagnosis of plant infection and also residue detection of pesticides. Despite of tremendous application scope of nanotechnology in plant disease management, there are certain demerits, risk and apprehensions in the use of NPs in agriculture which are required to be worked out on priority and before the commercial use of nanotechnology in agriculture. Foremost is the phytotoxic behaviour of the nanomaterials which needs to be thoroughly understood and ascertained at different plant growth stages such as seed germination, seedling growth, fruit setting and maturity. Effect of seed coating with NPs on the seed germination, root development and plant growth/productivity will be of immediate concern.

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