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CHAPTER : 10

Exploiting Nanotechnology for Plant Pathogen Detection and Management

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Abstract
In the present day, the demand for food has been rising rapidly. In this raising demand, the most recent advance is the use nanomaterials which have become a vital tool to enhance the crop production and productivity. The nanomaterials can also improve the growth and development of the plants. The crop yield loss of upto 20-40 % has been reported due to biotic stress. Till now, the biotic stress management primarily depended on using the chemicals which has a lot of environmental risks. To overcome some of the risks associated with the use of chemicals for managing biotic stress caused by both microbes and insects, nanotechnology has emerged as an alternative which is sustainable and eco-friendly Nanotechnology, nanoparticles and quantum dots (QDs) have become vital tools for the rapid and highly accurate detection of specific biological markers. Technologies such as biosensors, QDs, nanostructured platforms, nano-imaging and nanopore DNA sequencing significantly enhance the sensitivity, specificity and speed of pathogen detection. These tools also support high-throughput analysis, facilitate high-quality monitoring and contribute to crop protection. Additionally, nanodiagnostic kits enable quick and efficient detection of plant pathogens, helping specialists to assist farmers in preventing the outbreak of the epidemic diseases.

Keywords Detection, diagnosis, disease, identification, management, nanotechnology

1. Introduction

The farming community consistently aims to reduce agricultural input costs to boost profits. To achieve this, farmers enhance crop yields through the use of fertilizers, herbicides and fungicides. However, this approach has created a significant adjustment between increased crop productivity and the health of soil and groundwater due to the extreme application of agro chemicals. In recent decades, population growth has led to an unprecedented expansion of

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farmland. As farmland increases, so does the dependency on agrochemicals, resulting in heightened pollution of soil, water and air. This growing impact on the environment and its pollution is urging the farming as well as the scientific community to explore innovative ideas and technologies to protect the soil and the environment. In light of the awareness throughout the globe, the farmers are facing a lot of pressure to minimise the use of chemicals by adopting various alternative farming practices. Precision agriculture is seen as a viable alternative for farmers by minimizing the use of agrochemicals and delivering target specific and site-specific solutions which are tailored to suite each crop, ultimately leading to higher economic returns. These practices focus on enhancing the crop productivity while reducing the dependence on fertilizers, pesticides and herbicides. By integrating nanotechnology, precision farming exploits computers, global positioning systems (GPS) and remote sensing devices to monitor crop and environmental parameters effectively. By 2050, the global population is expected to reach approximately 10 billion, with around 800 million people facing hunger and 653 million undernourished by 2030. Reaching the estimated food demand by 2030 will be a big challenge. The present research advancements and disease management strategies are insufficient to satisfy the projected food needs by 2050 (FAO, 2017). The green revolution saw improved yields to overcome the food shortage, but the crop production has stagnated in the recent years though the demand for the food continues to rise. The present situation calls for another green revolution to meet the increasing food demand of the ever growing population.

The biotic stress in crop plants caused by various group of microbes leads to significant disease and crop loss. Every year approximately 20-40% of yield loss is reported from different pathogens and insect pests. The present methods to manage biotic stress greatly depends on the use of chemicals which lead to harmful effects to the environment as well as the consumer. Nanotechnology has emerged as a sustainable and eco-friendly alternative for managing the loss caused by the biotic stress (Khan *et al.*, 2021).

Nanotechnology has the prospective to modernize agriculture by offering effective disease management solutions. Though the field of nanotechnology is still in its early stages and requires further research, the incorporation of nanomaterials in agriculture could significantly decrease the reliance on poisonous chemicals for sustainable plant disease management (Cabral-Pinto *et al.*, 2019).

"Nano" refers to one-billionth of a meter, making nanotechnology a field focused on extremely small materials. Specifically, "nano" describes particles ranging from 0.1 to 100 nanometers in size (Khan *et al.*, 2019). The word nanotechnology was first coined by Taniguchi in 1974 to describe the developments related to nanoscale particles (1.0×10^{-9} m). When large materials are decreased to the nanoscale, they exhibit a high surface-to-volume ratio, which can enhance their sensitivity and reveal new properties (Cabral-Pinto *et al.*, 2014). In plant pathology, nanomaterials hold promise for controlling plant diseases and promoting plant growth. In 2010, approximately 260,000 to 309,000 metric tons of nanoparticles were produced globally, while the worldwide usage of nanomaterials ranged from about 225,060 metric tons to 585,000 metric tons between 2014 and 2019 (Cabral Pinto *et al.*, 2019; BBC, 2017).

The basic step in reducing the plant disease in both greenhouse and field conditions is to accurately identify the pathogen. The present day technologies, like Q-PCR (Quantitative polymerase chain reaction), requires a substantial amount of target tissue and involve multiple assays to distinguish different pathogens that infect the plants. Traditional diagnostic methods often face the drawbacks of being time-consuming and lacking high sensitivity. Therefore, there is a pressing need for economical methods that enhance the accuracy and speed of disease diagnosis. Nanotechnology, including nanoparticles and quantum dots (QDs), has evolved as a vital tool for the rapid and precise detection of specific biological markers. Tools such as biosensors, QDs, nanostructured platforms, nanoimaging and nanopore DNA sequencing have the potential to improve sensitivity, specificity and detection speed, enabling high-throughput analysis and effective monitoring and protection of crops.

Farmers employ various strategies to over come the effects of plant diseases. Traditionally, agricultural systems have relied heavily on chemicals to manage crop diseases and suppress both pre and post harvest phytopathogens. But, the excessive use of pesticides, herbicides and fungicides can lead to detrimental consequences for both the environment and human health. Research by Tilman *et al.* (2002) has shown that high pesticide use can foster resistance in pathogens and pests, hinder nitrogen fixation and result in the bioaccumulation of toxic substances. A notable example is the synthetic pesticide DDT (dichloro-diphenyl-trichloro-ethane), which was widely used to combat plant pathogens but has been linked to genotoxicity in humans and endocrine disorders. Additionally, the overuse and misuse of these chemicals contribute to water and soil pollution (Cohn *et al.*, 2007). As the demand to reduce synthetic chemical use grows, the negative impacts on wildlife, the environment and human health have prompted a search for alternative methods to control plant pathogens. Some plant pathologists are now focusing their interests on developing new alternatives to substitute chemical treatments (Figure 1 & 2).

In this book chapter we have focused on nano-based diagnostic techniques for identification of plant pathogens and application of nanotechnology for plant disease management.

2. Nano Detection and Diagnosis of Plant Pathogens

Current technologies, such as Q-PCR, require relatively large amounts of target tissue and depend on multiple assays to accurately identify specific plant pathogens. A popular drawback of traditional diagnostic methods is their time-consuming nature and lack of high sensitivity. Therefore, there

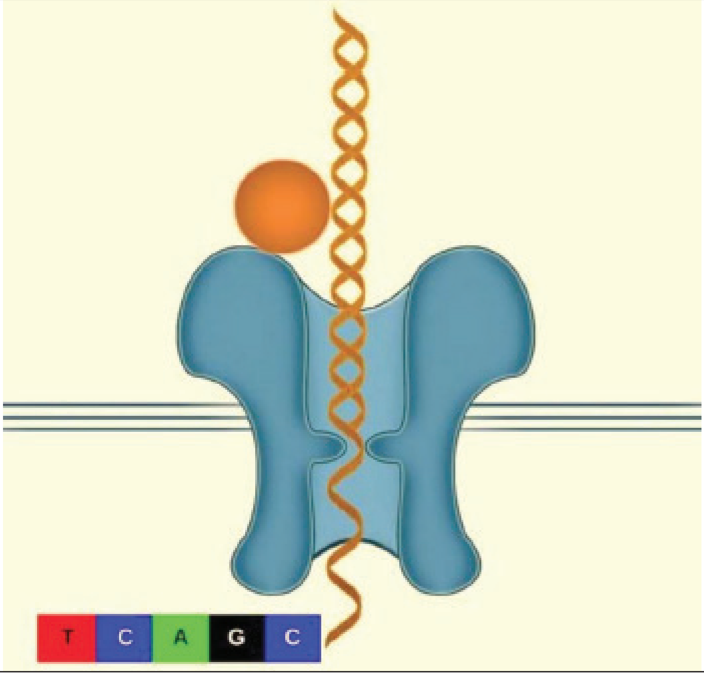

Figure 1: Diagram of a DNA molecule travelling through a protein nanopore (Adopted from Khiyami *et al.*, 2014)

is a need for cost effective methods that enhance the speed and accuracy of plant pathogen diagnosis. Nanotechnology, including nanoparticles and quantum dots (QDs), has become crucial for the rapid and precise detection of specific biological markers. Biosensors, QDs, nanostructured platforms, Nano imaging and Nano pore DNA sequencing tools offer the potential to improve the sensitivity, specificity and speed of pathogen detection, enable high-throughput analysis and support high-quality crop monitoring and protection. Additionally, Nano diagnostic kits can quickly and effectively detect major plant pathogens, assisting scientists in helping farmers prevent disease outbreaks (Khiyami *et al.*, 2014).

The application of molecular diagnostics at the nanoscale level holds significant promise for identifying pathogens. Nano molecular diagnostics refers to the use of Nano biotechnology for diagnosing plant diseases, commonly known as Nano diagnostics. Notably, various Nano devices and Nano systems are being utilized to sequence individual DNA molecules. These assays, which leverage nano-sized devices for DNA sequence analysis and disease diagnosis, are becoming increasingly rapid, versatile and sensitive (Jain *et al.*, 2003).

Nano-phytopathology is an advanced area that employs nanotechnology to detect, diagnose and manage plant diseases at early stages, contributing to the protection of crops from epidemic outbreaks. Modern plant pathologists aim to use their expertise in nanophytopathology to better understand the factors that manage plant diseases and to develop or evaluate eco-friendly diagnostic approaches. Contemporary monomolecular techniques are used to monitor and study pathogen population genetics, plant-microbe interactions and gene transfer between pathogens and hosts. Recently, nanoparticles like nanosized silica-silver have been used as antimicrobial and antifungal agents. Additionally, nanomaterials are being utilized for detection of mycotoxin, detoxification, enhancing plant resistance, forcast the plant diseases and nano-molecular diagnostics of plant pathogens.

3. Nano Diagnosis Technologies

3.1. Portable Genome Sequencer (Nanopore Sequencing System)

Many companies are exploring the potential of nanopore technology for DNA sequencing. However, two key challenges need to be addressed: (1) how to accurately differentiate nucleotides as DNA strand passes through the nanopore and (2) how to manage the speed of the DNA strand during this process (Niedringhaus *et al.*, 2011). Despite these challenges, nanopore sequencing is considered a promising platform due to its simplicity and the ability to theoretically generate very long reads from a small amount of nucleic acid. A protein nanopore and enzyme are engineered to guide a single strand of DNA through the nanopore, allowing direct electronic analysis as the strand passes through. The protein nanopore is embedded in a polymer bilayer membrane atop a microwell, with each microwell containing a sensor chip that measures ionic current as the DNA molecule passes through (Figure 1). However, the DNA strand currently moves too quickly for precise identification (Rai *et al.*, 2012). In response, IBM and Roche are collaborating to develop a new sequencing technology called the "DNA transistor," which could potentially record the nucleotide sequence as the DNA template is drawn through the nanopore sensor (Clarke *et al.*, 2009; Ozsolak, 2012). The portable genome sequencer, MinION, has successfully sequenced 10 kb of both sense and anti-sense DNA strands, bringing next-generation sequencing (NGS) within reach for many research groups and laboratories. When integrated into current diagnostic equipment, the nanopore platform has the potential to analyze entire genomes in minutes rather than hours. Nanotechnology's application in plant science research holds great promise for analyzing plant genomics, studying gene function, detecting pathogens and enhancing crop species.

3.2. Nanodiagonastic Kit

A laptop-sized kit is transported to the fields where crops are raised to detect pathogens that could potentially infect and lower crop yields. This process is both fast and accurate. The nanodiagnostic kit can swiftly identify major plant pathogens, enabling experts to assist farmers in preventing disease outbreaks. For instance, the 4mycosensor is a tetraplex competitive antibody-based assay in dipstick format, designed for real-time detection of ZEA, T-2/HT-2, DON and FB1/FB2 mycotoxins on a single strip in corn, wheat, oat and barley samples, at or below their respective (Pimentel, 2009; Lattanzio *et al.* (2012) European maximum residue limits (MRLs). The multiplex stripe scheme used in this study is illustrated in Figure 2.

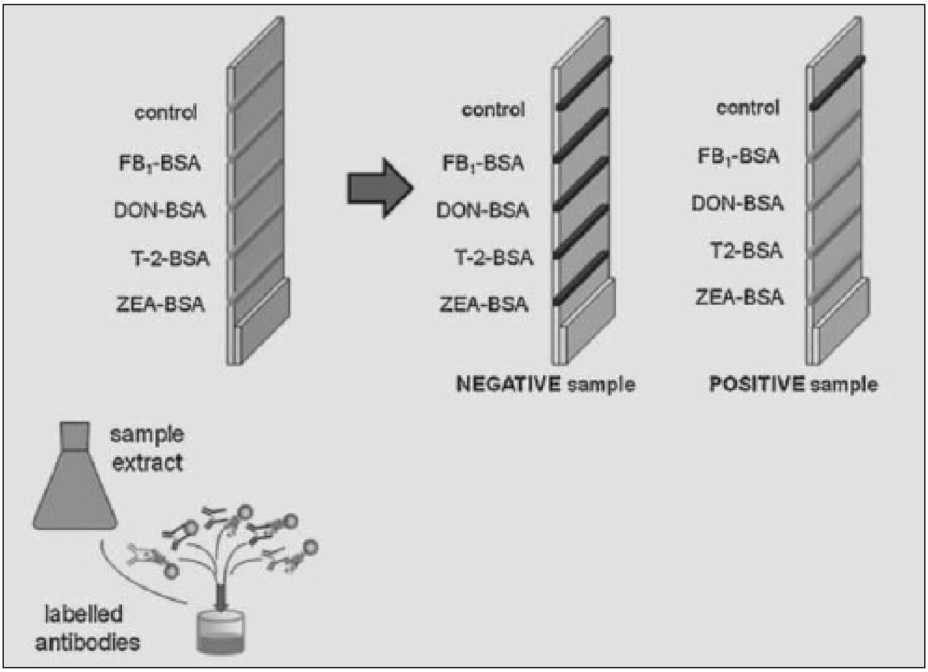

Figure 2: Schematic description of the 4mycosensor dipstick test (adapted from Lattanzio *et al.*, 2012)

3.3. Nano Biosensors

The use of nanomaterials or nanoparticles in biosensors has enabled the development of novel signal detection methods and devices. Various strategies, such as antibody-antigen interactions, adhesion-receptor binding, antibiotic recognition and complementary DNA sequence identification, have been developed for the specific detection of target phytopathogenic cells using bio-functionalized nanomaterials (Wang *et al.*, 2006).

Bio-nanosensors have the potential for enhanced sensitivity, leading to significantly reduced response times in detecting potential disease. These bio-analytical nanosensors have been used to identify and quantify trace amounts of contaminants, such as viruses, bacteria, fungi, toxins and other biohazardous substances, in agricultural and food systems. As a result, these biosensors could have a substantial impact on precision agriculture (Sekhon, 2010). Hashimoto *et al.* (2008) developed a new biosensor system for the rapid diagnosis of soil-borne diseases, comprising two biosensors. The system was designed using equal quantities of two distinct microbes, each separately immobilized on an electrode. Fluorescent silica nanoparticles

(FSNPs) combined with antibody molecules have been successfully used to detect plant pathogens like *Xanthomonas axonopodis* pv. *vesicatoria*, which causes bacterial spot disease in tomatoes and peppers. Copper oxide (CuO) nanoparticles and nanolayers were synthesized using sol-gel and spray pyrolysis methods, respectively. Both CuO nanoparticles and nanostructural layer biosensors were employed for detecting the *Aspergillus niger* fungus.

Gold nanoparticles serve as excellent markers in biosensors, as they can be adapted for various optical or electrochemical techniques to detect pathogens. Numerous nanoparticle-based experiments have been conducted to create biomolecular detection systems using DNA or protein-functionalized gold nanoparticles, which act as target-specific probes. Recent studies have highlighted several nanobiosensors designed for the molecular diagnosis of food-borne pathogens and agro-terrorism agents (Yang *et al.*, 2008). Wang *et al.* (2011) successfully and accurately measured salicylic acid using gold electrodes integrated with a copper nanoparticle (CuNP) sensor. This approach highlights the effectiveness of nanomaterial-based sensors in detecting specific biochemical compounds. Fan *et al.* (2003) reported that gold nanoparticles effectively quench the fluorescence of light-harvesting polymers, such as polyfluorene. This finding opens up new possibilities for enhancing the optical performance of nanobiotransducers for diagnostic applications.

Vaseghi *et al.* (2013) reported that DNA-gold nanoparticle probes can effectively detect infections caused by pathogenic varieties (pathovars) of *Pseudomonas syringae*. This method demonstrates the potential of using gold nanoparticles for sensitive and specific detection of plant pathogens.

Carbon nanomaterials have been developed to function as electrodes for electrochemical analysis (Sharon and Sharon, 2008). These materials hold the potential to be used as electrochemical sensors for detecting pesticide residues in plants. While no patents have been filed exclusively for the diagnosis of plant diseases using nanotechnology methods, the techniques developed for diagnosing animal diseases could also be applied to plants (Kalpana Sastry *et al.*, 2010). González-Melendi *et al.* (2007) reported the use of carbon-coated magnetic nanoparticles and microscopy methods at various levels of resolution to visualize and trace the transport and deposition of nanoparticles within the plant host.

Lavanya and Arun (2021) developed a visual detection method using functionalized gold nanoparticles (AuNPs) containing the *clccpi1* probe for the detection of begomoviruses in tomato, chili, common gram, green gram and black gram plants. This AuNP-based assay showed a higher detection efficiency (77.7%) compared to the conventional PCR technique, which achieved a detection rate of 49.4%. This demonstrates the potential of AuNP-based methods as a more effective and efficient alternative to traditional techniques for detecting plant viruses.

3.4. Nano based Micro Arrays

Nanochips are a type of microarray that contains fluorescent oligonucleotide capture probes, allowing for the detection of hybridization events. These probes enable precise identification of target molecules based on their complementary binding, making nanochips a powerful tool for molecular diagnostics. Nugaeva *et al.* (2005) demonstrated the use of micromechanical cantilever arrays for the detection of fungal spores, specifically *Aspergillus niger* and *Saccharomyces cerevisiae*. This technique allows for highly sensitive detection by measuring the mechanical responses of the cantilevers upon interaction with the fungal spores.

3.5. Quantum Dots

Quantum dots (QDs) are semiconductor nanoparticles that fluoresce when stimulated by an excitation light source. These QDs offer significant advantages over traditional organic fluorophores used as markers for nucleic acids or proteins in visual detection (Arya *et al.*, 2005). The mycosynthesis of semiconductor nanomaterials was first reported in unicellular yeast, which were shown to produce cadmium sulfide (CdS) crystallites in response to cadmium salt stress. Various microbes have also been used for the biosynthesis of CdS, though few studies have explored its luminescent properties. Highly luminescent CdSe QDs were successfully synthesized by the fungus *Fusarium oxysporum* when incubated with a mixture of $CdCl_2$ and $SeCl_2$ at room temperature (Kumar *et al.*, 2007).

Rad *et al.* (2012) reported that quantum dot (QD)-based sensors were highly effective in detecting witches' broom disease of lime, which is caused by *Candidatus phytoplasmaaurantifolia*. The QD sensor demonstrated a sensitivity and specificity of 100%, providing a high level of detection for *Ca. P. aurantifolia*. Additionally, Safarpour *et al.* (2012) observed that QD-based Förster resonance energy transfer (FRET) was effective in detecting disease vectors. For instance, *Polymyxabetae*, a vector for beet necrotic yellow vein virus (BNYVV), which is one of the most destructive diseases affecting sugar beet, was successfully detected using the QD-FRET-based sensor.

3.6. Nanobarcodes

The bio-barcode assay is an ultrasensitive method for amplifying and detecting proteins or nucleic acids. DNA bio-barcode tests use oligonucleotide-modified magnetic gold nanoparticles (AuMNPs) for signal amplification and easy separation of target proteins from the sample. The high b-DNA-to-recognition agent ratio enables significant signal amplification. This method shows promise for the rapid detection of multiple protein targets at low-attomolar concentrations and nucleic acids at high-zeptomolar levels under optimized conditions (Nam *et al.*, 2004). The bio-barcode assay offers a unique approach and represents a potential alternative to the PCR technique.

3.7. Nanodiagnostic/ Fabrication Imaging

Nanotechnology provides the opportunity to precisely tune and control the chemical and physical properties of contrast materials, addressing concerns related to toxicity, imaging time, tissue specificity and signal strength. In the 'mesoscopic' size range of 5-100 nm in diameter, nanoparticles possess large surface areas and functional groups that facilitate conjugation to multiple diagnostic tools. Consequently, advancements in nano-scale contrast agents will be crucial for enhancing our diagnostic imaging capabilities in the coming years (Nie *et al.*, 2013).

Cell biology scientists at Cornell University are researching nanofabrication technologies to understand how fungi and bacteria sense and navigate plant surfaces to initiate infection (McCandless, 2011). This work aims to uncover the mechanisms by which these pathogens interact with plants, potentially leading to new strategies for preventing infections.

For example, electron beam and photolithography techniques were utilized to fabricate topographies that replicate leaf surface features and the internal structures of plants. Subsequently, nano-imaging technologies were employed to investigate how bacteria and fungi invade and colonize the leaf. Lithography was also used to nanofabricate a pillared surface on silicon wafers, creating a lawn of miniature pillars ranging from 1.4 to 20 µm wide and spaced at various distances. This surface was used to study the movement of fungi, mimicking certain characteristics of the host (Mccandless *et al.*, 2005). Images of *Colletotrichum graminicola* crawling across the nanofabricated surface helped researchers determine that the fungus requires a minimum contact distance of at least 4.5 µm to initiate the development of appressoria (López, *et al.*, 2009).

4. Application of Nanoparticles in Plant Disease Control/ Management

Nanotechnology offers new methods for enhancing and changing the traditional crop management techniques. Plant nutrients and protective chemicals are typically applied to crops through spraying or broadcasting. However, challenges such as chemical leaching, degradation by photolysis, hydrolysis and microbial breakdown result in only a very low concentration of chemicals, much less than the required effective concentration, reaching the target site in crops. Consequently, repeated applications are often necessary to achieve effective control, which can lead to negative effects such as soil and water pollution. Nanoformulated agrochemicals should be designed with specific properties, including effective concentration, time-controlled release in response to certain stimuli, enhanced targeted activity and reduced ecotoxicity. These features, combined with safe and simple delivery methods, can help avoid the need for repeated applications (Green and Beetsman, 2007; Figure 3).

4.1. Silver Nanoparticles

Silver nanoparticles (NPs) are known to inhibit plant pathogens through various mechanisms (Clement and Jarret, 1994) and the disease management achieved using them could be more effective and safer than traditional chemical fungicides (Park *et al.*, 2006). Many fungi, including phytopathogens, have been found to be sensitive to silver nanoparticles (AgNPs). Notable examples include *Fusarium culmorum* (Kasprowicz *et al.*, 2010), the oak wilt pathogen *Raffaelea* sp. (Kim *et al.*, 2009) and sclerotium-

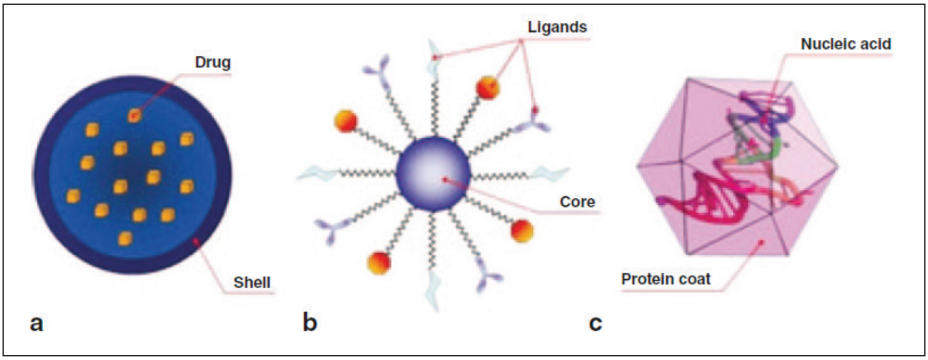

Figure 3: Pictorial representation of various nanodevices that could be effectively used to control plant diseases (Adopted from Pérez-de-Luque and Diego, 2009)

forming fungi such as *Rhizoctonia solani, Sclerotinia sclerotiorum* and *S. minor* (Min *et al.*, 2009). Additionally, *Bipolaris sorokiniana* and *Magnaporthe grisea* (Jo *et al.*, 2009) also exhibit sensitivity to AgNPs.

A study conducted by Mishra and Sharma (2017) reported the effects of silver nanoparticles (AgNPs) against *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris*, revealing the highest inhibition of *P. vulgaris* colonization and the lowest inhibition of *S. aureus*. Patra and Baek (2017) recorded a moderate inhibitory effect of AgNPs on the colonization of *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus, E. coli* and *Salmonella typhimurium*. Additionally, when AgNPs were combined with antimicrobial chemicals, the inhibitory effect of the resulting mixture was enhanced.

Kim *et al.* (2008) studied the antifungal effectiveness of a colloidal nano silver solution (with an average diameter of 1.5 nm) against rose powdery mildew, which is caused by *Sphaerotheca pannosa* var. *rosae*. Their research demonstrated the potential of nano silver as a viable treatment option for controlling this fungal disease in roses. Park *et al.* (2006) investigated the effectiveness of nanosized silica-silver on suppressing the growth of various fungi. They found that *Pythium ultimum, Magnaporthe grisea, Colletotrichum gloeosporioides, Botrytis cinerea* and *Rhizoctonia solani* exhibited 100% growth inhibition at a concentration of 10 ppm of nanosized silica-silver. In contrast, *Bacillus subtilis, Azotobacter chroococcum, Rhizobium tropici, Pseudomonas syringae* and *Xanthomonas campestris* pv. *vesicatoria* showed 100% growth inhibition at 100 ppm. They also reported that higher concentrations of nanosized silica-silver (3200 ppm) caused chemical injuries to cucumber and pansy plants when sprayed.

Green nanoparticles (NPs) have the potential to become a vital tools in modern crop production practices and environmental remediation. However, it is essential to conduct appropriate research to identify cost-effective production methods and ensure safe and sustainable applications. By focusing on these areas, green NPs can significantly contribute to improving agricultural productivity and mitigating environmental issues (Irshad *et al.*, 2024). Silver nanoparticles (Ag NPs) are widely used for sterilization processes, including wastewater treatment and water sanitization, due to their antimicrobial properties. By employing a green chemistry approach, Ag NPs can be synthesized to mitigate the harmful effects of certain fungal diseases. For example, Krishnaraj *et al.* (2012) tested the efficacy of Ag NPs at various concentrations against several fungal plant pathogens, including *Rhizoctonia solani, Macrophomina phaseolina, Alternaria alternata, Curvularia lunata, Botrytis cinerea* and *Sclerotinia sclerotiorum*, using green AgO NPs synthesized from the leaf extract of *Acalypha indica*. Remarkably, Ag NPs at a concentration of 15 mg demonstrated significant inhibitory activity against all the aforementioned pathogens in agricultural settings. In another study, Ag NPs prepared from a 5 mM AgNO3 solution using *Argemone mexicana* leaf extract at a concentration of 30 ppm were found to be highly toxic to the pathogenic fungus *Aspergillus flavus*.

4.2. Copper Nanoparticles

Fan *et al.* (2020) observed the antibacterial activity of copper (Cu) composites against *Xanthomonas euvesicatoria*. Huang *et al.* (2015) demonstrated the antifungal activity of copper oxide (CuO) nanoparticles against several fungal pathogens, including *Botrytis cinerea*, *Colletotrichum graminicola, Rhizoctonia solani, Colletotrichum musae, Magnaporthe oryzae, Penicillium digitatum* and *Sclerotium rolfsii*. Giannousi *et al.* (2013) further showed the antifungal properties of CuO and Cu_2O nanoparticles against *Phytophthora infestans*. Additionally, Sharma *et al.* (2015) reported both antifungal and antibacterial activities of magnesium oxide nanoparticles (MgO NPs) against the bacteria *Ralstonia solanacearum* and the fungus *Phomopsis vexans*.

Curcumin-copper oxide (Cur-Cu) nanoparticles have shown to enhance disease resistance in chickpea plants infected with *Fusarium oxysporum* f. sp. *ciceri*, a pathogen responsible for wilt disease. This nanomaterial-based approach highlights the potential of Cur-Cu NPs in strengthening plant defenses against fungal infections, offering an effective strategy for managing Fusarium wilt in chickpea cultivation (Sathiyabama *et al.*, 2020).

4.3. Zinc Oxide Nanoparticles

Jamdagni *et al.* (2016) found that zinc oxide nanoparticles (ZnO NPs) exhibit promising antifungal activity against several fungal pathogens, including *Alternaria alternata, Botrytis cinerea, Aspergillus niger, Fusarium oxysporum* and *Penicillium expansum*. Similarly, Navale *et al.* (2015) demonstrated the antifungal efficacy of ZnO NPs against *Aspergillus flavus* and *Aspergillus fumigatus*. Rajiv *et al.* (2013) further reported antifungal activity of ZnO NPs against *A. flavus, A. niger, A. fumigatus, Fusarium culmorum* and *F. oxysporum*. Gunalan *et al.* also found ZnO NPs to be effective against *A. flavus, Trichoderma harzianum, A. nidulans* and *Rhizopus stolonifer*. Dimkpa *et al.* (2013) showed the antifungal activity of ZnO NPs on *Fusarium* *graminearum*. Additionally, Jayaseelan *et al.* synthesized ZnO NPs using *Aeromonas hydrophila* and demonstrated their activity against the pathogenic bacteria *P. aeruginosa* and fungi, including *Candida albicans*, *A. flavus* and *A. niger*.

4.4. Titanium Nanaoparticles

Sar *et al.* (2017) reported that titanium dioxide nanoparticles (TiO_2 NPs) exhibit antifungal activity against *Fusarium oxysporum* f. sp. *radicislycopersici* and *Fusarium oxysporum* f. sp. *lycopersici*. Hamza *et al.* (2016) found that TiO_2 NPs are effective against the fungal pathogen *Cercospora beticola*. Additionally, Ardakani (2013) discovered the nematicidal activity of TiO_2 NPs against the *Meloidogyne incognita* nematode. Cui *et al.* (2009) further demonstrated the antibacterial activity of TiO_2 NPs against *Pseudomonas syringae* pv. *lachrymans* and *P. cubensis*.

4.5. Iron Nanomaterial

Iron is a highly reactive element and iron nanoparticles (Fe_3O_4 NPs) have demonstrated antiviral activity against the tobacco mosaic virus (TMV) in *Nicotiana benthamiana* plants. When treated with Fe_3O_4 NPs, the plant leaves showed activation of their resistance response to TMV, involving the stimulation of plant oxidants and the expression of salicylic acid (SA)-responsive pathogenesis-related (PR) genes. The iron nanoparticles successfully entered plant leaf cells, accumulated throughout the plant and contributed to increased dry and fresh weight, promoting overall plant health (Cai *et al.*, 2020).

4.6. Nickel Nanoparticles

Cucumber plants treated with nickel oxide nanoparticles (NiO NPs) exhibited antiviral activity against the cucumber mosaic virus (CMV). The treatment reduced disease severity and virus accumulation while inducing the expression of plant defense-related genes involved in salicylic acid (SA), jasmonic acid (JA) and ethylene signaling pathways in the infected plants. Additionally, the treatment with NiO NPs led to an increase in both the fresh and dry weight of cucumber leaves, as well as an increase in the number of leaves, contributing to improved plant health and growth (Gaikwad *et al.*, 2013).

4.7. Bio-Compounds Nanoparticles

The field of nanotechnology has recently explored a new class of nanoparticles known as bio-compound nanoparticles. These bio-compound nanoparticles are biodegradable, biocompatible and less toxic, offering a significant advantage by minimizing the environmental and biological damage often associated with various metallic and non-metallic nanoparticles. Their safer profile makes them a promising alternative for use in applications where conventional nanoparticles may pose risks.

Chitosan, a bio-compound derived from the deacetylation of chitin, is renowned for its strong antimicrobial properties. In an antiviral assay, chitosan/dextran nanoparticles (CDNPs) demonstrated effectiveness against the alfalfa mosaic virus (AMV) in *Nicotiana glutinosa* plants. The application of CDNPs reduced disease severity and virus accumulation in mechanically infected plants. Furthermore, CDNP treatment increased total carbohydrate and phenolic contents, while also triggering the activation of defense-related enzymes and genes, including peroxidase, phenylalanine ammonia-lyase (PAL) and pathogenesis-related protein 1 (PR-1) genes (Abdelkhalek *et al.*, 2021).

Curcumin, a highly potent and non-toxic bio-compound derived from the rhizome of turmeric (*Curcuma longa*), has shown promising antiviral properties. In a study, curcumin-milk protein nanoparticles demonstrated virucidal activity against Potato virus Y (PVY) in infected potato plants. The treatment with these nanoparticles activated key defense enzymes, such as peroxidase (POX) and polyphenol oxidase (PPO), helping the plants to combat the viral infection more effectively (Taha *et al.*, 2019).

Pomegranate peel (PP) extract and PP nanoparticles (PP NPs) were applied to *Datura metel* plants to combat tobacco mosaic virus (TMV). Dawi *et al.* (2021) observed that PP NPs were significantly more effective in reducing the number of local lesions and virus concentration in infected plants compared to the use of PP extract alone. This highlights the enhanced antiviral potential of nanoparticle formulations over traditional plant extracts (Dawi *et al.*, 2021).

Glycyrrhizic acid ammonium (GAS) salt and salicylic acid (SA) nanoparticles have shown potential in inhibiting the infectivity of Potato leafroll virus (PLRV) in potato plants. These nanoparticles work by attaching to the viral coat protein, effectively blocking the packaging of virus particles, thereby preventing the virus from spreading and replicating within the plant. This mechanism makes GAS and SA nanoparticles promising tools for antiviral strategies in plant disease management (Shoala *et al.*, 2021).

5. Mechanism Involved in Nanoparticles in Plant Disease Management

The restrictive action of nanoparticles on fungi and bacteria involves multiple mechanisms that ultimately lead to cell death. These mechanisms include pore formation in the cell membrane, disturbance of membrane potential, damage to the cell wall, direct attachment to the cell surface, DNA damage, cell cycle arrest, inhibition of enzyme activity and the generation of reactive oxygen species (ROS). The generated ROS damage cellular structures, contributing to the death of microbial cells (Singh *et al.*, 2019).

ROS generated by nanoparticles include both free radicals and non-radicals. Free radicals, such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), singlet oxygen ($^1O_2$), carbon dioxide radicals (CO_2^-), hydroxyl radicals (HO•), hydroperoxyl (HO_2•), carbonate (CO_3^-), peroxyl (RO_2•) and alkoxyl (RO•), play a critical role. Non-radicals like ozone (O_3), nitric oxide (NO), hypobromous acid (HOBr), hypochlorous acid (HOCl), hypochlorite (OCl^-), peroxynitrite ($ONOO^-$), organic peroxides (ROOH), peroxomonocarbonate ($HOOCO_2^-$), peroxynitrous acid (ONOOH) and peroxynitrate (O_2NOO^-) also contribute.

As nanoparticles accumulate in microbial cell membranes, they alter the permeability, disrupting the proton motive force (PMF). This oxidative stress, especially at higher concentrations, can lead to single- and double-strand breaks in DNA, along with lesions in nitrogen bases and pentose sugars. This combination of stress and damage to critical cellular structures results in the death of bacterial and fungal cells (Slavin *et al.*, 2017).

6. Nano Biostimulants

Plants have evolved innate immune responses, including the production of antioxidants, activation of defense enzymes and strengthening of cell walls, to defend against pathogen attacks (Kumaraswamy *et al.*, 2018). These natural defense mechanisms are non-specific and can be modulated to protect crop plants against a wide range of phytopathogens (Sathiyabama *et al.*, 2018).

Kumari *et al.* (2017b) synthesized silver nanoparticles (Ag NPs) with bactericidal properties, which can also enhance plant immunity. When tomato plants were pre-treated with 5 μg ml^{-1} of Ag NPs, a significant increase in the production of phenolic compounds and oxidative enzymes, along with a reduction in pathogen infection, was reported. This suggests that Ag NPs can stimulate plant resistance, preparing them to withstand pathogen invasion. Additionally, tomatoes pre-treated with Ag NPs showed a notable 23.52% increase in total chlorophyll content compared to plants infected by *Alternaria solani*, potentially supporting enhanced resistance by meeting the plants' energy demands. Several metal nanoparticles (NPs) have been reported to trigger plant defense responses against pathogens. Imada *et al.* (2016) reported that magnesium oxide (MgO) NPs exhibit strong bactericidal activity against *Ralstonia solanacearum in vitro*. However, significant inhibition of bacterial wilt development occurred only when MgO NPs were applied preventatively, suggesting they induced systemic resistance in tomato plants. The treatment with MgO NPs also resulted in the rapid production of reactive oxygen species (ROS), along with the activation of the salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signaling pathways, as well as the accumulation of β-1,3-glucanase and tyloses. Similarly, a 6% reduction in the severity of tomato early blight caused by *Alternaria solani* was observed with the joint foliar application of selenium (Se) NPs (20 mg/l) and copper (Cu) NPs (50 mg/l) as a preventative treatment. This led to an increase in both enzymatic and non-enzymatic antioxidant compounds in the leaves and fruit, helping the plants better tolerate pathogen-induced stress (Quiterio-Gutiérrez *et al.*, 2019).

7. Conclusion

In India, nanotechnology, which has already made significant strides in various fields of plant pathology, is the second most popular area of innovative research after biotechnology. While there are numerous examples of using nanosensors for detecting animal and human pathogens, the application of nanotechnology in plant pathogen detection is still in its early stages. However, there is a growing number of reports on the use of microbes and plant pathogens in the biosynthesis of nanoparticles, highlighting its potential in this area. The use of nanomaterials (NMs) in plant disease management holds great promise. NMs can offer protection not only as antimicrobial agents and biostimulants but also serve as delivery systems for active ingredients to suppress phytopathogens. Their enhanced efficacy, lower input costs and reduced toxicity to non-target organisms present significant advantages over conventional products and methods. However, there remains a gap in understanding the environmental fate of NMs, which limits their broader application.

8. References

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