REVIEW ARTICLE



Nanotechnology in crop protection: Status and scope

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ABSTRACT: Pests, including insects, mites, nematodes and pathogens, are the major limiting factor in profitable crop production. Frequent application of pesticides has resulted in development of pest and disease resistance, accumulating residues in produce and environmental pollution. So there is a need for alternative approach as to control pests and pathogens. Application of nanotechnology in crop protection holds a significant promise in management of insects and pathogens, by controlled and targeted delivery of agrochemicals and also by providing diagnostic tools for early detection. Nanoparticles are highly stable and are biodegradable; it can be successfully employed in production of nanocapsules for delivery of pesticides, fertilizers, and other agrochemicals. Nanoparticles display slow release of encapsulated functional molecules and reduce its frequent applications. Nanoparticles are smaller in size with more charge and larger surface area with higher stability and solubility, so behave differently from their bulk sized counterparts. The biological agents such as plants and microbes have emerged as cost effective and efficient candidates for the synthesis of nanoparticles by green synthesis approaches. They have advantages over conventional chemical methods which associated with eco toxicity. This review is focused on potential applications of nanomaterials in crop protection for a cleaner and greener agriculture.

Keywords : Crop protection, nanoparticles, nanotechnology, pathogens.

INTRODUCTION

Nanotechnology has developed as one of the most ground-breaking scientific fields in decades. Nanotechnology is the understanding and control of matter at dimensions between approximately 1 to 100 nanometers (nm) where unique phenomena involved novel applications. A nanomaterial is one billionth of a meter. Nanoscale ranges from 1 and 100 nanometers (http://www.nano.gov/node/241). Nanoscale materials show unusual physical, chemical and biological properties, which are completely distinct from their bulk materials and individual molecules (Li et al., 2001). These unique properties find its novel applications in all the fields. Nanoparticles have large surface to volume ratio, chemically alterable physical properties, and possess strong affinity to targets such as proteins (Kumar et al., 2010). Through the ever growing global food demand due to changing climate, urbanization and environmental issues such as run-off and accumulation of agrochemicals, there is an increasing need to feed an estimated population growth from the 6 billion to 9 billion by 2050 (Chen and Yada, 2011). With the limited natural resources such as land, water and soil fertility, demand for food has increased tremendously. The cost of chemical fertilizers, pesticides and other production inputs has been drastically increased due to limited reserves of natural gas and petroleum (Ditta, 2012). There is a need to overcome these constraints with the help of precision

farming practices and effective application of nanotechnology to agriculture.

Nanoscale systems like encapsulation and entrapment of agrochemicals such as fertilizers, pesticides, herbicides, plant growth regulators and other active substances by using polymers, dendrimers, surface ionic attachments and other mechanisms may be used in controlled and slow release of agrochemicals, which allow the slow uptake of active ingredients and in turn reduces the amount of agrochemical application by minimizing the input and waste. Importance of nanoscale delivery system in agriculture is because of its improved solubility and stability to degradation in the environmental factors. The nanoscale delivery vehicles increase effectiveness by binding firmly to the plant surface and reduces the amount of agrochemicals by preventing runoff into the environment (Johnston, 2010; Chen and Yada, 2011). Nanomaterials also play an important role in promoting sustainable agriculture and provide better foods globally (Gruère, 2012). In developing countries, nanotechnology has got important application for enhancing agricultural productivity, along with other emerging technologies such as biotechnology including genetics, plant breeding, disease control, fertilizer technology, precision agriculture, and other allied fields (Sastry et al., 2010; Jha et al., 2011). Nanotechnology can be used for combating the plant diseases either by

controlled delivery of functional molecules or as diagnostic tool for disease detection. Nanosensors and other field sensing devices can be used in detection and measurement of crop nutrient status, insects, pathogens, weeds, moisture level, soil fertility, temperature, etc. which helps in real time monitoring of the crop growth and provide essential data for precision farming practices leading to minimize agricultural inputs and maximizing resource output and yield (Scott and Chen, 2003). Nanosensors can also provide information about optimal times for planting and harvesting crops and provides useful information for timely application of agrochemicals like fertilizers, pesticides and herbicides.

With the global efforts to reduce generated hazardous waste and the growing demand for synthesis of safe nanomaterials, researchers adopted "green" synthesis methods. The synthesis of nanoparticles from the plant extracts and microbes is a boon for advance research in nanotechnology. Synthesis of extracellular nanoparticles with variety of locally available biological agents is a novel and economical concept for bioprospecting. It provides new avenues to exploit wide variety of biological species for product development (Sharma et al., 2010). Green synthesis practice helps in reducing generation of hazardous waste by using environmentally safe solvents and nontoxic chemicals. The effective use of nanoparticle synthesized by green synthesis is very important, as several pathogenic bacteria have developed resistance against various antibiotics. Because of the introduction of new populations and sexual recombination within the species, pathogens have become resistant to systemic bactericides and fungicides. For instance, Staphylococcus aureus has developed resistance to methicillin, Candida albicans is resistant to fluconazole and Phytophthora infestans developed resistance against metalaxyl (Schaller et al., 2003; Chowdappa et al., 2013). Among the recent advancement in agricultural sciences, nanomaterials play a significant role in crop protection because of its unique physical and chemical properties. Nanoparticles remain bound to the cell wall of pathogen and causes deformity due to high energy transfer leading to its death. Nanotechnological application in plant pathology targets specific agricultural problems in plant-pathogen interactions and provide new perceptions for crop protection. Nanocomposites fulfil the two most important criteria in disease management: efficacy with minimal ecological impact and less toxicity on humans. While there is no much evidence of harm to people or the environment at present, nanotechnology is a new and evolving area of study where the research and developments are still at bench-top scale. Social and

ethical implications of nanotechnology applications in agriculture have to be addressed and toxicity of nanomaterials has to be clearly understood before its commercialization and field application. The potential application of nanomaterials in different agricultural applications needs further research investigation with respect to synthesis, toxicology and its effective application at field level. In the field of agriculture, there are still many possibilities to explore with new nano products and techniques.

Here, we have highlighted some of the most promising and potential applications of nanotechnology in crop protection including plant growth enhancement, pest and pathogen control with biopolymer and metallic nanoparticles, post-harvest disease management, green synthesis of nanomaterials and we also focused on limitations and current issues of applying nanomaterials in agriculture. This review examines whether nanotechnology can provide the science and technological boost for the improvement of agriculture in a cost effective, and environmentally friendly greener way.

Nanoparticles in plant growth enhancement

With the goal to promote its use for agricultural applications, nano materials (NM) can be effectively used in plant germination and growth. Khodakovskaya et al. (2012) reported the application of carbon nanotubes as regulators of seed germination and plant growth. They have shown that multiwalled carbon nanotubes (MWCNTs) have the ability to enhance the growth of tobacco cell culture by 55-64% when compared to control at a wide range of concentrations from 5-500 µg/ml. At low concentrations, activated carbon enhanced cell growth while at higher concentrations it intensely inhibited the cellular growth. They demonstrated the correlation between the activation of cell growth exposed to MWCNTs and the up regulation of genes like aquaporin NtPIP1, CycB and NtLRX1involved in cell division/cell wall formation and water transport, compared to control cells. Penetration of nanomaterials into the seed plays a key role in increasing seed germination rate. Carbon nanotubes (CNTs) can regulate cell division and plant growth by a unique molecular mechanism that is related to the activation of water channels (aquaporins) and major gene regulators of cell division and extension. They have highlighted the novel positive effects of MWCNTs at the cellular level and have provided an understanding of the complex mechanism underlying the enhancement of plant growth. However, to consider the possible use of carbon nanoparticles in agriculture, the consequences of the introduction of carbon nanotubes

into the environment have to be thoroughly investigated. Some of the recent studies have shown enhanced germination rate by the use of NM in the seeds. Recently, Khodakovskaya et al. (2009) have shown that use of MWCNTs in tomato seeds resulted in 90% increase in the rate of seed germination (compare to control which showed only 71%). CNTs penetrate into the hard coat of germinating tomato seeds and enhanced growth due to increased water uptake. MWCNTs could find an application in delivering desired molecules into the seeds during germination, which in turn protect the seeds from diseases. Because of their growth promoting effect, they will not pose any toxic or adverse effect on the plant. Authors also insist on additional studies evaluating the resistance to pests by tomato plants germinated through CNTs. Canas et al. (2008) studied the effects of functionalized (poly-3-aminobenzenesulfonic acid) and non-functionalized single-walled carbon nanotubes on root elongation of six commercially important crop species such as cabbage, carrot, cucumber, lettuce, onion, and tomato (regularly used in phytotoxicity studies). Root growth measurement at different time intervals, concurred that the non-functionalized carbon nanotubes affected root length more than functionalized nanotubes. Scanning electron microscopy (SEM) images displayed the presence of nanotube sheets on the root surfaces with no visible uptake of nanotubes by plant.

Zheng et al. (2005) studied the effect of nano and non-nano TiO₂ on the growth of spinach seeds. They reported that plants produced by seed treatment of nano-TiO, had 73% more dry weight, increase in chlorophylla formation and three folds higher photosynthetic rate, compared to the control. Photo sterilization and photo generation of superoxide and hydroxide anions by nanoTiO₂ play an important role in increasing seed germination and growth of spinach seeds. Particle size plays an important role in behaviour, toxicity and reactivity of nanomaterials. Germination increases with the decrease in size of the nanomaterials. Recently Mahmoodzadeh et al. (2013) investigated effects of nano titanium dioxide particles on plant growth and development. They chose Canola seeds as the model system for their investigation and treated them with different concentrations of nanoscale titanium dioxide and observed their effect on seed germination and seedling vigor. The authors reported that the treatment of nanoscale TiO₂ (~20 nm) at 2000 mg/L concentration promoted both seed germination and seedling vigor when compared to control. Both positive and negative effects of nanoparticles were observed in living plants.

In view of the potential influence of nanoscale zinc oxide (ZnO) particles on growth and development of peanut, Prasad et al. (2012) reported the effects of zinc oxide nanoparticles (25 nm) at 1000 ppm concentration promoted the seed germination, seedling vigor and growth of peanut plant. Their study also focused on determination of early establishment of flowering, higher leaf chlorophyll content, increasing stem and root growth in treated plants. Particles were very effective in increasing pod yield and root growth of peanut. Field experiment on foliar application of ZnO nanoparticles showed determinable effect on pod yield at 15 times lower dose compared to control. Nanomaterials can be effectively employed in growth and germination of plant when used in controlled conditions. Investigation of the adverse effect of nanomaterials on the seed and plant properties increases the effective applicability of nanomaterials in crop production.

Nanotechnology: scope in pathogen control

A wide variety of bacterial and fungal pathogens spoils vegetables. Among them the most common bacterial agents are Erwinia carotovora, Pseudomonas spp., Corynebacterium and Xanthomonas campestris which attack most vegetables. Fungal pathogens causing spoilage of vegetables are species belonging to genera Alternaria, Aspergillus, Cladosporium, Colletotrichum, Phomopsis, Fusarium, Penicillium, Phoma, Phytophthora, Pythium, Rhizopus spp., Botrytis cinerea, Ceratocystis fimbriata, Rhizoctonia solani, Sclerotinia sclerotiorum, and some mildews. Some of these organisms are host specific whereas others affect a wide variety of vegetables causing huge economic losses. Some pathogens produce toxic metabolites and adversely affect human health. Many of these agents enter the plant tissue over mechanical or chilling injuries, and cause overwhelming losses (Tournas, 2005). With the estimated doubling in global food demand in next 50 years huge challenges have been posed in food production. In the year 2000 the pesticide production was about three million tons of active ingredients worldwide (Tilman et al., 2002). It is reported that very small amount (less than 0.1 %) of pesticide reaches the sites of action, due to loss of pesticide in air during application and as run-off, spray drift, off-target deposition and photodegradation affecting both the environment and application costs (Pimentel, 1995; Castro et al., 2013). With the growing demand of pesticide worldwide to control the pathogens and pests, there is an urgent need to tackle the excessive usage of pesticides and fertilizers by finding alternatives. Table 1 shows some of the nanomaterials used for control of plant pathogens.

Potential applications of nanotechnology in crop protection include controlled release of encapsulated pesticide, fertilizer and other agrochemicals in protection against pests and pathogens, early detection of plant disease and pollutants including pesticide residues by using nanosensors (Ghormade *et al.*, 2011). The potential applications of nanomaterials in crop protection, helps in the development of efficient and potential approaches for the management of plant pathogens. As an efficient and potential modern approach, nanotechnological research studies carried out related to management of agriculturally important pathogens are reviewed here.

Nanoparticles in disease management

Various nanoparticles employed in plant disease management are listed in Table 1.

Biopolymer nanoparticles: Development of nanoformulation for field application of agrochemicals requires the use of readily biodegradable, nontoxic, environment friendly, safe and low-cost materials. So, use of biopolymers produced by natural sources with good physical and chemical properties is a fascinating approach to prevent the use of petrochemical and toxic chemical substances in production of nanomaterial.

Chitosan: Chitosan nanoparticles have got various applications in biology due to its biodegradable and nontoxic properties. In acidic condition the free amino groups of chitosan protonates and contributes to its positive charge (Phaechamud and Ritthidej, 2008). The inhibition mode of chitosan against fungi is defined by the following three mechanisms.

i) The positive charge of chitosan interacts with negatively charged phospholipid components of fungi membrane, which in turn alter cell permeability of plasma membrane and causes the leakage of cellular contents, which consequently leads to death of the cell (García-Rincón *et al.*, 2010).

ii) Chitosan chelates with metal ions, which has been implicated as a possible mode of antimicrobial action (Rabea *et al.*, 2003). On binding to trace elements, it interrupts normal growth of fungi by making the essential nutrients unavailable for its development (Roller and Covill, 1999).

iii) It is suggested that chitosan could penetrate fungal cell wall and bind to its DNA and inhibit the synthesis of mRNA and, in turn, affect the production of essential proteins and enzymes (Sudarshan *et al.*, 1992; Kong *et al.*, 2010).

In search of natural antimicrobials to avoid harmful synthetic chemicals, chitosan and chitosan nanoparticles are found to be more effective against plant pathogens like Fusarium solani. Inhibitory effect was also influenced by particle size and zeta potential of chitosan nanoparticles. The chitosan therefore could be formulated and applied as a natural antifungal agent in nanoparticles form to enhance its antifungal activity (Ing et al., 2012). Chen et al. (2010) studied antibacterial activities of low molecular weight chitosan products (molecular weight ranges from 6.9 kDa to 22.4 kDa) with average nanoparticle sizes of 117 nm to 965 nm. They emphasised that antimicrobial activity of chitosan nanoparticles depends on its zeta potential, which plays a significant role in binding with negatively charged microbial membrane. Antimicrobial activity of chitosan, chitosan derivatives bound metal ions and nanoparticles are well studied (Sanpui et al., 2008; Jagadish et al., 2012; Kaur et al., 2012).

Metallic nanoparticles: Metallic nanoparticles possess unique chemical and physical properties, small size, huge surface to volume ratio, structural stability and strong affinity to their targets (Kumar *et al.*, 2010). Metal nanoparticles can be used as new antimicrobial agents and an alternative to synthetic fungicide to delay or inhibit the growth of many pathogens species because of its multiple mode of inhibition.

Silver nanoparticle: Due to emerging plant diseases, agricultural production is reduced worldwide. Every year millions of dollars have been spent to control plant diseases. Various natural and artificial control measures have been used for plant protection. Use of pesticides is the most prevalent method for disease control at present. Scientists are searching for alternative measures against pesticide application, due to its environmental hazards and residual problem. As an alternative to chemical pesticides, use of silver nanoparticles as antimicrobial agents has become more common (Jo et al., 2009; Kim et al., 2012). Silver has been used as an antimicrobial agent since ancient civilizations; it has been used extensively due to its broadspectrum and multiple modes of antimicrobial activity (Wei et al., 2009). Its specific antimicrobial mechanisms are still unclear. Silver exhibits higher toxicity to microorganism and lower toxicity to mammalian cells. The application of silver nanoparticles as antimicrobial agents is because of its economical production and multiple modes of inhibitory action to microorganisms (Clement and Jarrett, 1994). Silver nanoparticles are the most studied and utilized nano particles in bio-system

because of its strong inhibitory and antimicrobial activities. Kim *et al.* (2008) evaluated the antifungal efficacy of colloidal nano silver solution, against rose powdery mildew. Nano silver colloid is more adhesive on bacterial and fungal cell surface; hence act as better fungicide because of its well dispersed and stabilized silver nanoparticles solution. Nano silver is classified as pesticide (Baier, 2009). Since silver acts as an excellent antimicrobial agent it is now an accepted agrochemical replacement. It acts as plant-growth stimulator and reduces unwanted microorganisms in soils and hydroponics systems (Sharma *et al.*, 2012).

Silver in ionic or nanoparticle forms has a high antimicrobial activity and is therefore widely used for various sterilization purposes including materials of medical devices and water sanitization. Relatively few studies were reported on the applicability of silver in controlling various plant pathogens in a relatively safer way compared to synthetic fungicides (Park et al., 2006). Since nanoparticles efficiently penetrate into microbial cells, lower concentrations of silver nanoparticles are sufficient for microbial control. This would be effective, especially for those organisms that are less sensitive to antibiotics because of poor penetration of some antibiotics into microbial cells (Samuel and Guggenbichler, 2004). Lamsal et al. (2011a) showed the effective usage of silver nanoparticles instead of commercial fungicides. They evaluated the effect of silver nanoparticles against six Colletotrichum species associated with pepper anthracnose under different culture conditions and found that, application of 100 ppm concentration of silver nanoparticles inhibited the growth of fungal hyphae as well as conidial germination in vitro when compared to the control. Silver nanoparticles showed significantly high inhibition of fungi in field conditions when applied on the plants before disease outbreak. Recently, Aguilar-Méndez et al. (2011) studied the dose-dependent fungistatic activity of the silver nanoparticles on Colletotrichum gloesporioides. Jo et al. (2009) tested various forms of silver ions and nanoparticles to examine their antifungal activity on two plant-pathogenic fungi, Bipolaris sorokiniana and Magnaporthe grisea. The in vitro and in planta evaluations of silver showed that both silver ions and nanoparticles effect colony formation of spores and disease progress of fungi. Kim et al. (2012) reported the inhibitory effect of three different silver nanoparticles (WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R) against eighteen different commercially important plant pathogenic fungi on potato dextrose agar (PDA), malt extract agar, and corn meal agar. They found that

inhibition of fungal pathogens with silver nanoparticles is concentration dependent and also on type of silver nanoparticles used. Most fungi showed a good inhibitory effect at 100 ppm concentration of silver nanoparticles on PDA, compared with others. WA-CV-WA13B showed the highest inhibition effect compared to other silver nanoparticles. Effect of silver nanoparticles on the growth of sclerotium-forming species Rhizoctonia solani, Sclerotinia sclerotiorum and S. minor, revealed that silver nanoparticles effectively inhibit the hyphal growth in a dose-dependent manner. Further, the microscopic observation of hyphae exposed to silver nanoparticles showed severe damage and resulted in the separation of layers of hyphal wall and collapse of fungal hyphae (Min et al., 2009). A recent study on in vitro and in vivo efficacy of silver nanoparticles against powdery mildew before and after disease outbreak in plants under different cultivation conditions, showed maximum inhibition of fungal hyphae and conidial germination with less concentration of nanoparticle on cucumbers and pumpkins (Lamsal et al., 2011b).

Silica nanoparticle: Silicon (Si) increases disease resistance and stress resistance in plants (Brecht et al., 2004). It also stimulates the physiological activity and growth of plants (Carver et al., 1998). Table 2 reports some of the recent applications of nanomaterials in agrochemical and genetic material delivery to plants. Torney et al. (2007) used honeycomb mesoporous silica nanoparticle (MSN) system with 3nm pores to deliver DNA and chemicals into plant cells and intact leaves. They loaded the gene and its chemical inducer into the MSN system and capped the ends with gold nanoparticles and studied the release pattern of chemicals and induction of gene expression in the plants under controlled-release conditions. Their study showed an application of silica nanoparticles in target-specific delivery of proteins, nucleotides and chemicals in plant biotechnology.

Copper nanoparticle: Copper-based fungicides produce highly reactive hydroxyl radicals which can damage lipids, proteins, DNA, and other biomolecules. It plays an important role in disease prevention and treatment of large variety of plants (Borkow and Gabbay, 2005). Complexation of copper with chitosan nanogels was shown to have strong synergistic effect between chitosan and copper in inhibiting the growth of phytopathogenic fungus *Fusarium graminearum*. Because of its bio-compatibility, these nanohydrogels are included as a new generation of copper-based bio-pesticides and it could also be developed into an efficient delivery system for copper based fungicides for plant protection (Brunel *et al.*, 2013). Low melting point soda-lime glass powder containing copper nanoparticles showed efficient antimicrobial activity against gram-positive, gram-negative bacteria, yeast and fungi, key reason for the increased antimicrobial activity is because of inhibitory synergistic effect of the Ca²⁺ lixiviated from the glass (Esteban-Tejeda *et al.*, 2009).

Zinc nanoparticle: Mechanism of action of zinc nitrate derived nano-ZnO on important fungal pathogen Aspergillus fumigatus showed hydroxyl and superoxide radicals mediated fungal cell wall deformity and death due to high energy transfer (Prasun Patra. and Goswami, 2012). Zinc oxide nanoparticles (ZnO NPs) could be used as an effective fungicide in agricultural and food safety applications. Recent study by He et al. (2011) showed significant inhibition of two postharvest pathogenic fungi Botrytis cinerea and Penicillium expansum with ZnO NPs with sizes of approximately 70 nm at less concentration, their mode of action was confirmed by SEM and Raman spectroscopy. ZnO nanoparticles cause deformation of fungal hyphae and prevent the conidiophores and conidial development which ultimately leads to the death of fungal hyphae.

Nano composites: Silver has been studied as an antibacterial agent and less as an antifungal agent. Pinto *et al.* (2013) described preparation and antifungal activity of composite films of pullulan and Ag nanoparticles (NP) against *Aspergillus niger* as a model system. They found that these composite films show strong inhibitory action on fungal sporulation, which was confirmed by disruption of the spores cells when observed under SEM. Silver in an ionic state exhibits high antimicrobial activity (Thomas and McCubbin, 2003). Park *et al.* (2006) developed a new nano-sized Silica-Silver composite for control of various plant diseases. Composite showed good antifungal activity where pathogens disappeared from the infected leaves within three days of spraying and the plants remained healthy thereafter. They also

attempted to determine the effective concentration of composites and also used it effectively for suppression of growth of many pathogens. Nano composites showed 100% growth inhibition of *Pythium ultimum*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides*, *Botrytis cinerea* and, *Rhyzoctonia solani* at 10 ppm concentration. Whereas, *Bacillus subtilis*, *Azotobacter chrococuum*, *Rhizobium tropici*, *Pseudomonas syringae* and *Xanthomonas compestris pv. vesicatoria* showed 100% growth inhibition at 100 ppm concentration.

In our recent study, we have used chitosan–silver nanoparticle (chitosan -Ag Np) composite (size distribution from 10 nm to 15 nm) for inhibition of conidial germination in *Colletotrichum gloeosporioides*. We observed complete inhibition of spore at 100μ g/ml concentration of the composite, but chitosan alone did not show significant inhibition at the same concentration. We also found the shrinking of spores and other morphological changes in the spores treated with CS-Ag nanocomposite (Fig.1) (Chowdappa *et al.*, unpublished data).

Nanotechnology in pest management

Globally insect pests cause a huge crop loss of 14% and plant pathogens cause an estimated loss up to 13% with a value of US \$2,000 billion per year (Pimentel, 2009). Nano materials are used efficiently for safe administration of pesticides, herbicides, and fertilizers at low er doses (Kuzma and VerHage, 2006). Pesticides cause adverse effects on human health and on pollinating insects. So, nanomaterials play an important role in decreasing toxicity and in turn help in increasing the efficacy of pesticides (Mousavi and Rezaei, 2011). Nano pesticide formulations increase the solubility of poorly soluble active ingredient and helps in releasing the active ingredient slowly. The bioavailability of poorly watersoluble agrochemicals can be increased through the use

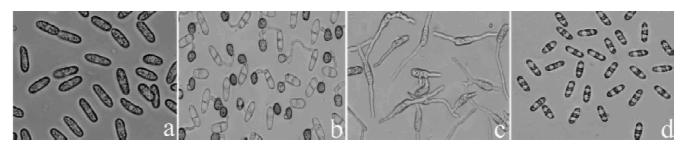


Fig 1. Effect of chitosan-AgNP composite on conidial germination of *Colletotrichum gloeosporioides*a) Normal conidia b) Water control with 0.1% (v/v) acetic acid (appressoria formation) c) Conidial germination in chitosan (100 μ g/ml) with 0.1% (v/v) acetic acid d) Complete inhibition of conidial germination by chitosan-AgNP composite at concentration of 100 μ g/ml.

of additives or by nanoparticulate formation of agrochemicals (Kah et al., 2012). Nanoparticles are loaded with pesticides and released slowly based on environmental trigger (Lauterwasser, 2005). Because of their high reactivity at nanoscale compared to their bulk counterparts, lesser quantity of nanocides show enhanced effect in crop protection (Debnath et al., 2011). Silva et al. (2011) synthesized chitosan alginate nanoparticles (635 nm) for delivering herbicide -paraquat. They show the association of paraquat with nanoparticles and also noted significant differences between the release profiles of free paraquat and the loaded alginate/chitosan nanoparticles. Loading of paraquat into nanoparticle has greater significance in reducing negative impact caused by paraquat. Insects synthesize some natural nanostructures which possess temperature dependent ferromagnetic resonance. In insects, magnetic material is present in the head, thorax and abdomen region. In social insects these magnetic nanoparticles act as geomagnetic sensors (Esquivel, 2007). Nanoencapsulation of chemical such as an insecticide and pesticide for slow and efficient release to a particular host plant for insect pest control allows the chemical to be properly adsorbed by plants (Scrinis and Lyons, 2007).

Rotenone, a water-insoluble botanical insecticide used to control aphids, trips, acari from decades, however its effective utilization has limited due to its poor water solubility, stability, degradation and isomerization when exposed to sunlight. Lao et al. (2010) successfully synthesized nanoparticles by embedding octadecanol-1glycidyl ether to amino groups and sulphate to hydroxyl groups with novel amphiphilic chitosan derivatives N-(octadecanol-1-glycidyl ether)-O sulphate chitosan (NOSCS), with particle sizes of 167.7 to 214.0 nm and zeta potential of -45.0 to -51.9 mV. They found that loading of insecticide rotenone into a nanoparticles was increased up to 13000 times compared to free rotenone in water and solubility in NOSCS micelles aqueous solution was increased, their study showed a way to encapsulate and slow release of water-insoluble agrochemicals in pant protection. As a cheap and reliable alternative for control of insect pests, nanostructured alumina was successfully employed in controlling two major stored insect pests Sitophilus oryzae L. and Rhyzopertha dominica (F.), on wheat (Stadler et al., 2010). Debnath et al. (2011) attempted to determine the efficacy of surface functionalized silica nanoparticle against rice weevil Sitophilus oryzae. They found that silica nanoparticle treated stored rice was not affected by pest even after 2 months of treatment, they also concurred that amorphous form of nanoparticles were

found to be highly effective in controlling this insect pest. Additionally Kah et al. (2012) have reviewed application of various nanoformulations used in enhancing the effect of pesticides such as microemulsion, nanoemulsion, nanodispersion, polymer-based nanoparticle, solid lipid nanoparticle, clay, porous hollow silica nanoparticles, layered double hydroxides and metal based nanoparticles for plant protection. Encapsulation of biocides into liposomes may prolong the action of these agents on plants. Liposomes may prolong the action of active biocides in plants by reducing the fast washing from the plant surface and can be effectively used to deliver essential nutrients and biocides to plant (Lasic, 1993). Wang et al. (2007) demonstrated the potential use of water-insoluble pesticide, β -cypermethrin (β -CP), into the nano and microemulsion system to increase the stability of sprayed solution compare with commercial β -CP. This system is found to be very effective in reduction of active pesticidal ingredients in waterinsoluble pesticide delivery. Barik et al. (2008) summarized the recent developments, application and mechanism of action of nanosilica as nanopesticide. Goswami et al. (2010) studied the effects of oxides nanoparticles like aluminium oxide, zinc oxide, titanium dioxide and silver nanoparticles against insect pests and pathogens. Nanosilica showed 100% mortality against insect pests whereas nanosulfur inhibited the sporulation and growth of fungi. For the first time Stadler et al. (2010), reported the insecticidal effect of nanostructured alumina on two globally important stored insect pests Sitophilus oryzae L and Rhyzopertha dominica (F.). They showed significant mortality rate on treated wheat after 3 days of continuous exposure. They also suggested that inorganic nanostructured alumina can be used as a cheap and reliable alternative for pest management. Pesticides can be effectively loaded into nanoparticles and can be slowly released related to an environmental trigger, they have uniform and extremely small droplet sizes (Forgiarini et al., 2001). Nanomaterials have low viscosity, high kinetic stability and optical transparency which make them smart and efficient delivery systems for many industrial applications (Lee and Tadros, 1982). The use of nanoparticles was found to be a viable alternative to conventional pesticides in combating pests which have developed pesticide resistance.

Nanoparticles in post-harvest disease management

Ever increase in human population, depleting natural resources and emergence of new resistant pathogens has made the supply of sufficient and healthy food a daunting

task. This problem might be magnified several folds in near future. Now, there is a need to increase production efficiency and decrease post-harvest wastage with application of emerging technologies like biotechnology and nanotechnology in post-harvest products. Nanotechnology has been effectively applied in agricultural and horticultural products by increasing shelf life, controlling growth of microorganisms by nanofilms and coatings, controlling influence of gases and the harmful rays (UV), using Nano biosensors for detection of quality and spoilage (Yadollahi et al., 2009). Nanotechnology can be applied in postharvest operations such as drying, storage and preservation of agricultural products. Chitosan, a deacetylated derivative of chitin, is found to be very effective in reducing postharvest decay of fruit and vegetables (Liu et al., 2007). Chitosan at a concentration of 1 g /L has found to be very effective in reducing the growth of several phytopathogenic fungi causing post-harvest spoilage of fruit and vegetables (Hirano, 1997). Yu et al. (2012) investigated the effect of 1% chitosan film with 0.04% nano-silicon dioxide on the qualitative properties of harvested jujube after 32 d of storage under ambient temperature, they studied the related defence enzymes in fruits and found that coated sample showed lower red indices, decay incidence, respiration rate, and weight loss. Shi et al. (2013) studied the novel chitosan/nanosilica hybrid film and its effect on preservation quality of longan fruits under ambient temperature. Coating extended shelf life, reduced browning index, retarded weight loss and inhibited the increase of malondialdehyde amount and polyphenoloxidase activity in fresh longan fruit.

Liu et al. (2009) investigated the effects of nano silver (with 2-5 nm diameters) on post-harvest shelf life of cut gerbera (Gerbera jamesonii) cv. Ruikou flowers. They observed that, pulsing for 24 h with 5 mg/L nano solution extended vase life and inhibited the bacteria growth in vase solution for initial 2 days when observed in vitro under microscope. A postharvest treatment of nano silver was extensively studied. Nano silver was found to be very significant in prolong the vase life by inhibiting the growth of bacteria on several cut flowers with different cultivar variety, including Rose, Gerbera and Acacia holosericea, (Lü et al., 2010; Li et al., 2012; Liu et al., 2012; Mohsen Kazemi., 2012; Nazemi Rafi and Ramezanian, 2013). In our recent study, we have evaluated the applicability of the CS-Ag Np composite as a fruit coating material to inhibit the growth of C. gloeosporioides associated with mango anthracnose. We found that nanocomposites showed a significant effect in reducing the percentage of rotting fruit tissue (71.28% at 1% concentration). The addition of 0.1% non-ionic surfactant tween 80 enhanced the wettability and adhesion property of coating solution and exhibited significant disease reduction compared to control (84.55% at 1% concentration). Thus, these nanocomposites can be utilized as coating material in preventing quiescent infections of *C. gloeosporioides* on mango to prevent post-harvest losses (Chowdappa *et al.*, unpublished data). Nanomaterial has important implication in management of postharvest diseases. Research findings showed the better applicability and advantages of nanopacking materials over conventional normal packing material on physicochemical and physiological quality of stored fruits, vegetables and other horticultural crops.

Going green with nanomaterials

With the potential adverse effects of agro-chemicals on human health and ecosystem the use of green technology to prevent the environmental damage has become major concern by research community. Green synthesis reduces the use of hazardous substances during the synthesis and protects the environment (Table.3). Nanotechnology is an important contribution to green chemistry; it helps in development of microscopic and submicroscopic devices with less cost and provides huge savings in materials (Badami, 2008). A group of researchers have successfully used, photosynthesis protein units (PSI) isolated from leafy vegetables and plants to form a biohybrid photoelectrochemical cell that converts light energy into electrical energy, it was interesting to note that this biohybrid devices display remarkable stability at ambient conditions up to one year (Ciesielski et al., 2010). Nanotechnology has a revolutionary impact on agriculture including pest management in future. Nanoencapsulation ensures promise in crop protection against insect pests and pathogens. nanotechnology plays an important role in green revolution (Bhattacharyya et al., 2010). With the effective application of emerging technologies like microemulsions, liposomes and nanoemulsions in agrochemicals formulations, use of petrochemical solvents can be reduced in effective delivery of herbicides, fungicides and pesticides (Castro et al., 2013). Recently, Tokarek et al. (2013) reported one-step green synthesis of chitosan-stabilized copper nanoparticles.

Application of silver nanoparticles in agriculture has made researchers to focus on synthesis of silver nanoparticles by chemical, electrochemical,

Nanomaterial	Application	Functions	Reference
Nano silver	Antibacterial activity of nano- silver against <i>Xanthomonas</i> <i>campestris</i> pv.campestris towards the control of cabbage black rot in the pot.	Dose dependent study of nanosilver on the <i>X.campestris</i> pv.campestris showed significant reduction of cabbage black rot in the pot experiment.	Gan <i>et al.</i> (2010)
Vålidamyc in loaded nano sized calcium carbonate	Controlled release of validamycin loaded nano sized calcium carbonate (50 to 200 nm) against <i>Rhizoctonia solani</i> .	The formulation showed better germicidal efficacy Tagainst <i>Rhizoctonia solani</i> compared to conventional technical validamycin when studied after7 days. The nanoformulation showed the release of validamycin upto 2 weeks.	Qian <i>et al.</i> (2011)
Thiamine di-lauryl sulfate (TDS) nanoparticles	Antifungal activity of TDS nanoparticles (258.6 nm) against <i>C. gloeosporioides</i> associated with pepper anthracnose.	Nanoparticles at 100 ppm concentration showed 80% growth inhibition of <i>C. gloeosporioides</i> compared to the control. TDS nanopaticles penetrated inside the hyphal cell membrane and destructed the cells.	Seo <i>et al.</i> (2011)
Chitosan nanoparticles (CS NPs)	Efficacy of CS NPs on fungal growth and chilli seed quality.	CS NPs at a concentration of 0.6% (w/v) significantly delayed mycelial growth of <i>Rhizopus</i> sp. <i>Colletotrichum capsici</i> , <i>C. gloeosporioides</i> , and <i>Aspergillus niger</i> when compared with control.	Chookhongkha et al. (2012)
Nanoc opper	Antibacterial activity against <i>Xanthomonas</i> axonopodis pv. punicae, the causative of pomegranate bacterial blight	Nanocopper inhibited the growth of <i>Xanthomonas axonopodis</i> at 0.2 ppm, i.e., >10,000 times lower than that usually recommended for Cu-oxychloride.	Mondal and Mani (2012)
Light activated nanoscale formulations of TiO ₂	Nanoscale for mulations of TiO ₂ with Ag and Zn on <i>Xanthomonas</i> <i>perforans</i> towards the control of bacterial spot of tomato	Compared to control, TiO_2/Ag and TiO_2/Zn had high photocatalytic activity against <i>X. perforans</i> and the combination significantly reduced bacterial spot severity without causing any adverse effects on tomato yield.	Paret <i>et al.</i> (2012)
Copper nan oparticles	Copper nanoparticles Cu-based NPs (11–55 nm) were tested on tomato (<i>Lycopersicon esculentum</i>) against <i>Phytophthora infestans</i> for their antifungal activity.	The synthesized Cu-based NPs were more effective than the commercial agrochemicals at lower concentrations and drastically reduced the active ingredient rate. The particles were not showed phytotoxicity on treated plants.	Giannousi et al. (2013)

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Table 1. Nanomaterials for control of plant pathogens

DNA directed silver nanoparticles on graphene oxide	Antibacterial activity of nanoparticles against <i>Xanthomonas perforans</i>	Nanopartic les at 100 ppm concentration severely reduced the bacterial spot disease compared to untreated tomato transplants in greenhouse condition without causing phytotoxicity.	Ocsoy et al. (2013)
Light-ac tivated nanoparticle formulation of Titanium dioxide with zinc	Nanocomposite for management of bacterial leaf spot on Rosa 'Noare'	Field applications of TiO_2/Zn nanoformulation at H'500 to 800 ppm on Rosa 'Noare' significantly reduced bacterial spot severity compared with the untreated control and other commercial bactericides.	Paret <i>et al.</i> (2013)
Chitosan based nanoparticles (chitosan, chitosan- saponin and Cu-chitosan nanoparticles)	In vitro evaluation of Chitosan based nanoparticles against Alternaria alternata, Macrophomina phaseolina and Rhizoctonia solani.	Cu-chitos an nanoparticles were most effective at 0.1% concentration and showed 89.5, 63.0 and 60.1% growth inhibition of <i>A. alternata</i> , <i>M. phaseolina</i> and <i>R. solani</i> , respectively. Nanoparticles were very effective in inhibiting spore germination.	Saharan <i>et al.</i> (2013)

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Table 2. Application of nan	Table 2. Application of nanomaterials as delivery vehicles in plants	plants	
Nanomaterial	Application	Functions	Reference
Honeycomb Mesoporous silica nanoparticles (MSN)	Delivery of DNA and chemicals into plants	MSN system (3nm pores) loaded with gene and its chemical inducer can be successfully delivered to plant cells and triggered gene expression in the plants under controlled release conditions.	Torney <i>et al.</i> (2007)
Fluorescence starch- nanoparticle coated with poly-L-lysine	As plant transgenic vehicle	DNA-nanoparticle (50–100 nm) complexes bind and transport genes across the cell wall of plant cells by inducing instantaneous pore channels in cell wall, cell membrane and nuclear membrane with the help of ultrasound treatment.	Liu <i>et al.</i> (2008)
Single-walled carbon nanotubes (SWNT)	Molecular transporters for walled plant cells	SWNT penetrated the cell wall and cell membrane of intact plant cells and successfully delivered different cargoes into different cell organelles.	Liu <i>et al.</i> (2009)

Magnetic carbon-coated nanoparticles	Absorption and translocation of nanoparticles through the root of different crop plants	Nanoparticles were penetrated through the root of pea, sunflower, tomato and wheat. Particles reach the vascular cylinder, through transpiration and spread through the aerial part of the plants in less than 24 hours	Cifuentes <i>et al.</i> (2010)
Ultra small Anatase TiO ₂ Alizarin Red S Nanoconjugates	Study of uptake and distribution of nanoconjugates in the plant model system- <i>Arabidopsis thaliana</i>	Nanoconjugates traversed cell walls, entered into plant cells, and accumulated in specific subcellular locations such as cell vacuoles and nuclei.	Kurepa <i>et al.</i> (2010)
ZnS nanoparticles modified with positively charged poly-L-lysine(PLL)	Delivering DNA into plant cell	Successfully delivered β -glucuronidase encoding plasmid DNA into tobacco cells by means of ultrasound assisted method.	FU <i>et al.</i> (2012)
Gold functionalized mesoporous silica nanoparticle (Au-MSN)	Protein and plasmid DNA codelivery to plant cells Via- biolistic method	Protein-loaded Au-MSN (10 nm pores) was coated with plasmid DNA and delivered into plant tissues through particle bombardment. The delivery and release of protein and plasmid DNA was detected in the same plant cells.	Martin Ortigosa et al. (2012)
Functionalized mesoporous silica nanoparticles	Gene delivery	Foreign DNA is delivered into intact <i>Arabidopsis thaliana</i> roots without applying any mechanical force. Gene expression was observed in the epidermal layer and in the endodermal root tissues, conformed by both fluorescence and antibody labelling.	Chang <i>et al</i> . (2013)
Mesoporous silica nanoparticles functionalised with amine cross-linked fluorescein is othiocyanate.	Delivery of biomolecule into plants	The uptake and distribution of nanoparticles (20 nm) were observed in wheat, lupin and <i>Arabidopsis</i> during seed germination, in roots of plants grown in a hydroponic system and in whole leaves of plants via vacuum infiltration.	Hussain <i>et al.</i> (2013)
Plant Bio-transformable HMG-CoA reductase gene loaded calcium phosphate nanoparticle (Cap)	Encapsulation of plasmid DNA into nanoparticle to enhance the stability and frequency of the genetic transformation in plants	DNA releasing in acidic media showed, initial slow release followed by fast release of Cap nanoparticles. It was stable at ambient temperature and humidity. It significantly enhanced the transformation efficiency and antioxidant activity of the biotransformed plants compared to normal plant	Sohadi <i>et al.</i> (2013)

Nanoparticle	Microrganism	Reference
Cadmium sulfide	Schizosaccharomyces pombe	Kowshik et al. (2002)
nanoparticles	E. coli	Sweeney et al. (2004)
	Rhodopseudomonas palustris	Bai et al. (2009)
Gold nanoparticles	Verticillium	Mukherjee et al. (2001)
	Fusarium oxysporum	Mukherjee et al. (2002)
	Thermomonospora	Ahmad et al. (2003)
	Trichothecium	Ahmad et al. (2005)
	Rhodopseudomonas capsulata	He et al. (2007)
	Pseudomonas aeruginosa	Husseiny et al. (2007)
	Bacillus licheniformis	Kalishwaralal et al. (2009)
	Penicillium	Du et al. (2011)
	Shewanella oneidensis	Suresh et al. (2011)
Gold and Silver	Brevibacterium casei	Kalishwaralal et al. (2010)
Nanoparticles	Bacillus Subtilis	Reddy et al. (2010)
	Chrysosporium tropicum	Soni and Prakash (2012)
Magnetite	Actinobacter	Bharde et al. (2005)
nanoparticles	Theroanaerobacter ethanolicus and Shewanella	Roh et al. (2006)
Selenium nanoparticles	Klebsiella pneumoniae	Fesharaki et al. (2010)
Silver	Fusarium semitectum	Basavaraja et al. (2008)
	Alternaria alternata	Gajbhiye et al. (2009)
	Staphylococcus aureus	Nanda and Saravanan (2009)
	Escherichia coli and Aspergillus niger	Kathiresan et al. (2010)
	Aspergillus clavatus	Verma et al. (2010)
	Phytophthora infestans	Thirumurugan et al. (2011)
	Penicillium	Nameirakpam et al. (2012)
	Trichoderma	Devi et al. (2013)
TiO ₂ nanoparticles	Lactobacillus and Sachharomycescerevisae	Jha et al. (2009)
	Aspergillus flavus	Rajakumar et al. (2012)
Titanium nanoparticles	Lactobacillus	Prasad et al. (2007)
Zinc oxide nanoparticles	Aspergillus aeneus	Jain et al. (2013)

 Table 3. Green synthesis of metallic nanomaterials by microorganisms

photochemical, and now via green synthesis route. Chemical methods of synthesis are simpler than physical methods, but they leave toxic residual agents in the final product and cause environmental toxicity. As a convenient and cleaner approach, green synthesis has a potential impact in safe delivery of agrochemicals for crop protection. Recently, Hettiarachchi and Wickramarachchi (2011) reported the synthesis of chitosan stabilized silver nanoparticles using gamma ray irradiation. Singh et al. (2010) demonstrated the cost effective and environment friendly technique for green synthesis of silver nano particles (30nm) by conversion of silver nitrate solution into silver nanoparticles with weed leaf extract of Argemone maxicana as reducing and capping agent. The antimicrobial activity of synthesized nanoparticles was commendable. They also found that, reduction of silver ions into silver nanoparticles was due to the participation of leaf proteins and metabolites. Stable silver nanoparticles can also be synthesized with the leaf extract of Eucalyptus hybrid and Cycas, (Dubey et al., 2009; Jha and Prasad, 2010). MubarakAli et al. (2011) reported the plant extract mediated synthesis of silver and gold nanoparticles. They also studied its antibacterial activity against clinically isolated pathogens Staphylococcus aureus and Escherichia coli. Apart from plant extracts, microorganisms can also be effectively used in synthesis of silver nanoparticles in a cost effective, eco-friendly, economic and efficient way. Bacteria and fungi can be effectively used to control the synthesis of metallic nanoparticles (Mandal et al., 2006). Pathogenic organisms such as Cryphonectria and Phytophthora infestans were exploited for the synthesis of silver nanoparticles (Thirumurugan et al., 2009; Dar et al., 2013). Sharma et al. (2009) reviewed the green synthesis of silver nanoparticles with different synthesis procedures such as mixed-valence polyoxometallates, polysaccharide, tollens, irradiation, and biological method. They also discussed the mechanism of silver nanoparticles bactericidal activity and their interaction with bacterial cell membranes. Table 3 shows some of the recent advancements in application of green synthesis methods to synthesize metallic nanomaterials by different microorganisms. Metal oxide semiconductor nanostructures help in degradation of organic pesticides and industrial pollutants into nontoxic and useful components through photo catalysis in a greener way. It offers great dividends in detection and removal of contaminants from water and soil sources (Baruah and Dutta, 2009).

The synthesis of stable metal based nanoparticles with environment friendly and convenient green route by

using biopolymers, plant extracts and microorganisms has attracted the researchers to adopt green nanotechnology, because of its nontoxicity, cost effective eco-friendly synthesis at large-scale and can be used very effectively against phytopathogenic microorganisms. It acts as a viable alternative for traditional chemical synthesis procedures.

Limitations and issues

Nanotechnology has a significant role to play in agriculture, food processing, food packaging, food security and water purification. But it may pose negative effects on the environment, ecosystem, and humans. The potential risks associated with releasing nanomaterials into the environment (soil and water organisms) are still unclear by scientists. Recent findings showed the potential harmful effects of nanomaterials on the digestive systems of a beneficial soil organism -earthworm (Ruitenberg, 2013). Xu et al. (2010) summarized the increased safety concerns over application of nanomaterials in food and agriculture. They emphasized their study on main exposure routes and determinants of nano toxicities involving particle size, surface, structure, chemical composition, and dosage. Griffitt et al. (2008) reported the toxic effect of metallic nanomaterials on aquatic organisms. They have taken zebrafish, daphnids, and an algal species as a model organism and exposed them to silver, copper, aluminum, nickel, and cobalt in both soluble salts and nanoparticle form. They noted that, nanosilver and nanocopper cause toxicity in all tested organisms at median lethal concentrations. With the technological advancement, large amount of engineered nanomaterials are reaching environment through consumer and commercial products. Colman et al. (2013) studied the adverse impact of nano-silver on plants and microorganisms. They found that nanosilver treatment led to changes in microbial community composition, biomass, extracellular enzyme activity, and affected some ground plant species. All nanostructures will not cause the same toxicity risks. Biopolymers, liposomes and natural organic compounds based nanocapsules such as lipids and chitin are potentially less hazardous than heavy metals nanoparticles (Perez-de-Luque and Rubiales, 2009). Current important limitation in application of nanocarriers to agriculture is the production scale and cost. Large scale manufacturing of nanomaterials and its effective application to agriculture will bring down the cost to a drastic range.

There are many gaps and unresolved problems and new challenges concerning the biological effects of nanoparticles. Monica and Cremonini (2009) reported

both positive and negative effects of nanoparticles on higher plants. With the rapid growth in nanotechnology, there is an urgent need for right regulation over the indiscriminate use of nanomaterials and their effective disposal. With the increasing nanomaterial production and their subsequent release into the environment, a concern has been increased on their effect on ecosystem health. Many commercial nanomaterials pose greater toxicity risks than the same materials in their larger particle form, if a substance has been approved in bulkform; it remains legal to sell it in nano form. The potential application of nanomaterials in different agricultural applications needs further research investigation with respect to synthesis, toxicology and its effective application at field level. Nanotechnology has got applicability in various fields, but research and development is still at bench-top scale. Great efforts are required in commercialization of nanomaterials for agricultural applications, which requires proper protection needs, testing priorities, risk assessment and regulatory guidance at global level (Chen and Yada, 2011).

CONCLUSION

Nanotechnology holds the promise of controlled delivery of agrochemicals to improve disease resistance, plant growth enhancement and nutrient utilization. Nanoencapsulation shows the benefit of more efficient and targeted use of pesticides, herbicides and insecticides in environment friendly greener way. Research and development in post-harvest nanotechnology can help in preservation of the freshness and quality and prevent diseases in a relatively safer way. With the advancement of nanotechnology, application of green chemistry in synthesis of nanomaterials by using plant extracts and living cells has reduced the use of toxic solvents and guarantees ecoprotection. Nanotechnology in conjunction with biotechnology has significantly extended the applicability of nanomaterials in crop protection and production. Even though the toxicity of nanomaterials has not yet clearly understood, it plays a significant role in crop protection because of its unique physical and chemical properties. The application of nanomaterials is relatively new in the field of agriculture and it needs further research investigations. Barring the miniscule limitations, nanomaterials have a tremendous potential in making crop protection methodologies cost effective and environmental friendly.

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