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NANOTECHNOLOGY AND ITS APPLICATIONS IN PLANT DISEASE MANAGEMENT: A REVIEW

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ABSTRACT

Nanotechnology is the creation of materials, devices or system with new properties or functions through manipulation of matter at the nano scale. Application of nanotechnology in the field of agriculture is achieved through fertilizers that increase plant growth and yield and nanopesticides for the management of pests and diseases; and nanosensors to monitor soil quality and plant health. As nanotechnology requires less inputs and producing less quantity of wastes than conventional products, it enhances the efficiency and sustainability of agricultural practices. Nanoparticles are the new weapons for battling plant diseases and provide crop protection by acting either as protectants or carriers. Metal nanoparticles such as silver, copper, zinc oxide, and titanium dioxide have been intensively researched for their antibacterial, antifungal and antiviral properties. This review paper discusses the green synthesis, characterization and potential applications of various nanoparticles in detection of plant pathogens and plant disease management. Additionally, it mentions the research gap or obstacles that must be overcome to fully harness its potential for sustainable plant disease management.

Key words: Disease management, Nanoparticles, Nanotechnology, Pathogens.

Introduction

World population is increasing rapidly and has been expected to reach about 10 billion by 2050 so to feed increasing mouth require increase in food production by 50%. At present, large quantity of agricultural inputs are used to maximize agricultural production. One data suggests that globally annual production exceeds 3 billion tonnes and requires 2.7 trillion cubic meters of water, 187 million tonnes of fertilizer, 2 quadrillion British thermal units (BTU) of energy, and approximately 4 million tonnes pesticides (Kah *et al.,* 2019). Conventional pesticides are strongly associated with environmental degradation and health hazards whereas nanotechnology provides promising responses to these multiple challenges due to higher efficacy of nano-active ingredients that allows the reduction of pesticide volumes, thus lowering costs and increasing yields. Nanoparticles are divided into different categories on the basis of their properties and the materials from which they are derived, but at present, in agriculture, nanoparticles of metals, non-metals, metallic oxide, metalloids, carbon nanomaterials, quantum dots, liposomes, dendrimers have been used (Elmer and White, 2018). There are several nanoparticles that are utilized in plant disease management among them only silver, copper and zinc have received much attention. Silver and copper nanoparticles are toxic to pest and pathogens as they act directly as antimicrobial agents while boron, silicon, copper and zinc nanoparticles activate defense mechanisms by altering the nutritional status of the host plant. Significance of nanotechnology has been realized at national and international level and in several countries have set committee and groups to support and monitor nano-technological advancements and to harness its benefits for the mankind. The Indian government has set up a Nano Research Project during the Eleventh Five Year Plan (2007-2012).

History of Nanotechnology

The ideas and concepts of nanoscience and nanotechnology was first given by renowned physicist Richard Feynman in his famous talk "There's Plenty of Room at the Bottom, on 29 December, 1959 at California Institute of Technology. Due to his contribution in nanotechnology, he is also known as "The Father of Nanotechnology" and awarded with noble prize in Physics in 1965. The term nanotechnology was first given by Norio Taniguchi in 1974 (Agrawal and Rathore, 2014). Term "Nano" is derived from the Greek word meaning "dwarf" or very small.

Synthesis of Nanoparticles

Nanopartcles is a microscopic particle where size is measured in nanometers. Nanoparticles are synthesized by three methods namely physical, chemical, and biological through two fundamental approach i.e. top down approach and bottom up approach. Top down approach involves creation materials at nano-scale through physical or chemical breaking down of larger materials. It is having long execution time whereas bottom up approach is the process of assembling nano materials atom-by-atom or molecule-by-molecule or arrangement of smaller components into more complex form. Bottom up approach is having short execution time. The physical and chemical methods for syntesizing nanopaticles are time consuming and tedious. Chemical methods include the use of hazardous chemicals that may cause adverse effects on the users. So, synthesis of nanoparticles must be achieved through ecofriendly, easy and rapid methods. The biological agents such as plants species and microbes (fungi, yeast, bacteria, plant virus) and algae have emerged as a cost effective, easy, ecofriendly and efficient candidates for the synthesis of nanoparticles by green synthesis approach as it elliminates the use of toxic chemicals (Bora *et al.,* 2022; Munir *et al.,* 2023). Because of its non-toxicity, cost effective, eco-friendly synthesis at large scale green nanotechnology attracted the researcher and can be used effectively for the management of several phytopathogens. It may act as a viable alternative for traditional chemical synthesis procedure.

Plant extracts are well known for suppressing plant pathogens and managing plant diseases because plants are rich source of several bioactive compounds like phenols, flavonoids, ascorbic acids, ketones, aldehydes, carboxylic acids, terpenoids, and amides. These compounds act as the reducing agents and stabilizers in the green synthesis of nanomaterials (Singh *et al.,* 2018). So, extracts different plants like *Ocimum tenuiflorum, Solanum tricobatum, Citrus limon, Azadirachta indica, Avena sativa, Centella asiatica, Helianthus annus, Brassica juncea, Medicago sativa, citrus sinensis* etc. are currently utilized for the synthesis of gold, silver, zinc, cobalt, copper, and nickel nanoparticles (Marchiol, 2012). Potential antimicrobial activities were also reported against both gram-positive and gram-negative bacteria when treated with copper oxide (CuO) nanoparticles synthesized from *Malva sylvestris (*Awwad *et al.,* 2015). Silver nanoparticles synthesized from plant extract of *Acalypha indica* were found effective against soil-borne pathogens (Krishnaraj *et al.,* 2010).

Although a large number of microorganisms are used for biological synthesis of nanoparticles, fungi are more precisely utilized as natural 'nanofactories' because of its easy downstream processing, easy in handling, and less amenable to genetic manipulation compared to prokaryotes and their ability to secrete a large number of enzymes. Bacterial species like *Pseudomonas stuzeri, Desulfovibrio desulfuricans, Clostridium thermoaceticum* and *Klebsiella aerogans* are involved in synthesis of several nanoparticles. Similarly, plant viruses mainly of spherical/icosahedral group are considered as the examples of naturally occurring nanomaterials or nanoparticles. Their ability to infect, deliver nucleic acids genome to a specific site in host cell, replicate, package nucleic acid and come out of host cell precisely in an orderly manner make them appropriate to be utilized in the field of nanotechnology. Also, algae belonging to the class Cyanophyceae, Chlorophyceae, Phaophyceae, and Rhodophyceae are used in the synthesis of silver, gold and other metallic nanoparticles. Due to the presence of bioactive compounds such as pigments and antioxidants in their cell extracts, algae are utilized in the synthesis of diverse nanomaterials.

Characterization of Nanoparticles

Characterization of nanoparticles after synthesis is also necessary to establish an understanding and control of its synthesis and applications. It is characterized by using several techniques such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIS), Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Energy-dispersive spectroscopy, atomic force microscopy (AFM), UV-visible spectrophotometry, Thermo gravimetric analyzer on the basis of its origin, shape, size, dimension, color, atomic composition, dispersivity, surface area, surface morphology, surface charge, magnetic properties, crystallinity and several other physical, chemical and biological properties of the synthesized nanoparticles (Ahmad *et al.,* 2019; Banerjee *et al.,* 2021). In a colloidal suspension Brownian movement of nanoparticles is determined by dynamic light scattering (DLS). The electrostatic interaction of nanoparticles with bioactive compounds depends on the surface charge, and the zeta potential value. XRD provides data about the crystalline structures of the nanoparticles. Different structural attributes of nanoparticles are determined by using nuclear magnetic resonance (NMR). UV-visible spectroscopy gives an idea about nanoparticles' optical properties by measuring the light reflected by the nanoparticles and also determines the stability of nanoparticles in the colloidal suspension (Mourdikoudis *et al.,* 2018).

Nano-formulations

Nano-formulations like nanoemulsions, nanoencapsulates, nanocontainers and other nano delivery techniques are used in plant protection. Nanoparticles are highly stable and are biodegradable and it can be successfully employed in production of nanocapsules for delivery of pesticides, fertilizers, and other agrochemicals. Nanoparticles displays slow release of encapsulated functional molecules and reduce its frequent applications.

Potential applications in Plant Pathology

Plant pathogens are well known threats and predominantly targets plants products, thus nanoparticles play a crucial role in early detection and plant disease management. Its application in plant pathology has brought advances in detection and eradication of pathogens even while they are in minute densities.

Detection of plant pathogens

Detection of infected plant parts as well as pathogen at initial stage is very important for the success of any sanitation practice that can save tons of food from possible damages by diseases (Elmer and White, 2018). Nanotechnology offers significant advances in this area of diagnosis through development of faster and more sensitive pathogen probes. Recently, the use of portable nanodevices helps to detect different environmental pollutants, pathogens, insects, diseases, and agrochemicals; consequently, it decreased the use of pesticides, fungicides, and antibiotics (Sharon *et al.,* 2010). Rapid detection of diverse plant pathogens such as bacteria, virus, fungus and nematodes can be achieved by the use of nanoparticles as a diagnostic tool (Yao *et al.,* 2009). In this context, superparamagnetic iron oxide nanoparticles (Ahmadov *et al.,* 2014), magnetic nanoparticles (as it attaches the biological tissues or DNA), nanosensors, nanochips and quantum dots (QDs) have been used as diagnostic tool for detection of phytopathogens. Magnetic nanoparticles including superparamagnetic iron oxide and QDs can be used as a disease diagnostic tool as both of these found to be attached with hyphae. QDs have emerged as pivotal tools for detecting pathogen with extreme accuracy.

Nanobiosensors are small, portable, rapid response and processing, accurate which can overcome the deficits of present sensors. Basically, biosensors are made up of a signal receptor that receives signals from the surrounding environment and a signal transducer. There are three components of nanobiosensors namely probe, bioreceptor, and transducer. Furthermore, biosensors act as an important diagnostic tool in the field of plant pathology as it produces an electric signal on coming in contact with a pathogen, electric signal increase with increase in density of the pathogen and this interaction is converted into a digital output. Nano-based biosensors increased the sensitivity of pathogen detection and disease diagnosis in comparison to conventional ELISA techniques in plants (Baac *et al.*, 2006; Perdikaris *et al.*, 2011). Different studies have reported the potential of antibody-based biosensors for the detection of the presence of tobacco mosaic virus (TMV), lettuce mosaic virus, and cowpea mosaic virus (Chartuprayoon *et al.*, 2013; Lin *et al.*, 2014). Similarly, QDs and FRET-based biosensors were used to detect the beet necrotic yellow vein virus in *Polymyxa betae* (Safarpour *et al.*, 2012). Bio-sensor consisting of nano-Au with single-stranded oligonucleotides were used for the detection of genomic DNA of *Ralstonia solanacearum* in the soil (Khaledian *et al*., 2017). In stress conditions plant accumulates several stress-related hormones. An investigation of Shang *et al.*, (2019) reported the detection of plant pathogenic fungus by using gold electrode nanosensor and copper nanoparticles by sensing salicylic acid concentrations in the soil. Nanobiosensors combined with a global positioning system (GPS) was used for real-time monitoring of the agricultural field. This technology offers early detection of the pathogen and gives data about crop growth and environmental pollutants (Bergeson, 2010; Khot *et al.*, 2012). So, nanobiosensors supports sustainable agriculture by increasing crop yield and suppressing diseae development. Nanochips are type of microarrays that contains florescent oligo capture probes through which the hybridization can be detected (Lopez *et al.*, 2009). It is known for its high sensitivity and

Table 1: Green Synthesis of Nanoparticles.

Plant-based nanoparticles		
Nanoparticles	**Plant name and plant parts used for synthesis**	**References**
Silver nanoparticles	*Argemone mexicana* (Leaf extract)	Singh *et al.*, 2010
	Cashew apple (Anacardium occidentale L.) Juice	Mukunthan and Balaji, 2012
	Emblica offcinalis (Fruit extract)	Ramesh *et al.*, 2015
Gold nanoparticles	*Musa paradisiaca* (Peel extract)	Banker *et al.*, 2010
	Coleus amboinicus Lour. (Leaf extract)	Narayanan and Shakthivel, 2010
	Allium cepa (Plant extract)	Parida *et al.*, 2011
Palladium nanocrystal	*Curcuma longa* (Tuber extract)	Sathishkumar *et al.*, 2009
Copper nanoparticles	*Punica granatum* (Peel extract)	Kaur *et al.*, 2016
Zinc oxide nanoparticles	*Solanum nigrum* (Leaf extract)	Ramesh *et al.* 2015
Silver and gold nanoparticles	*Aloe barbadensis* (Plant extract)	Chandran *et al.*, 2006
	Chenopodium album (Leaf extract)	Dwivedi and Gopal, 2010
	Azadirachta indica (Leaf extract)	Kora *et al.*, 2012
Nickel, Silver, Gold and Copper oxide nanoparticles	*Medicago sativa* (Whole plant)	Mittal *et al.*, 2013
Fungi-based nanoparticles		
Nanoparticles	**Fungal species**	**References**
Gold nanoparticles	*Verticillium* sp.	Mukherjee *et al.*, 2001
	Trichothecium	Ahmad *et al.*, 2005
	Penicillium sp.	Zhang *et al.*, 2009; Du *et al.*, 2011
Silver nanoparticles	*Pleurotus* spp.	Gade *et al.*, 2007
	Fusarium semitectum	Basavaraja *et al.*, 2008
	Pleurotus sajor caju	Nithya *et al.*, 2009
	Alternaria alternata	Gajbhiye *et al.*, 2009
	Penicillium sp.	Singh *et al.*, 2014
	Aspergillus oryzae (MTCC No. 1846)	Phanjom and Ahmed, 2015
	Trichoderma harzianum	Guilger *et al.*, 2017
Silver, gold and bimetallic nanoparticles	*Neurospora crassa*	Castro *et al.*, 2011
Bacteria-based nanoparticles		
Nanoparticles	**Bacterial species**	**References**
Gold nanoparticles	*Rhodococcus* sp.	Ahmad *et al.*, 2003
	Pseudomonas aeruginosa	Husseiny *et al.*, 2007
Silver nanoparticles	*Clostridium versicolor*	Sanghi and Verma, 2009
	Escherichia coli	Gurunathan *et al.*, 2012
Gold and silver nanoparticles	*Brevibacterium casei*	Kalishwaralal *et al.*, 2010
	Bacillus subtilis	Reddy *et al.*, 2010
Plant-virus based nanoparticles		
Nanoparticles	**Plant virus**	**References**
Gold nanoparticles	*Cowpea chlorotic mottle virus* (CCMV)	Slocik *et al.*, 2005
	Brome mosaic virus	Sun *et al.*, 2007
Iron and platinum nanoparticles	*Cowpea mosaic virus* (CMV)	Shah *et al.*, 2009
Algae-based nanoparticles		
Nanoparticles	**Algae species**	**References**
Silver chloride nanoparticles	*Sargassum plagiophyllum*	Dhas *et al.*, 2014
Silver nanoparticles	*Cystophora moniliformis*	Prasad *et al.*, 2013
	Chlamydomonas reinhardtii	Barwal *et al.*, 2011
Gold nanoparticles	*Sargassum muticum*	Namvar *et al.*, 2015
	Chlorella vulgaris	Annamalai and Nallamuthu, 2016
Iron nanoparticles	*Euglena gracilis*	Brayner *et al.*, 2012
Magnetic iron oxide nanoparticles	*Sargassum muticum*	Mahdavi *et al.*, 2013

specificity in detecting single nucleotide change in bacteria and viruses.

Plant Disease Management

Continuous efforts are made by plant pathologists to find an effective solution that can protect food and agricultural products from various phytopathogens. Nanotechnologists are also playing their important role in plant disease development by improving existing crop protection protocols in short to medium terms (Kah *et al.*, 2013). It is reported that very small amount (less than 0.1%) of pesticides in air during application and as run-off, spray drift, off-target deposition and photo-degradation affecting both the environment and application costs (Pimentel, 1995). Currently, plant diseases are managed through an efficient use of fungicides and insecticides (viral diseases). These chemicals although provides rapid response, easy availability, and reliability in their application. However, chemical pesticides also affect nontarget organisms, and may lead to disease resistance and resurgence of the pest population. Further, pesticides have no long-lasting effects and 90% of used pesticides are lost during or after application (Ghormade *et al.*, 2011). So, it is required to develop cost-effective, efficient pesticides that are less toxic to the environment. Application of nanotechnology has significant potential in crop protection, especially in the plant disease management by controlled and target delivery of several agrochemicals (nanopesticides) and also by providing diagnostic tools for early disease detection.

Nanopesticides are a new field of agriculture technology utilizing nanomaterials for smart delivery of pesticides in protecting plant against diseases and damages caused by pests and pathogens. Nanopesticides are formed by the combination of numerous surfactants, polymers, and metal nanoparticles at nanoscale dimensions (Sekhon, 2014). Nanoparticles may act upon the pathogens in a way similar to chemical pesticides or these nanomaterials can be used as a carrier for active ingredients of pesticides, double-stranded RNA (dsRNA), host defense chemicals for their use as spray application, drenching/soaking onto seeds and over other tissues like root and leaves (Worall *et al.*, 2018). Because of ultra-small size, nanoparticles may hit target virus particularly and may open a new field of virus control in pants. Nanoencapsulated pesticide formulations are applied in crop protection with an intention to pesticide dosage, and prevent their exposure to human health and environment (Nuruzzaman *et al.*, 2016). Further application of nanopesticides is associated with conservation of both water and energy as they are required in lesser amount and less frequently than other agrochemicals used as pesticides. In this way, nanopesticides usages over other agrochemicals are economically safe as they enhance crop productivity by increasing the pesticide use efficiency, reducing the wastages and labor cost. The slow degradation and controlled release of active ingredients in presence of suitable nanoparticles or other nanomaterials can control pests effectively over longer period of time (Chippa, 2017). The solubility and mobility of the active ingredients formulated as nanopesticides are favored and increased by soil-inhabiting microorganisms and therefore, causing lesser drastic impacts on environment than harmful pesticides (Kah and Hofmann, 2014).

Metal Nanoparticles

Metal nanoparticles can be used as new and alternative means against synthetic fungicides because of its multiple mode of inhibition.

Silver nanoparticles (Ag NPs):

Use of nanosize silver particles as antimicrobial agents becomes more common and most studied and utilized nanoparticles with its strong inhibitory, bactericidal as well as broad spectrum of antimicrobial activities (Swamy and Prasad, 2012). Further, Ag NPs were first used in management of plant disease (Richards, 1981). Although the mechanisms of Ag NPs toxicity are not fully characterized but it is believed the Ag^+ ions bind to cysteine-containing proteins on plasma membrane, causing both physiological and biochemical damage that compromise membrane integrity. Effect of Ag NPs on the growth of sclerotium-forming species *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor,* revealed that Ag NPs effectively inhibit the hyphal growth in a dose-dependent manner. Further, the microscopic observations of hyphae exposed to Ag NPs showed severe damage and resulted in the separation of layers of hyphal wall and collapse of fungal hyphae (Min *et al.* 2009). Ag NPs have been deployed as antifungal agent against *Rhizoctonia solani, Alternaria alternata, Macrophomina phaseolina, Curvularia lunata, Sclerotinia sclerotiorum*, and *Botrytis cinerea* (Rafique *et al.*, 2017).

Since silver displays various modes of inhibitory action of plant pathogens, it may be used for controlling various plant pathogens in a moderately safer way compared to synthetic fungicides (Oh *et al.*, 2006; Park *et al*., 2006).

Zinc oxide nanoparticles (ZnO NPs):

With lower toxicity and secondary benefits on soil fertility, ZnO NPs has clear advantage over silver nanoparticles for controlling fungal pathogen (Dimpka *et al.*, 2013). Furthermore, in many studies, Zn nanoformulations have been found very effective against different bacteria as well as fungal plant pathogens including *Botrytis cinerea, A. alternata, Mucor plumbeus, F. oxysporum, R. solani, Penicillium expansum, S. sclerotiorum* and *Rhizopus stolonifera* (He *et al.,* 2011; Indhumathy and Mala, 2013; Sardella *et al.,* 2017). ZnO NPs application leads to deformation of fungal hyphae, prevention of development of conidiophores and conidia which ultimately leads to the death of fungal hyphae. Maximum inhibition of mycelia growth of *Fusarium oxysporum* and *Penicillium expansum* was reported (Yehia and Ahmed, 2013).

Silica nanoparticles:

Application of silicon enhances resistance in plants against various disease and stress (Brecht *et al*., 2004). It also stimulates the physiological activity and growth of plants. Mesoporous silica nanoparticles were used to deliver DNA and chemicals inside plant cells and intact leaves (Torney *et al*., 2007).

Copper nanoparticles (Cu NPs):

In addition to the antimicrobial activity of copper oxide nanoparticles ((CuO NPs) towards many plant-pathogenic bacteria and fungi, Cu NPs could operate by a nutritional mechanism. Copper is an essential micronutrient that plays a pivotal role in growth as well as defense against plant diseases. Copper based fungicides damages lipids, proteins, DNA, and other

Table 2: Nanomaterials for Plant Disease Management.

Types of Nanoparticles	Pathogen/ disease	Effect	References
Silver nanoparticles	*Sphaerotheca pannosa* var. *rosae*	Antifungal effect was shown against pathogen causing powdery mildew in roses.	Kim *et al.*, 2008
	Sclerotium cepivorum	90% inhibition in growth of pathogen (*in vitro*) causing white rot in green onion.	Jung *et al.*, 2010
	Colletotrichum species	Inhibition of growth, conidial germination, and hyphae of pathogen causing anthracnose in pepper.	Lamsal *et al.*, 2011
	Bipolaris sorokiniana	Complete inhibition of conidial germination of given pathogen causing spot blotch in wheat.	Mishra *et al.*, 2014
	Sun-hemp rosette virus	Application Ag NPs on leaves, complete suppression of virus.	Jain and Kothari, 2014
	Bean Yellow Mosaic Virus (BYMV)	Faba bean shown better result against BYMV after 24 hours of inoculation.	Elbeshehy *et al.*, 2015
	Rhizoctonia solani	Inhibition of sclerotia formation and mycelial growth (*in vitro*) and suppression of lesion development in leaves by pathogen causing sheath blight in rice (*in vivo*).	Soltani *et al.*, 2016
	Colletotrichum musae	70% disease suppression in banana fruit.	Jagana *et al.*, 2017
	Tomato spotted wilt virus (TSWV)	Inhibition of local lesions and reduction in TSWV infection.	Shafie *et al.*, 2018
	Pectobacterium carotovorum	controlled soft rot disease in pepper (*Capsicum annum*).	Ayisigi *et al.*, 2020
	Xanthomonas campestris and *X. axonopodis*	Effective against given bacterial pathogen in *Vigna unguiculata.*	Vanti *et al.*, 2019
	Alernaria alternata and Sclerotium rolfsii (in tomato), and *Xanthomonas oryzae* (in rice)	Antifungal activity noticed with 21mm zones of inhibition against *A. alernata*, 17 mm against *S. rolfsii* while antibacterial activity against *X. oryzae* with 15 mm zones of inhibition.	Rana *et al.*, 2023
ZnO, CuO and Ag nanopariicles	*Botrytis cinerea*	Suppression of Grey mold disease in *Prunus domestica* fruits.	Malandrakis *et al.*, 2019
Double stranded DNA directed Ag nanopariicles	*Xanthomonas perforans*	Controlled bacterial spot disease in tomato plant.	Ocsoy *et al.*, 2013

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Fluconazole mixed with Ag nanopariicles	*Cladosporium berbarum*, *Alternaria alternata* and *Fusarium oxysporum*	Exhibited enhanced fungicidal activity in given pathogen.	Bholay *et al.*, 2013; Fajar *et al.*, 2020
Silver and silica (mixture) nanoparticles	*Pseudomonas syringae* pv. *tomato*	Activated defense response & resulted in 25% disease resistance in *Arabidopsis thaliana*.	Chu *et al.*, 2012
Poly dispersed gold nanoparticles	*Barley mosaic virus*	Induced resistance in plantes by melting and dissolving the virus particles.	Alkubaisi *et al.*, 2015
Zinckicide, a formulation of Zinc nanoparticles	*Xanthomonas citri*	Suppressed citrus canker disease.	Graham *et al.*, 2016
	Sclerospora graminicola	Plasmolysis and inhibition of spore germination, 35% reduction in incidence of downy mildew in pearl millet.	Nandhini *et al.*, 2019
Copper nanoparticles	*Xanthomonas axonopodis* pv. *punicae*	Reduction in severity of bacterial blight in Pomegranate (*Punica granatum* L) crop.	Mondal and Mani, 2012
Bordeaux mixture made out from copper nanoparticles	*Xiphinima index*	Inhibited the nematode, causing disease in grape plant.	Elmer and White, 2018
	Xanthomonas oryzae pv. *oryzae* and *X. campestris* pv. *phaseoli*	Highly effective in controlling bacterial blight of rice (*Xanthomonas oryzae* pv. *oryzae*) and leaf spot of mung (*X. campestris* pv. *phaseoli*).	Gogoi *et al.*, 2009
Sulphur nanoparticles	*Fusarium solani, F. oxysporum, Aspergillus niger* and *Venturia inaequalis*	Inhibition of *Fusarium solani* in potato, Effective fungicide and provided protection against given pathogen.	Boxi *et al.*, 2016
TiO_2/Zn nanoparticles	*Xanthomonas* spp.	Suppression of bacterial leaf spot disease in rose.	Paret *et al.*, 2013
Magnesium oxide nanoparticles	*Ralstonia solanacearum*	Controlled bacterial wilt disease by inducing systemic resistance in tomato (*Solanum lycopersicum*).	Imada *et al.*, 2016
Aluminum oxide nanoparticles	*Fusarium oxysporum*	Suppressed *Fusarium* root rot disease in tomato.	Shenashen *et al.*, 2017
Nickel oxide nanoparticles	*Cucumber mosaic virus* (CMV)	Reduction in disease severity and CMV concentration.	Derbalah and Elsharkawy, 2019
Chitosan nanoparticles	*Fusarium* sp., *Botrytis* sp., *Pyricularia grisea*	Shown antimicrobial activity against Fusarium crown, root rot in tomato, Botrytis bunch rot in grapes, and *P. grisea* in rice.	Kashyap *et al.*, 2015
	Alternaria solani and *Fusarium oxysporum*	Inhibition of mycelial growth and spore germination of fungi causing early blight (*A. solani*) and wilt (*Fusarium oxysporum*) in tomato.	Saharan *et al.*, 2013
	Bean mild mosaic virus (BMMV), Tobacco mosaic virus (TMV), Tobacco necrosis virus (TNV)	Shown antiviral effect in beans against BMMV, and in tobacco against TMV and TNV.	Malerba and Cerana, 2016
Fungicide-Loaded Chitosan-Carrageenan Nanoparticles	*Alternaria alternata* and *Sclerotinia sclerotiorum*	Controlled infection potatoes and tomatoes under *in vivo* condition.	Kumar *et al.*, 2022
Thiamine di-lauryl sulfate (TDS) nanoparticles	*Colletotrichum gloeosporoides*	Shown antifungal effect against pathogen causing anthracnose in pepper.	Seo *et al.*, 2011

biomolecules by producing highly reactive hydroxyl radicals. Because of its bio-compatibility, these nano-hydrogels are included as a new generation of copper-based biopesticides and it could also be developed into an efficient delivery system for copper-based fungicides for plant protection (Brunnel *et al.*, 2013). Fungicides formulated from Cu NPs can inhibit the progression of *Phytophthora infestans* in the tomato plants.[88] Application of CuO NPs enhanced the synthesis of different defense-related enzymes, including polyphenol oxidase, in plants (Wang *et al.,* 2013; Sarkar *et al.,* 2020). It was reported that, copper nanoparticles synthesized biologically from *Streptomyces griseus*, successfully suppressed root rot disease in tea caused by *Poria hypolateritia* (Ponmurugan *et al.,* 2016).

Non-metal, metalloids, metallic oxide nanoparticles:

Very little studies are done on application of non-metal, metalloids and metallic oxides in plant disease management. Still there are some reports which ensure the disease resistance potentials of these nanoparticles. Among non-metal nanoparticles, nano S (sulfur) and nano Ti (titanium) are the most studied. Nano S is mostly used for formulating nano fungicides. It was found that the applications of S nanoparticles can protect plants against *Fusarium solani, F.oxysporum, Aspergillus niger*, and *Venturia inaequalis* (Rao and Paria, 2013). Metalloids Si (silicon) nanoparticles have several impacts on disease management. Application of nano Si with Ag inhibited powdery mildew (Park *et al.,* 2006). Nano manganese oxide (MnO) is a metallic oxide nanoparticle to control wilt disease in eggplant and watermelons (Elmer and White, 2018). CuO, TiO_2, Fe_2O_3, and MgO are some other metallic oxide nanoparticles that are used in plant disease management.

Carbon Nanomaterials:

Carbon nanoparticles are made out of carbon, having several unique physical properties and functions in different research fields. Till now, very little information is available on how these nanoparticles contribute to plant disease management. However, recent researchers have revealed that engineered carbon nanoparticles influences plant growth and can eradicate some plant pathogens. The allotropic character of carbon provides a wide array of nanomaterial morphologies and structures that have vast applications in development of each biological and chemical sensors and their applications in agriculture and medicine (Kurbanoglu and Ozkan, 2018). There are mainly three types of carbon nanoparticles that have demonstrated the potential to affect plant health: graphene oxide (oxidized and reduced forms), carbon nanotubes (single wall or multiwall), and fullerenes, which in turn suggests an important disease management tool could be exploited. The mechanisms for inhibition may be physical, with the sharp nano-edges directly damaging cell walls of the pathogens (Berry *et al.,* 2014; Hao *et al.,* 2017).

Graphene oxide can induce 95% disease resistance in *Xanthomonas oryzae* pv *oryzae* against bacterial pathogen of rice (Chen *et al.*, 2013). Antifungal activity of reduced graphene oxide (rGO) nanosheets was evaluated against *Aspergillus niger, A. oryzae* and *Fusarium oxysporum* and inhibition of mycelial growth of the tested fungi was noticed and it was believed that this was due to sharp edges of the rGO (Sawangphruk *et al.*, 2012).

In vitro studies have shown that carbon nanotubes can restrict growth of many plant pathogens, including *Aspergillus* spp., *Botrytis cinerea* and *Fusarium* spp. The mechanism of antifungal effects of carbon nanomaterials was evaluated against two important fungal plant pathogens (*Fusarium graminearum* and *F. poae*) and it was noticed that the antifungal mechanism of carbon nanotubes multiwalls (CNMs) was deduced to target the spores in three steps:

(i) deposition on the surface of the spores,

(ii) inhibition of water uptake and

(iii) plasmolysis (Wang *et al*., 2014).

Currently, there are no field studies with carbon nanomaterials and plant disease, so much additional work is needed, serious investigation should be targeted at these materials as a management tools.

Chitosan nanoparticles:

Chitosan is a natural biopolymer obtained from the shells of snails and prawns is one of the most popular biopolymer nanoparticles, having several biological activities. Recently chitosan nanoparticles are gaining more interest as it is biodegradable, ecofriendly, cost-effective with non-allergenicity and antimicrobial activity. So, it could be formulated and used as a natural antifungal agent in the form of nanoparticles. A recent study demonstrated that the use of chitosan nanoparticles induced disease resistance in the plant. It is a very useful vehicle for drug-delivery and gene transfer as its preparation is very simple (El Hadrami *et al.,* 2010). Application of chitosan nanoparticles induces disease resistance in the tea plant by elevating the defense-related enzymes such as polyphenol oxidase (PO), phenylalanine ammonia-lyase (PAL) and β-1,3-glucanase (Chandra *et al.,* 2015).

Potential mechanisms that are responsible for antimicrobial effects in chitosan, such as agglutination, disruption of the cell membrane, inhibition of H^+-ATPase activity, inhibition of toxin production and microbial growth, inhibition of the synthesis of mRNA and proteins, and blockage of nutrient flow was reported (Malerba and Cerana, 2016). In addition, chitosan nanoparticles can enhance plants immune responses, indicating that it is a unique and potential therapeutic agent for plant disease management.

Dendrimers:

They are tree-like nanoparticles, mainly used as vehicles for transferring chemicals or DNA to achieve disease resistance. Dendrimers facilitate transportation of disease inhibiting chemicals into those tissues generally where these chemicals fail to reach (Elmer and White, 2018).

Liposomes:

Liposomes are mainly used for crop irrigation treatment as they are very stable in water. It is made up of phospholipid bilayers and has a spherical structure forming a central hollow core. Liposomes ligated with different antimicrobial agents and other disease suppressing chemicals have a potential function in plant disease management (Matouskova *et al.*, 2016).

Nanotechnology in agriculture provides a new generation of pesticides and other actives or nanomaterials for plant disease management. Protection in plants against pests and pathogens occurs through two different mechanisms:

(i) nanotechnology themselves providing crop protection, or

(ii) acting as carriers for existing pesticides, such as double-stranded RNA (dsRNA). There are several benefits of applying nanoparticles as carriers, like

(a) enhanced shelf life,

(b) improved solubility of poorly soluble pesticides,

(c) reduced toxicity,

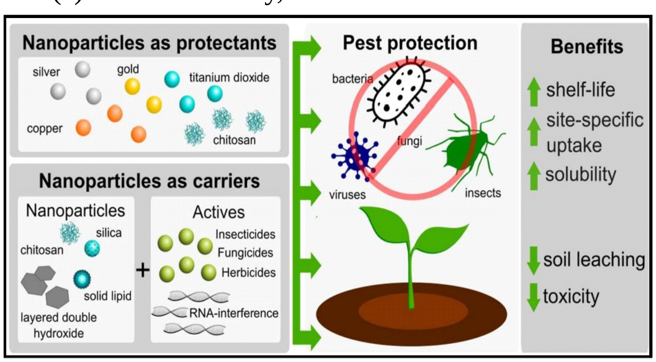

Fig. 1: Nanomaterials as protectants or carriers to provide crop protection and their benefits (Worrall *et al.*, 2018).

and boosting site-specific uptake on target pests (Hayles *et al.*, 2017). Another possible benefits of nanocarriers includes increase in efficacy of the activity and stability of the nanopesticides under environmental pressure (UV and rain), significantly reducing the number of applications, thereby decreasing toxicity and reducing their costs (Fig. 1) (Worrall *et al.*, 2018).

Gene transfer via nanoparticles: A molecular approach in plant protection

The amalgamation of biotechnology with nanotechnology develops a new tool for gene transfer and drug delivery. Manipulation in gene expression is successfully achieved by researchers by utilizing nanofibers, nanocapsules, and nanoparticles (Agrawal and Rathore, 2014). The conventional methods of gene transfer face several challenges during transfer of genetic materials by viral vectors whereas nanotechnology-based gene transfer provides a more convenient approach. Application of DNA coated silver nanoparticles in addition to ethylene glycol successfully transferred plasmatic DNA into the isolated protoplast of the petunia plant (Rad *et al.*, 2013). CRISPR/Cas9 system is a revolutionary invention in molecular biology that changes the biotechnological practice forever. CRISPR/Cas9 is an RNA-mediated genome editing system that comprises two components, a small stretch of repeated sequence and Cas9 protein.

Transfer of CRISPR/Cas9 single guide RNA (sg-RNA) through nanotechnology opens a new era for plant disease management (Miller *et al.*, 2017). Application of nanoparticles in delivery of CRISPR/Cas9 system improves its specificity and efficiency for the target genome editing (Mout *et al.*, 2017). Chitosan, a natural biopolymer having several bioactivities, including antimicrobial properties against several pathogenic microbes. Chitosan NPs are good choices for drug delivery and genome transfer as their transfection efficiency can be modified using various chemical agents such as polyethylene glycol (PEG). One of the benefits of using chitosan NP is that they can easily bind to RNA and can cross the plasma membrane, enabling their use as an excellent gene transfer vehicle. Chitosan nanoparticles can transfer si-RNA, improving disease tolerance against various pests and pathogens in crop plants (Zhang *et al.*, 2010; Gaur *et al.*, 2020). SiO_2NP mediated gene transfer has been carried out in *Nicotiana tabacum* and *Zea mays* which induced significant disease resistance (Galbraith, 2007). Gene modification in various crops can be achieved using DNA-coated nanoparticles mediated gene gun technology (Vijayakumar *et al.*, 2010). In another study, genome editing has been done in *Zea* *mays* immature embryo by using mesosporous silica nanoparticles (MSNPs). The MSNPs are used to carry Cre-recombinase which is correctly recombined in the chromosomal DNA by lox P in maize (Martin-Ortigosa *et al.,* 2014). Recently a study was conducted where carbon nanotubes were used for si-RNA mediated gene transfer in *Triticum aestivum*, *Nicotiana benthamiana*, *Gossypium hirsutum* and *Eruca sativa*. Among which 95% si-RNA mediated gene silencing has occurred in *N. benthamiana* (Demirer *et al.,* 2019).

Antimicrobial mechanism of nanoparticles

Lemire *et al.,* (2013) proposed five theories about the antimicrobial mechanism of metal nanoparticles as:

(1) release of toxic ions (Cd^{2+}, Zn^{2+}, Ag^{+}) that can bind to sulphur-containing proteins, this accumulation prevents the proteins from properly functioning in the membrane and interfere in cell permeability;

(2) genotoxic – toxic ions that can destroy DNA which leads to cell death;

(3) interruption of electron transport, protein oxidation and membrane potential collapse due to its contact with CeO_2 or nC_{60};

(4) generation of ROS (reactive oxygen species) – ROS-mediated cellular damage, and different metal-catalyzed oxidation reactions could underline specific types of protein, membrane or DNA damage;

(5) interference with nutrient uptake.

Nanoparticles disrupt the polymer subunits of pathogen's cell membrane. It was reported that antimicrobial property of metal nanoparticles largely depends upon several parameters including size, shape and the surface charge of the particles. Treatment of fungal pathogen with nanoparticles causes deformations in hyphae and led to the death of fungal hyphae. In case of Ag NPs several studies demonstrated that positive charge on the Ag^{+} ion is crucial for its antimicrobial activity through the electrostatic attraction between the negative charges on the nanoparticles. Ag ions inactivate the proteins with SH-groups (Sulphydryl group) and prevent the ability of DNA to replicate (Feng *et al.,* 2000).

Accumulation of NPs and ions on cell wall by forming pores leading, out flow of intracellular components and shrinkage of fungal mycelium. It attacks membrane lipid bilayer and disturbs the membrane potential, inhibit the germination of conidia and suppress conidial development. Nanoparticles affect mitochondrial membrane potential that increases the transcript level of stress response genes such as SOD_2, $ShSOD_2$ and Shgst1 leading generation of oxidative burst. It also causes genotoxic effect and destroys DNA. Interaction with proteins containing SH-groups cause protein denaturation. All this activity causes fungal cell death.

Bacterial infection in agroecosystem leads to prolonged contamination and mass mortality. For treatment of bacterial infection antibiotics are used as they are readily available and effectively control bacterial phytopathogens. But some bacteria have developed resistance to almost all kind of antibiotics. Nanoparticles have the ability to prevent resistance in bacteria because mode of action of nanoparticles is directly linked with bacterial cell wall. So, it would be possible that new NPs-based materials can perform antibacterial activity (Wang *et al.,* 2017). Antibacterial properties of nanoparticles are reported by many researchers (Cabiscol *et al.*, 2007; Gurunathan *et al.,* 2009). and it was due to physical destruction of the bacterial cell wall or by oxidative stress through generation of ROS.

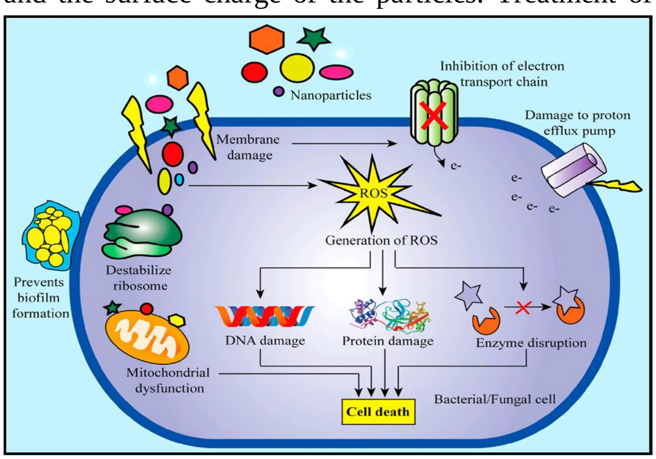

Fig. 2: Schematic representation of antimicrobial (fungal and/ or bacterial) mechanisms of various nanoparticles (Sharmin *et al.*, 2021).

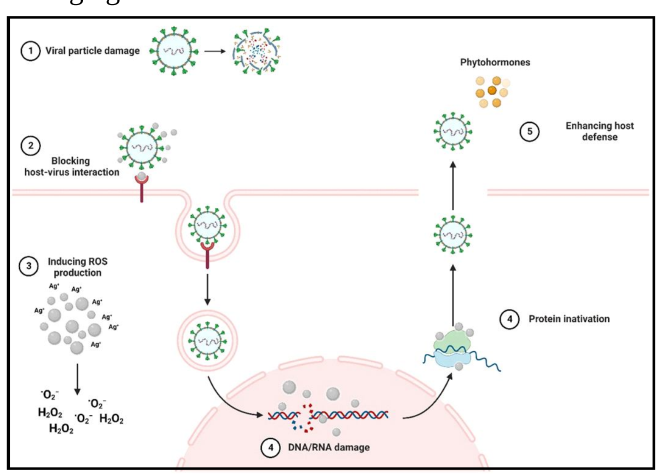

Fig. 3: Schematic representation of antiviral mechanisms of various nanoparticles (dos Santos *et al.*, 2024).

Plant viruses are responsible for 47% of all crop losses brought by plant diseases. Nanoparticles can also be used to precisely detect plant viruses using nanobiosensors or as biostimulants. Nanophytovirology is the application of nanotechnology in plant virology, and it involves biostimulation, drug transport, genetic manipulation, therapeutic agents, and introduction of plant defenses. The antiviral mechanism of nanoparticles involves inactivation and denaturation of capsid protein, nucleic acids (RNA or DNA), and other protein constituents are involved in the underlying mechanism (Warghane *et al.,* 2024).

Conclusions

Application of nanotechnology is providing the significant opportunities to treat phytopathogens by minimizing excess use of chemical pesticides. Innovations always result in both drawbacks and benefits for human and environmental health. As application of nanotechnology in agriculture is in its initial phase, so before using nanoparticles or nanopesticides in plant disease management, further studies and extensive experimental trials are needed to evaluate the effects of nanopesticides on host plant, pathogens, host-pathogens interaction, disease development, disease diagnosis, beneficial microbes, animals, human beings and environment that will help in developing strategies to manage disease through nanopesticides that are cheap ecofriendly and less harmful than conventional pesticides.

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