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Nanotechnology in Plant Disease Management

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Abstract

The mesmerizing science of nanotechnology is the process of manipulating atoms and molecules to produce materials characterized by their minuscule dimensions, including nanoparticles ranging from 1 to 100 nanometers. Despite being relatively new areas of study, nanoscience and nanotechnology are rapidly emerging as the forefront of research, continually generating the latest discoveries. Every year, nearly 20-40% crop losses occur mainly due to diseases and pests. The only method currently used to control plant diseases are toxic pesticides and fungicides, which pose risks to both the human well-being and the ecosystem. To reduce these problems the only needed solution is nanotechnology. It employs the use of nanoparticles synthesized by various methods. Plant diseases are managed effectively by using diverse nanoparticles, like silver nanoparticles, copper nanoparticles and zinc oxide nanoparticles. The rapid detection of plant pathogens, the biosensor-based control of pests and diseases, soil management and other areas are all greatly impacted by nanotechnology.

Keywords: Characterization, Nanofungicides, Nanoparticles, Nanotechnology, Plant disease management, Synthesis

Introduction

The word nanotechnology was derivated from a Greek word 'nano' meaning dwarf and it represents one billionth of a meter. Nanotechnology is the fastest growing science in the 21st century. Nano industry is going to be the next industrial revolution. Nanoparticles, spanning a scale of 1 to 100 nm, can be found in various forms such as nonmetals, metallic oxides, metalloids and carbon nanomaterials. Additionally, they manifest as customised liposomes, quantum dots and dendrimers (Elmer and White, 2018). Due to their diminutive size, expansive surface area and heightened reactivity, nanoparticles exhibit promise in applications as bactericides, fungicides, nanopesticides and nanofertilizers (Elmer and White, 2016). It can be employed to develop nanosensors and biosensors aimed at early detecting plant diseases and employing them as molecular tools.

To meet the escalating demand and sustain the growing population, recent studies have revealed that the global production of food must be doubled by the year 2050 (Tilman *et al.*, 2011). However, achieving this target is impeded by various challenges. These include the adverse impacts of climate change, encompassing phenomena like floods, droughts, cyclones and others, which disrupt the crop life cycle. Moreover, the persistence of pesticide and fungicide residues in the soil, as well as the contamination of water bodies, poses significant risks to human health. Additionally, the development of resistance and the risk of new strains of plant pathogens against conventional chemicals further compound the predicament.

Nanotechnology emerges as the key solution for addressing these multifaceted issues in disease management of plants. Despite being in its early stages, the utilization of nanotechnology in the field of crop protection, genetic transformations and diagnostics has recently gained attention in the plant pathology literature (Dutta *et al.*, 2022). A very few patents have been registering in India in this field mainly due to the lack of awareness that how this nanotechnology can be applied in order to control the plant diseases. In fact, there are more possibilities regarding these that nanomaterials can be applied as nanofungicides and

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nanopesticides for the plant diseases and pests management and nanofertilizers can also be used to improve the plant health (Elmer and White, 2018).

Definition, Properties and Synthesis

According to the US Environmental Protection Agency, nanotechnology is explained as the examination of comprehending and managing matter at scales approximately 1-100 nm (Concha-Guerrero *et al.*, 2017), where unique physical features enable innovative uses. However, this definition might be considered overly stringent in terms of size dimensions, potentially overshadowing the emphasis on the materials problem-solving capabilities.

Nanoparticles, ranging between 1 to 100 nanometers in size, have their physical and chemical properties influenced by factors including shape, size, surface charge and crystallinity. They find applications across various industries, owing to their miniature size and large surface area-to-volume ratio, enabling efficient reactions, binding, absorption and transportation of substances like DNA, RNA, proteins, small-molecule drugs and probes (Albanese *et al.*, 2012; Elmer and White, 2018).

Besides their surface area, nanoparticles display distinct physical properties compared to their heavier bulk counterparts. For example, gold, which is inert and retains its golden appearance in its bulkier form, becomes reactive and red at the nano level. Similarly, ZnO and TiO, typically white, appear colorless at the nanoscale (Elmer and White, 2018).

Nanoparticles can occur naturally, for example, in volcanic dusts and oceanic salt sprays (Kadar *et al.*, 2014) or be engineered artificially to specific dimensions. They find applications across diverse fields, encompassing high sensitivity biomolecular detection, the development of medicinal and antibacterial chemicals and disease diagnostics, showing promising potential for improving agricultural productivity *via* cost-effective production methods (Dutta *et al.*, 2023; Dutta *et al.*, 2022; Singh *et al.*, 2022).

Two primary approaches are commonly utilized for synthesizing the nanoparticles: the top-down approach and the bottom-up approach (Sahoo *et al.*, 2022). The bottom-up approach is further subdivided into three types: 1) physical methods, 2) chemical methods and 3) biological methods. Among these, the biological method (green synthesis) has gained substantial prominence for nanoparticle synthesis. This method involves the use of various biological sources, comprising plants and their different parts (leaves, stems, fruits and roots), fungi, bacteria, algae, yeast and actinomycetes. Notably, numerous plant pathogenic fungi species, including *Aspergillus*, *Penicillium*, *Fusarium* and *Verticillium*, have been harnessed for the production of nanoparticles.

Various analytical techniques can be employed to classify nanoparticles, including Scanning electron microscopy (SEM), Atomic force microscopy (AFM) and Transmission electron microscopy (TEM), aids in size determination; while Nuclear magnetic resonance spectroscopy (NMR) and Differential mobility analyzer (DMA) are instrumental in assessing surface area. For compositional analysis, X-ray photoelectron spectroscopy is frequently employed. Surface morphology is typically investigated using imaging methods of electron microscopy, such as TEM and SEM. Zeta potential analysis is often conducted using a zeta potentiometer to evaluate charges on the surface and the durability of scattering in a solution (Oprică and Bălășoiu, 2020). Powder X-ray, electron or neutron diffraction methods are utilized in nanoparticle crystallography to determine the structural arrangement, while condensation particle counters are commonly used to measure nanoparticle concentration (Ealias and Saravanakumar, 2017).

Table 1: Potential applications of various nanoparticle types in plant pathology

Types of nanoparticles	Applications in plant pathology	References
Metalloids	Nanofertilizers, Nanobactericides/ nanofungicides, Delivery vehicle (antimicrobials and genetic material)	Elmer and White, 2018
Metallic oxides	Nanofungicides, Nanopesticides, Nanofertilizers	Saikia *et al.*, 2022
Nonmetals and their composites	Nanofungicides, Nanopesticides	Elmer and White, 2018
Carbon nanomaterials	Diverse uses	Ealias and Saravanakumar, 2017
Liposomes	Acts as delivery vehicles (Genetic or antimicrobial products)	Pinto-Alphandary *et al.*, 2000
Dendrimers	Transporters for genomic or antimicrobial substances	Saeedi *et al.*, 2019
Nanobiosensor	Disease identification	Dutta *et al.*, 2023
Quantum dots	Diagnostics, Disease management	Dutta *et al.*, 2023

Nanoparticles in Plant Disease Management

A diverse range of nanoparticles finds application in the plant disease management. They are mainly silver nanoparticles, copper nanoparticles and zinc oxide nanoparticles.

Antifungal Activity of Nanoparticles

The initial exploration of plant disease treatment using silver nanoparticles was prompted by their well-established antimicrobial activity (Richards, 1981). In a study by Kim *et*

Table 2: Categories of the nanoparticles synthesized from top down approach

Methods	Nanoparticles	References
Sol-gel	Titanium dioxide NPs	Barcelos and Gonçalves, 2023
Pyrolysis	Ceramic NPs	D'Amato *et al*., 2013
Biosynthesis	Silver NPs	Keat *et al*., 2015
Spinning	Magnesium hydroxide NPs	Tai *et al*., 2007
Chemical vapour deposition (CVD)	Silica NPs	Rezaei *et al*., 2014

al. (2008), the efficacy of colloidal Ag nanoparticles versus rose powdery mildew was examined. They created a double-capsulated nanoscale variant of Ag through the reaction of Ag-ions with a reducing agent, while incorporating a stabilizing agent. Notably, the authors observed a gradual reduction in *Sphaerotheca pannosa* colonies on the leaves after spraying a concentration of 10 µg mL^{-1} onto powdery mildew-infected rose plants, with pathogen absence detected only seven days later.

In another study, Jagana *et al.* (2017) advocated that postharvest treatments should make the use of nano-Ag. In order to control *Colletotrichum musae* post-harvest, they used plant extracts to make nano-Ag and applied it to banana fruits at various rates. Compared to the untreated control, which had a severity of 75.6%, the disease severity was reduced to 6.7% at the highest rate of 2,000 µg mL^{-1} nano-Ag (Elmer and White, 2018; Jagana *et al.*, 2017). Ag nanoparticles were produced by Moussa *et al.* (2013) in the *Serratia* spp. supernatant culture. In detached leaf assay and on-leaf infection in greenhouse studies, they discovered that doses as low as 2 µg mL^{-1} could totally stop the conidial germination of the wheat-infecting pathogen *Bipolaris sorokiniana*. Histochemical staining demonstrated that the nano-Ag treatment had caused an artificial build-up of lignin in the vascular bundles.

Furthermore, silver nanoparticles have exhibited efficacy against foliar fungal pathogens, with prophylactic use demonstrating significant effectiveness (Mishra *et al.*, 2014). Additionally, silver nanoparticles exhibited potent control against *Rhizoctonia solani* at various concentrations, with the highest radial growth inhibition percentage recorded at 200 ppm (Dutta *et al.*, 2021). They have also demonstrated efficacy in controlling soil-borne diseases brought on by *Fusarium* spp., *Meloidogyne* spp. and *Phytophthora parasitica* (Sahoo *et al.*, 2022). Notably, green-synthesized AgNPs have displayed inhibitory effects against *F. oxysporum* (Dutta *et al.*, 2020).

Research by Kaman and Dutta (2019) demonstrated that silver nanoparticles impeded the radial growth of four fungal pathogens, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *S. rolfsii* and *Rhizoctonia solani*, with the inhibition percentage increasing as the concentration of silver nanoparticles increased. Mallaiah (2015) found that Fusarium wilt in *Crossandra* spp. was inhibited in pot culture by applying Ag nanoparticles at 800 µg mL^{-1}; the wilt decreased from 75% in the untreated control group to 55% in the treated group. Notably, there has been potential in mixing nanoscale Ag with biocontrol agents. When Ag was mixed with the biological control agents *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma viride*, Mallaiah (2015) noted additional improvements in the inhibition of Fusarium wilt. Compared to the control group, the flower production rose when Ag was combined with these treatments.

Additionally, the first study on nano-Cu as a bactericide/fungicide was carried out by Giannousi *et al.* (2013), developed nano-CuO, Cu_2O and Cu/Cu_2O composites and compared them with commercially registered Cu-based fungicides like Kocide 2000 35 WG, Kocide Opti 30 WG and Ridomil Gold Plus (Elmer and White, 2018). Their findings indicated that the nano-Cu/Cu_2O composite and CuO nanoparticles at 150-340 µg mL^{-1} were effective in suppressing leaf lesions caused by *Phytophthora infestans*. Importantly, the nano-Cu products exhibited performance on par with or superior to the commercially available Cu-based products, with lower application rates and no observed phytotoxicity.

Furthermore, Elmer and associates discovered a novel use for Cu nanoparticles in disease management through their research on the use of Cu nanoparticles as supplements or nanofertilizers to improve disease resistance (Elmer *et al.*, 2018; Elmer and White, 2016). Their research showed that Cu nanoparticles were particularly effective in preventing Fusarium wilt of tomatoes and Verticillium wilt of eggplants. When CuO nanoparticles were applied foliarly at a concentration of 500 µg mL^{-1} on young seedling leaves before being transplanted into potting mix contaminated with *F. oxysporum* f. sp. *lycopersici* or *Verticillium dahliae*, disease severity ratings consistently indicated lower severity than controls and bulked oxide counterparts (Elmer and White, 2018).

Utilising biologically synthesized nano-Cu from *Streptomyces griseus* cultures, Ponmurugan *et al.* (2016) successfully treated the red root rot disease of tea crop in India, which is caused by the fungus *Poria hypolateritia*. They compared bulked CuO equivalents or the fungicide carbendazim with increasing rates of nano-CuO (1.5 L $bush^{-1}$) sprayed on *P. hypolateritia*-infected plants. The highest dose of nano-Cu (2.5 µg mL^{-1}) showed a 53% reduction in disease severity, while a 57% decrease with carbendazim. Applying nano-Cu resulted in increased edaphic qualities and leaf yield, which were probably caused by improved root health (Elmer and White, 2018).

Numerous investigations have demonstrated the antifungal efficacy of CuO nanoparticles against a diverse of pathogens, including *Phoma destructiva*, *Alternaria alternata*, *Botrytis cinerea* and *Curvularia lunata* (Kanhed *et al.*, 2014; Ouda, 2014). Likewise, the antimicrobial properties of Zn nanoparticles against plant pathogens have been explored in different laboratories, mirroring the research conducted on Ag and Cu (Graham *et al.*, 2016; Indhumathy and Mala, 2013).

To control Cercospora leaf blight of sugar beet, researchers in Egypt used a combination of six bacterial biocontrol agents, nano-silica, diatomaceous earth and the conventional fungicide tetraconazole (Elmer and White, 2018). The foliar application of ZnO nanoparticles at 500 μg mL^{-1} was compared by Derbalah *et al.* (2013). The application of nano-Zn was found to be the second most effective treatment, following tetraconazole, in enhancing root yield, sugar content and leaf dry weight while simultaneously reducing disease severity, over a two-year study.

Moreover, the inhibitory effects of various CuNPs treatments synthesized from Allamanda, night jasmine and yellow oleander were observed against fungus growth, demonstrating significant effectiveness comparable to commercial fungicides. Additionally, CuNPs-treated cut flowers exhibited delayed discoloration of the flower head and the first fall of petals, potentially due to the anti-ethylene properties of CuNPs, which slow down the process of flower senescence. CuNPs derived from night jasmine were particularly effective against *Corynespora dendranthema* and exhibited a notable increase in the vase life of chrysanthemum cv. Snowball (Saikia *et al.*, 2022).

Antibacterial Activity of Nanoparticles

The first strain of *Pseudomonas stutzeri* AG259 that was obtained from a silver mine, was the source of the discovery that bacteria could synthesize silver nanoparticles (Dutta and Kaman, 2017; Haefeli *et al.*, 1984). At concentrations as low as 2.5 μg mL^{-1}, Nano-Ag exhibited inhibition of *Citrobacter freundii*, *Erwinia cacticida* and all the tested xanthomonads *in vitro* (Liang *et al.*, 2017). In the agricultural context, silver nanoparticles have proven effective in controlling plant pathogens at lower doses compared to chemicals, consequently reducing cultivation costs and promoting environmental sustainability (Dutta and Kaman, 2017).

Studies have suggested that the amalgamation of nano-Ag with silica and its application to *Arabidopsis thaliana* at 1-10 g mL^{-1} as a pre-treatment, resulted in elevated levels of PR1, PR2 and PR5. Additionally, compared to the water control treatment, it produced disease resistance contrary to the bacterial pathogen *Pseudomonas syringae* pv. tomato (Chu *et al.*, 2012). However, considering that Si can independently produce these defense compounds (Datnoff *et al.*, 2007); it remains crucial to thoroughly investigate the specific consequences of nano-Ag.

CuO nanoparticles effectiveness against xanthomonads that cause plant damage has been amply demonstrated (Strayer-Scherer *et al.*, 2018). One could argue that any approach involving Cu may worsen its utilization as a management tool, given the extensive employment of Cu in agricultural field and the emergence of Cu-tolerant bacterial strains. Nevertheless, the counterargument to this perspective is based on the observation that the quantity of Cu provided in nano form is currently noticeably less than that in traditional Cu-based products.

Researchers have consistently demonstrated the inhibitory effects of nano-Zn on bacteria, with more attention focused on the management of bacterial diseases compared to diseases caused by other pathogens (Graham *et al.*, 2016; Indhumathy and Mala, 2013; Kaushik and Dutta, 2017). In fact, a Zn-based nano-product (Zinkicide™) is presently in the process of being registered for controlling citrus canker (Young *et al.*, 2017).

Additionally, Imada *et al.* (2016) confirmed that utilizing magnesium oxide nanoparticles as a preventative measure enhances tomatoes resistance to systemic disease caused by *Ralstonia solanacearum*. Reactive oxygen species were produced in the roots quickly as a result of the treatment, and genes linked to systemic resistance, ethylene, jasmonic acid and PR1 were also upregulated. Moreover, histochemical investigations on plants exposed to nano-MgO showed that the vascular tissues of the hypocotyls contained tyloses and β-1,3-glucanase (White and Elmer, 2018).

Moreover, research by Chen *et al.* (2013) illustrated that graphene oxides at 250 μg mL^{-1} were effective in eliminating 95% of *Xanthomonas oryzae* pv. *oryzae* bacterial cells, outperforming the bactericide bismerthiazol, which resulted in 13.3% mortality. Matouskova *et al.* (2016) encapsulated antimicrobial components like chitosan and herbal extracts containing phenolics in liposomes to suppress gram-negative bacteria. The antibacterial efficacy of nanoparticles was discovered to rely on factors such as nanoparticle concentration, microbial physiology, metabolism, selective intracellular membrane permeability and cell type (Khan and Rizvi, 2014).

Antinematodal Activity of Nanoparticles

The application of nano-Ag in the suppression of plant-parasitic nematodes has shown encouraging outcomes. Several researches have investigated the effects of nano-Ag on *Meloidogyne* species, demonstrating its efficacy in rendering all stage two juveniles of *Meloidogyne* spp. completely inactivated within a relatively short period, typically around 6 hours (Abdellatif *et al.*, 2016; Ardakani, 2013; Cromwell *et al.*, 2014; Nassar, 2016).

In a specific case, biweekly applications of nano-Ag at 90.40 mg m^{-2} on a commercial putting green infected with *Meloidogyne graminis* improved the quality of the turfgrass and decreased the development of root galls (Cromwell *et al.*, 2014). Additionally, studies have successfully suppressed *Meloidogyne javanica* and *Meloidogyne incognita* using low concentrations of nano-Ag, achieving control levels equivalent to those provided by conventional nematicides (Abdellatif *et al.*, 2016; Ardakani, 2013).

Conclusion

The technologies presently employed in numerous fields, including as agriculture, could be completely transformed by nanotechnology. With the recent advancements and the trends following in nanotechnology, it was paving a great way for the early detection and management of plant diseases. Although nanotechnology was still in infant stage but it unraveled a greater scope of applications in plant disease management. The use of different nanoparticles against various group of pathogens were proven successful in controlling the plant diseases. The green synthesis mediated nanoparticles were environmentally safe and good for the application. There is a need of in depth research in this field about to control the plant diseases and to check their phytotoxicity levels.

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