

Review Article

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Application of Nano-technology in Plant Pathogen Managements: Knowledge and Gaps

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ABSTRACT

Nano-particles have wide potential in plant disease diagnosis and as an eco-friendly mode of disease management. Nano-scale platforms, biological sensors, miniature detection devices and nano-sensors could play a significant role in pathogen detection and disease management in the future. In this article various nano-particles utilized in disease management and the possibility of large scale adaptability of nano-particles by integrating into present practices and avoiding crop losses reviewed. Study identifies existing efficacy of anti-microbial activities of silver, silicon-silver, Zinc-iron-magnesium oxides, copper, validamycin, chitosan and iron nano-particles. Seven different types of gaps were identified for use of these particles in pathogen managements and these were pertains to pathogen types, hypothetical mode of actions, application of these particles at phyllosphere and rhizosphere, environmental stability and economics of nano-particles.

Keywords

Knowledge and Gaps
Nano-technology, Nano-particles, Plant protection, Plant disease management, Mode of actions

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Introduction

Plant diseases caused by parasitic and non-parasitic agents is one of the major factors limiting crop production and productivity. Among the total crop losses caused by different sources, 14.1% are lost due to plant disease along the total annual worldwide crop loss from plant disease is about \$220 billion. Commercial agricultural relies heavily upon high input of agrochemicals to protect crops

against pathogens and pests. The continuous and unchecked use of pesticides and fungicides has caused the resistance in pests and plant pathogens, thus leading to serious effects (Patel *et al.*, 2014). The pathogen resistance against fungicides is increasingly becoming a serious threat to crops. So, now is the time to seriously think of the use of these practices because of their critical health and environmental effects. Also, with the increased access to digital technology,

consumers have developed more awareness about the use of fungicides and their negative attributes. Thus, scientists and farmers across the globe are trying hard to minimize such hazardous effect of fungicides and other chemical control measures and switching over to other safer technologies.

Nano-biotechnology is a new branch of biology that has originated due to the compatibility of nano-sized inorganic and organic particles with biological functions. 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 (Sharon *et al.*, 2010, Sharma *et al.*, 2012, Mathur, 2014 and Roy *et al.*, 2014). Nano-particles are highly stable and biodegradable, and can be successfully employed in production of nano-capsules for delivery of pesticides, fertilizers, and other agrochemicals (Jha *et al.*, 2009).

Nano-particles can either be directly modified for use in pathogen detection or for a diagnostic tool to detect compounds indicative to a diseased condition (Ghormade *et al.*, 2011). Nano-particles display slow release of encapsulated functional molecules and reduce their frequent applications. The disease diagnosis, pathogen detection and residual analysis may become much more precise and quick with the use of nano-sensors (Mathur and Vyas, 2013, Khan and Rizvi, 2014). Thus, in crop protection, uses of nano-particles can be bifurcate into application of these for pathogen detections and for management of plant pathogens.

In present review article an effort has been carried out to summarize the uses of various types of nano-particles for plant pathogen management and their related knowledge and gaps.

Existing Knowledge

Plant diseases can be controlled using nanotechnology by controlled release of encapsulated fungicides/pesticide, and other agrochemicals in protection against pests and pathogens. The potential application of nano-materials in crop protection helps in the development of efficient and potential approaches for the management of plant pathogens. Several studies were conducted using nano-sized particles to control fungal pathogens such as *Pythium ultimum*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides*, *Botrytis cinerae* and *Rhizoctonia solani*, as well as pathogenic bacteria including *Bacillus subtilis*, *Azotobacter chroococcum*, *Rhizobium tropici*, *Pseudomonas syringae* and *Xanthomonas compestris* pv. *vesicatoria* (Park *et al.*, 2006).

Silver Nano-Particles

Silver can affect various microorganisms and their biological processes (Donnell and Russell, 1999; Sondi and Sondi, 2004; Pal *et al.*, 2007) and also inhibits protein expression (Yamanaka *et al.*, 2005). The use of nano-sized silver particles as antimicrobial agents has become more common as technological advances make their production more economical. Silver has potential applications in management of plant diseases. Since silver exhibits multiple modes of inhibitory action to microorganisms (Park *et al.*, 2006; Roe *et al.*, 2008) it may be used for controlling various plant pathogens in a relatively safer way as compared to synthetic fungicides. In a study Ki-Jo *et al.*, (2009) found it to inhibit colony formation of both *Bipolaris sorokiniana* and *Magnaporthe grisea*. Silver ions and nano-particles effectively reduced leaf spot and gray leaf spot on perennial ryegrass without noticeable phytotoxicity. The inhibitory effect on colony formation significantly diminished after silver cations were neutralized with

chloride ions. Kim *et al.*, (2009) studied the antifungal effectiveness of colloidal nano-silver (1.5 nm average diameter) solution, against rose powdery mildew caused by *Sphaerotheca pannosa* var. *rosae*. The nano-silver colloidal solution at the concentration of 5000 ppm sprayed at large area of 3306 m² polluted by rose powdery mildew. Two days after the spray more than 95% of rose powdery mildew faded out and did not recur for a week. The antifungal activity of the silver nano-particles was evaluated on the *Colletotrichum gloeosporioides*, which is responsible for anthracnose in a wide range of fruits. Silver nano-particles significantly reduced the mycelia growth of *Colletotrichum gloeosporioides* in a dose-dependent manner (Aguilar *et al.*, 2011).

Antifungal properties of silver nano-particles, silver ions, acrylate paint and cotton fabric impregnated with silver nano-particles were assessed against *Aspergillus niger*, *Aureobasidium pullulans* and *Penicillium phoeniceum* (Khaydarov *et al.*, 2011). Bioassay of elemental and nano-sulphur against *Aspergillus niger* showed that nanosulfur was more efficient than its elemental structure (Choudhury *et al.*, 2010). Lamsal *et al.*, (2011) applied Silver nano-particles (10, 30, 50, and 100 ppm) on the pepper infected with *Colletotrichum* 3~4 wk before the disease outbreak. 100 ppm concentration of silver nano-particles produced maximum inhibition of the growth of fungal hyphae as well as conidial germination. The highest antifungal properties were observed in the case of treatment with 50 ppm silver nano-particles in field trials and 100 ppm silver nano-particles *in vitro*. Kim *et al.*, (2012) applied three types of silver nano-particles (AgNPs) at concentrations of 10, 25, 50, and 100 ppm concentration for controlling *Alternaria laternata*, *A. brassicicola*, *A. solani*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Corynespora cassiicola*,

Cylindrocarpon destructans, *Didymella bryoniae*, *Fusarium* spp., *Glomerella cingulata*, *Monosporascus cannonballus*, *Pythium aphanidermatum*, *P. spinosum* and *Stemphylium lycopersici* presented on various hosts. They reported that WA-CV-WB13R types of AgNPs showed maximum inhibition of most fungi on PDA with 100 ppm of AgNPs. Elamawi *et al.*, (2013) sprayed silver nano-particles in concentrations 0, 25, 50, 100 and 200 ppm on rice seedling (*Magnaporthe grisea*) leaves at three times (3 hours before inoculation, 1 and 5 days after artificial inoculation with spore suspension) and found significant reduction in colony formation. Othman *et al.*, (2014) showed that AgNP synthesized through *Aspergillus terreus* (KC462061) effectively controls the all five isolates of *A. flavus* and maximum inhibition was achieved with 150 ppm.

Cavity slide experiment to quantify antagonistic potential of AgNPs against *Bipolaris sorokiniana* causing spot blotch disease in wheat with their conidial concentration of 2,4 and 10 µg/ml was conducted with Mishra *et al.*, (2014) and reported that 2, 4 and 10 µg/ml concentrations of bsAgNPs accounted for complete inhibition of conidial germination, whereas in the absence of bsAgNPs, conidial germination was 100%. Mendes *et al.*, (2014) accessed five different concentrations (i.e., 5, 50, 180, 270 and 540 µg ml⁻¹) of silver colloidal NP against *Phomopsis* sp in soyabean seeds. They reported that as the concentration of silver nano-particles increases, colony formation was reduced. 90% inhibition was observed with a 180 µg ml⁻¹ concentration while 270 and 540 µg ml⁻¹ showed 100% reduction in the radial growth of fungal mycelia. Ouda (2014) tested antifungal properties of AgNPs, CuNPs and Ag/CUNPs (0, 1, 5, 10 and 15 mg L⁻¹ of nano-particles were applied for 2, 3, 5 and 6 incubation days) for *Alternaria alternate* and *Botrytis cinerea*. 15 mg L⁻¹ concentration

of silver nano-particles produced maximum inhibition of the growth of fungal hyphae. Additionally AgNPs had a detrimental effect on sugar, protein, n-acetyl glucosamine and lipid of culture filtrate and cell wall components of both pathogens. Dose-dependent study of nano-silver on *Xanthomonas campestris* pv. *campestris* (causing cabbage black rot) showed significant reduction of cabbage black rot in the pot experiment (Gan *et al.*, 2010).

Sunhemp rosette virus treated with solutions of the silver nano-particles at a concentration of 50 mg/L. After 30 sec, a 10 μ L droplet was deposit on a carbon coated nickel TEM grid and exposed to a 2.5% solution of PBS/glutaraldehyde vapours for 30 min. Leaves inoculated with (SHRV) showed complete suppression of the disease. Results suggested that silver nano-particles bind to the virus body and inactivates the virus by inhibiting virus replication in host plant Jain and Kothari (2014).

Silicon Nano-Particles

Silicon (Si) is known to be absorbed into plants and to increase disease resistance and stress resistance (Mao *et al.*, 2001 and Brecht *et al.*, 2004). Aqueous silicate solution, used to treat plants, is reported to exhibit excellent preventive effects on pathogenic microorganisms causing powdery mildew or downy mildew in plants thus used for treating diseased plants.

Moreover, it promotes the physiological activity and growth of plants and induces disease and stress resistance in plants. But, since silica has no direct disinfection effects on pathogenic microorganisms in plants, it does not exhibit any effect on established diseases. Further, the effects of silica significantly vary with the physiological environment and thus, it is not registered as an

agricultural chemical. As mentioned above, silver is known as a powerful disinfecting agent. It kills unicellular microorganisms by inactivating enzymes having metabolic functions in the microorganisms by oligodynamic action (Kim *et al.*, 2009).

A new composition of nano-sized silica-silver for control of various plant diseases has been developed by Park *et al.*, (2006), which consists of nano-silver combined with silica molecules and water soluble polymer, prepared by exposing a solution. Park *et al.*, (2006) also studied the 'effective concentration' of nano-sized silica-silver on suppression of growth of many fungi; and found that, *Pythium ultimum*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides*, *Botrytis cinere* and, *Rhizoctonia solani*, showed 100% growth inhibition at 10 ppm of the nanosized silica-silver. Whereas, *Bacillus subtilis*, *Azotobacter chroococcum*, *Rhizobium tropici*, *Pseudomonas syringae* and *Xanthomonas compestris* pv. *vesicatoria* showed 100% growth inhibition at 100 ppm. They have also reported chemical injuries caused by a higher concentration of nano-sized silica-silver on cucumber and pansy plant.

Ocsoy *et al.*, (2013) observed that DNA-directed silver nano-particles grown on graphene oxide (GO) composites effectively decrease cell viability in culture and on plants of *Xanthomonas perforans* causing bacterial spot of tomatoes in Florida while the pathogen has developed resistance to Cu fungicides.

These compounds (Ag@dsDNA@GO) show excellent antibacterial activity in culture at a very low concentration of 16 ppm with higher adsorption rate. Severity of tomato bacterial spot is significantly reduced by application of Ag@dsDNA@GO at 100 ppm in greenhouse when compared to untreated and showed no phytotoxicity.

Zinc, Titanium and Magnesium oxide Nano-Particles

The potential biocidal efficacy of ZnO and ZnTiO₃ nano-powders against the fungus *Aspergillus niger* was assessed by Rufolo *et al.*, (2010). ZnTiO₃ nano-powder showed higher growth inhibition efficiency than ZnO. Wani and Shah (2012) and utilized Nano-suspension (0.0 to 0.5 ml) of Zinc and Magnesium oxide against *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus*. They concluded that Nano-Mgo at highest concentration was most effective in reducing the spore germination followed by nano-Zno. Hafez *et al.*, (2014) utilized 30, 15, 7.5, 3.75, 1.87 and 0.938 mg/ml concentration of zinc nano rods towards controlling bacterial strains of *Pectobacterium carotovorum* subsp. *Wasabiae*, *P. atrosepticum*, *Dickeya chrysanthemi*, *D. solani* and *D. dianthicola*. They found that 30 mg/ml ZnO suspension concentration showed largest inhibition zone. Paret *et al.*, (2013) reported that light-Activated Nanoscale Formulations of TiO₂ significantly reduced disease incidence of Bacterial Spot of Tomato (*Xanthomonas perforans*).

Copper Nano-Particles

Antibacterial properties of nano-copper (20, 16, 8, 4, 2, 0.5, 0.4, and 0.2 ppm concentrations of nano-coppers) against pomegranate bacterial blight (*Xanthomonas axonopodis* pv. *Punicae*) was studied by Mondal and Mani (2012) and reported that nano-copper suppressed xap growth at 0.2 ppm, i.e., >10,000 times lower than that usually recommended for Cu-oxychloride.

Validamycin Nano-Particles

Controlled release of validamycin loaded nano-sized calcium carbonate (50 to 200 nm)

against *Rhizoctonia solani* was tested by Qian *et al.*, (2011). Nano-encapsulation of thiamine dilauryl sulfate (TDS), a vitamin B1 derivative, (at 50 and 100 ppm concentrations for 0 to 24 hours) for inhibition of the spore germination and mycelia growth of *Fusarium oxysporum* f. sp. *Raphani*. Study showed highest mycelia growth inhibition activity with 100 ppm TDS (Chao *et al.*, 2013). Entrapping TDS solution in the nano size of 150 nm or smaller increases the surface area acting on the spores of *F. oxysporum*, as well as the spore penetration capability of TDS, thus generating a control effect greater than that of general pesticides acting on mycelia as reported by Seo *et al.*, (2011).

Chitosan Nano-Particles

Chookhonghka *et al.*, (2013) tested Chitosan nano-particles for four pathogens namely *Rhizopus* sp. *Colletotrichum capsici*, *C. gloeosporioides*, and *Aspergillus niger*. Chitosan polymer and chitosan nano-particles at a concentration of 0.6% w/v significantly delayed mycelial growth of *Rhizopus* sp. *Colletotrichum capsici*, *C. gloeosporioides*, and *Aspergillus niger* when compared with 0.15% w/v captan, 0.2% w/v chitosan nano-particles, and the control (PDA). Similarly Pabobn-Baquero *et al.*, (2015) studied the chitosan NP (0.5, 1.0, 2.0, and 4.0 mg /ml⁻¹) on *Fusarium equiseti* and *Curvularia lunata* present on *Latropha curcas*, and their study revealed that Chitosan completely inhibited sporulation of *C. lunata* and spore germination of *F. equiseti*. Inoculation with *F. equiseti* and *C. lunata* reduced seed germination of *J. curcas* by 20 and 26.6 %, respectively. However, application of chitosan before inoculation inhibited pathogenic activity. Therefore, chitosan did not affect seed germination and caused inhibitory effects on *F. equiseti* and *C. lunata*. Chowdappa *et al.*, (2013) observed an inhibition of conidial germination in *Colletotrichum*

gloeosporioides with chitosan-silver nano-particle (chitosan-Ag Np) composite (size distribution from 10 nm to 15 nm).

Iron Nano-Particles

“Smart Delivery Systems” for agriculture can possess timely controlled, spatially targeted, self-regulated, remotely regulated, pre-programmed, or multi-functional characteristics to avoid biological barriers to successful targeting. In order to develop smart treatment-delivery system in plant, Gonzalez-Melendi *et al.*, (2008) worked with *Cucurbita pepo* plants, which were treated with carbon-coated Fe nano-particles in vitro. The magnetic core consisting of Fe nano-particles allow themselves to be guided to a place of interest in the body (affected part) of an organism using small magnets that create a magnetic field. The carbon coating provides biocompatibility and acts as a surface for adsorption where various types of molecules of interest (drug/DNA/chemical/enzyme) can be adsorbed.

Some Mode of Actions of Nano-Particles Knowledge and Gaps

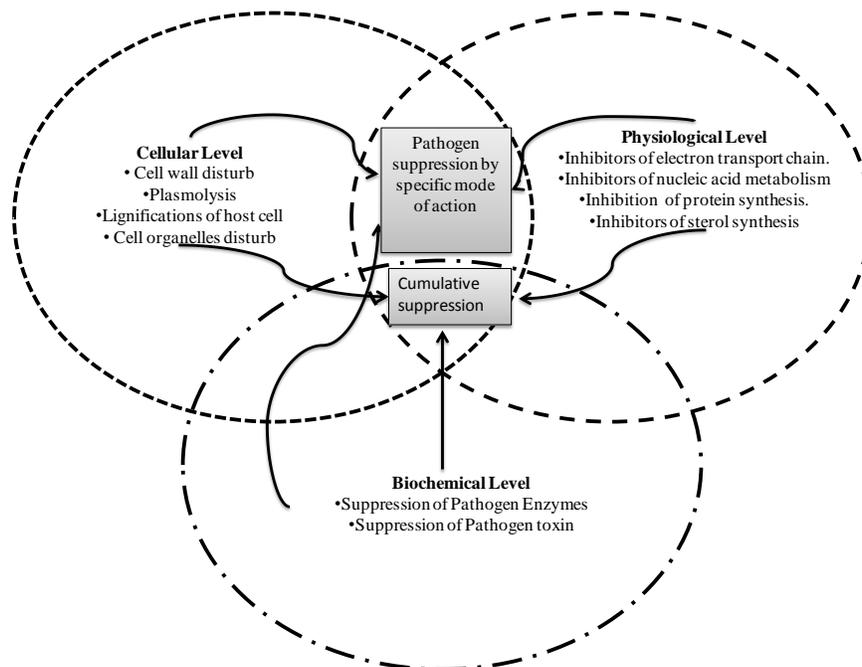
Nano-particles can be applied directly in soil or these particles can be utilized as carriers of some chemicals like pheromones, SAR inducing chemical, polyamine synthesis inhibitors or even (Khand and Rizvi, 2014).

Silver Nano-Particles

Nanometer-sized silvers possess different properties, which might come from morphological, structural, and physiological changes (Nel *et al.*, 2006). Indeed, several lines of evidence support the enhanced efficiency of silver nano-particles on antimicrobial activity. Silver nano-particles are highly reactive as they generate Ag⁺ ions while metallic silver is relatively un-reactive

(Morones *et al.*, 2005). It was also shown that the nano-particles efficiently penetrate into microbial cells, which implies lower concentrations of nano-sized silver would be sufficient for microbial control. This would be efficient, especially for some organisms that are less sensitive to antibiotics due to the poor penetration of some antibiotics into cells (Samuel and Guggenbichler, 2004). Silver has strong bactericidal and inhibitory effects. Silver nano-particles, which have high surface area and high fraction of surface atoms, have high antimicrobial effect as compared to the bulk silver. A previous study observed that silver nano-particles disrupt transport systems including ion efflux. The dysfunction of ion efflux can cause rapid accumulation of silver ions, interrupting cellular processes at their lower concentrations such as metabolism and respiration by reacting with molecules. Also, silver ions are known to produce reactive oxygen species via their reaction with oxygen, which is detrimental to cells, causing damage to proteins, lipids, and nucleic acids (Hwang *et al.*, 2008). Study suggested that silver nano-particles can significantly delay mycelial growth of *C. gloeosporioides* in a dose-dependent manner in vitro (Aguilar-Mendez *et al.*, 2011). Antifungal efficiency of silver nano-particles was observed at 24 h after inoculation, suggesting that direct contact of silver with spores or germ tubes is critical in inhibiting disease development (Young *et al.*, 2009). Moreover, antifungal efficiency of silver was also observed at 5 days after inoculation, suggesting that silver nano-particles could have penetrated the plant cell wall and inhibited the disease development. (Elamawi and Shafey, 2013). Elamawi *et al.*, (2013) reported that AgNP severely damaged *Magnaporthe grisea* hyphal walls, resulting in the plasmolysis of hyphae. Considering many cellular effects of silver ions, silver nano-particle-mediated collapse in pathogen hyphae is probably not only by damaging hyphal walls, but also other cellular effects.

Fig.1 Potential mode of actions of different nano-particles



The preventative and post-inoculation application of the silver nano-particles effectively reduced disease severity on plants at all concentrations. A mechanism of this antifungal activity is suggested by the direct effect on germination and infection process in the fungi. *M. grisea* can cause foliar disease and reproduce as asexual conidia.

Lamsal *et al.*, (2011) evaluated different concentrations of AgNPs against powdery mildew and reported that the treatment should be given to the plants before appearance of the symptoms on the host plants. According to Kim *et al.*, (2012) inhibition efficiency of AgNP positively associates with concentrations of AgNPs. They hypothesized that this could be due to the high density at which the solution was able to saturate and cohere to fungal hyphae and to deactivate plant pathogenic fungi. Ag NPs synthesized thorough *Aspergillus terreus* (KC462061) inhibited the growth of *A. flavus* by disturbing cellular functions which caused deformation in fungal hyphae. AgNPs cause decrease in

spores number, abnormality and hypertrophy, these special effects lead to destroyed and damaged of spores (Othman *et al.*, 2014). Dose dependent impact of nano-silver on *Xanthomonas campestris* pv. *campestris* could destroy the cell membrane and increase the cell conductivity of the tested bacteria (Gan *et al.*, 2010). Mishra *et al.*, (2014) synthesized AgNPs by using *Serratia* sp. and evaluated these AgNPs against spot blotch disease in wheat caused by *Bipolaris sorokiniana*. They hypothesized that the treatment of bsAgNPs enhanced lignifications which could have worked as a hindrance against pathogen attack in plants treated with pathogen and bsAgNPs in combination (B4), while least lignin deposition in pathogen challenged plants (BC) favoured pathogen attack. Ocoy *et al.*, (2013) on their study found leaf-spot disease caused by *Xanthomonas perforans* (Cu resistant) can be inhibited by DNA-directed silver (Ag) nano-particles (NPs). The *in vitro* studies and nano-particle-treated plants demonstrated that at 16 ppm the growth was inhibited, which provides

evidence of remarkable antibacterial activity against *X. perforans*. The disease was significantly reduced, when 100 ppm Ag@dsDNA@GO was applied in green house experiment.

Zinc, Titanium and Magnesium oxide Nano-Particles

Wani and Shah (2012) correlated the anti-fungal properties of ZnO and MgO against *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus* with suppression of pathogen enzymes and toxins. For Bacterial strain *Pectobacterium carotovorum* subsp. *Wasabiae*, *P. atrosepticum*, *Dickeya chrysanthemi*, *D. solani* and *D. dianthicola*, Hafez *et al.*, (2014) had hypothesized that the synthesized ZnO nano-rods may be diffused to enter the bacterial cells through the cell walls and the pelli, and inhabited the mitochondrial DNA and the ribosomes as well.

Numerous studies on the mechanism of action of TiO₂ identified three possible modes of action, including (i) direct oxidation of coenzyme A, which inhibits cell respiration leading to cell death; (ii) change in cell permeability; and (iii) cell wall damage followed by cytoplasmic membrane damage. Plant cells might also be expected to be susceptible to this chemical effect. Paret *et al.*, (2013) theorize that the physical size of plant cells and, in particular, the thickness of their cell walls may exceed the lethal radius of diffusion of the free radicals generated by TiO₂, affording selectivity compared with their pathogens. *X. perforans* has a cell wall thickness of ≈10 nm and overall cellular dimensions of roughly 500 by 1,500 nm. In an interesting study, Palmqvist *et al.*, (2015) used Titanium NPs to understand the interaction between *Bacillus amyloliquefaciens*, a plant growth promoting bacterium and the host plant *Brassica napus*

for providing protection against *Alternaria brassicae*.

Copper Nano-Particles

Cioffi *et al.*, (2004) studied antifungal activity of nano-copper against plant pathogenic fungi. They screened the antifungal activity of the three nano-composites performed on *Saccharomyces cerevisiae* yeast using a two-step protocol. Antifungal activity correlates with the electrothermal atomic absorption spectroscopy (ETAAS) analysis of the copper released by the nano-composites in a yeast-free culture broth. Result demonstrated that the releasing properties of such nano-composites can also be controlled by a proper modulation of the CuNPs loading. It was also found that comparable amounts of Cu. Copper nano-particles have been reported an important role in pathogen inhibition. It is reported that antifungal activity of copper nano-particles against *Fusarium oxysporium* and found antifungal activity of bavistin increases in combination with CuNPs in the cases of *Fusarium oxysporium*. Sahar *et al.*, (2014) reported antifungal activity of silver (AgNPs), copper (CuNPs) and silver/copper (Ag/CuNPs) nano-particles against two plant pathogenic fungi *Botrytis cinera* and *Alternaria alternate*.

Chitosan Nano-Particles

Increased activity of defence enzymes in leaves of chitosan treated turmeric plants may play a role in restricting the development of disease symptoms. The eliciting properties of chitosan make chitosan a potential antifungal agent for the control of rhizome rot disease of turmeric. Increase in chitinase and chitosanase activity may play a role in enhanced resistance in turmeric plants against *P. aphanidermatum* infection. (Anusuya and Sathiyabama, 2014). Interestingly, Anusuya and Sathiyabama (2014) applied chitosan

nano-particles to induce antifungal hydrolyses in turmeric plant (*Curcuma longa*) found correlation between reduction on incidence of rhizome-rot and enhanced activity of defense enzymes such as peroxidases, polyphenol oxidases, protease inhibitors and β -1, 3-glucanases.

Gaps and Conclusion

Majority of the studies conducted for pathogens belonging to phylum Ascomycota (includes *Aspergillus niger*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Colletotrichum gloeosporioides*, *Corynespora cassicola*, *Curvularia lunata*, *Cylindrocarpon destructans*, *Didymella bryoniae*, *Fusarium* Spp., *Magnaporthe grisea*, *Monosporascus cannonballus*, *Penicillium phoeniceum*, *Saccharomyces cerevisiae*, *Sclerotinia sclerotiorum* and *Sphaerotheca pannosa* var. *rosae*). Few studies on genera belonging to Proteobacteria like *Dickeya chrysanthemi*, *Pectobacterium carotovorum*, *Xanthomonas campestris* pv. *Campestris* and *Xanthomonas perforansi* and Zygomycota genera like *Mucor plumbeus* and *Rhizopus stolonifer* were conducted. While *Pythium aphanidermatum* (Oomycetes) and *Rhizoctonia solani* (Basidiomycota) were studied in one representative studies each.

These trends suggested that majority of the research works focused on fungi that producing specialized external asexual conidia and thus, forcing to conclude that nano-particles might choosing the easiest site in their target pathogen. However, such generalized statement need to explore. Gaps were also pertains to implication of this novel technology for management of obligate pathogens likes rust fungi and *Phytophthora*, facultative saprophytes like *Ustilago* spp., bacteria like *Pseudomonas* and ds DNA and RNA viruses, phytoplasma etc.

Most of the suggested modes of actions of nano-particles are hypothetical therefore; efforts should be directed toward exploration of their suppression behaviour at physiological, biochemical and cellular levels (figure 1).

Further role of these highly reactive particles as elicitors for inducing host resistance also need exploration for different host-pathogen complex.

At field level, applications of nano-particles or nano coated materials at phyllosphere or at rhizosphere and their subsequent behaviour on these applied sites should be figure out. As far as present status is concern, in most research these particles were applied as curative one, however, research required for production of some preventive nano-coated material so that epidemic avoidance can be attempted.

Also as observed, that the use of Carbon Nano Tubes have enhanced the plant growth in tomato, while another study using carbon nano-tubes depicted that it had inhibitory effect on root elongation in tomato whereas in onion and cucumber it showed enhancement in root elongation (Can~as *et al.*, 2008).

Thus, large-scale application of CNTs still needs review and further experimentation. Some other studies have also depicted the toxic effect of multi-walled carbon nano-tubes (MWCNT's) in plant cells and application of MWCNTs was found to be responsible for accumulation of reactive oxygen species (ROS) and subsequently decreased cell proliferation and cell death (Tan *et al.*, 2007 and 2009). Based on the positive as well as negative effects of CB NPs it can be stated that the response of plants or plant cells to NPs varies with the plant species, stages of growth and the nature of the NPs. Further research on nano-sciences is needed to reveal

the most efficient and useful NPs combinations for betterment of agriculture.

Despite the numerous potential advantages of nanotechnology in plant protection it has not yet made its way commercially in our diseased fields. Firstly, Nanotech products require high initial investments and secondly large scale field use is a pre requisite for its application. And there are numerous reports of nano-materials biosynthesis from plant pathogens. Nanotechnology can play as a catalyst for enhancing agricultural growth rate. Many countries across the globe are pursuing Research & Development for nano-technological application in agriculture to nullify the toxic effects of chemicals used in field. The broad implication of nanotechnology for society can be grouped into two categories, namely environmental, health and safety implication and societal dimensions.

In the future, nano-scale devices with novel properties could be used to make agricultural systems “smart”. A rapidly growing body of toxicological studies suggest that a number of nano-materials may be toxic (Kahru and Dubourguier, 2010), yet there are currently no regulations that limit nano-material exposures (Powell *et al.*, 2008). It is widely recognized that the environmental impacts of nano-materials need to be understood, although a number of laboratory trials have measured acute toxicity and sub lethal effects of engineered nano-particles on organisms. Some of these nanotechnologies may help reverse environmental degradation or may replace the more toxic technologies currently in use. Others may end up substituting a new problem for an old one. It is important that we develop the scientific tests and models that can distinguish the alternatives. Though, exact a priori predictions of all environmental impacts from controlled laboratory conditions cannot be determined. The toxicity of nano-

materials has to be clearly understood before its commercialization and field application. The potential application of nano-materials in different agricultural applications needs further research 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. Barring the miniscule limitations, nano-materials have a tremendous potential in making crop protection methodologies cost-effective and environmental friendly.

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