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NANOTECHNOLOGY IN PLANT PATHOLOGY: INNOVATIVE TOOLS FOR EARLY PATHOGEN DETECTION

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ABSTRACT

Plant pathogens are critical factors that restrict crop yields, causing substantial reductions in agricultural productivity worldwide. They pose serious threats to food security and remain a major global challenge for agriculture. While chemical treatments remain the primary method for controlling plant diseases, repeated use can lead to reduced pathogen sensitivity. Excessive application can also harm the environment and disrupt soil microbiota. To promote agricultural sustainability and safeguard food security, it is essential to develop efficient diagnostic methods for the early and rapid plant pathogens detection. Various conventional and molecular techniques have been designed for the quick detection of plant pathogens. However, these methods are usually costly, time-consuming, dependent on skilled operators, and not ideal for in-field analysis. Plant protection becomes achievable with the use of nanotechnology tools such as microneedle patches, nanopore sequencing, nano barcoding, nano biosensors, quantum dots. These tools offer significant potential to enhance the sensitivity, accuracy, and speed of plant pathogen detection while enabling high-throughput analysis.

Keywords: Nanotechnology, Plant Pathology, Pathogen Detection, Nano Biosensors, Quantum Dots, Microneedle Patches, Nanopore Sequencing, Bio-barcode Assay, Plant Disease Diagnosis and Sustainable Agriculture.

INTRODUCTION

At present, more than a billion individuals around the world face hunger to varying degrees due to a shortage of essential staples and inadequate nutrition (Debnath, 2013). This issue is primarily

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caused by the ongoing rise in global populations and the decline in agricultural productivity, driven by social, economic and environmental factors. Crop disease caused by pathogen infection and pest attack is one of the main constraints of agricultural production and has become one of the critical global issues (Strange and Scott, 2005). Globally, insect pests and plant diseases are estimated to be responsible for 14% and 13% crop loss, respectively. The annual cost of crop loss was estimated to be 2000 billion dollars (FAO, 2018). It is estimated that pathogen attacks on key crops such as potatoes, maize, peanuts, and soybeans result in a yearly loss of 10–25% of total production (Kaminski and Christiaensen, 2014; Kumar and Kalita, 2017). Currently, several plant diseases are identified by looking at a plant's visual characteristics and leaf features. Additionally, by culture method which is used for isolating and growing pathogens and microscopy which is used to identify bacterial cells, fungal spores and viral particles are used to identify potential pathogens. Traditional molecular detection methods, such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and other classic techniques like colony counting and fluorescence in situ hybridization (FISH), are commonly used in laboratories worldwide to accurately and precisely identify plant pathogens. However, many molecular biology reagents, such as enzymes and primers, are costly and have a short shelf life. Moreover, these techniques require specialized instruments and laboratory setups, which limits their accessibility in developing countries (Mark *et al*., 2014).

Nanotechnology organizes individual atoms, molecules, or clusters of molecules into structures that allow materials to carry out unique or remarkably diverse tasks (Joseph and Morrison, 2006). The National Science and Technology Council described nanotechnology as a field that relates with seeing, assessing and manipulating matter at atomic, molecular and supramolecular level (Muktar et al., 2015). Nanotechnology works with small sized nanoparticles or molecules with a size of approximately 1 to 100 nm, at least in one dimension (He *et al*., 2019). The use of nanotechnology in agriculture can transform agricultural research by providing advanced tools for the early detection of plant diseases and pathogens. It has great potential to recognize pathogens more quickly and accurately than other currently available plant pathogens detection methods.

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Table 1: Comparison of different methods for the detection of plant pathogens

Method	Detection limit	Time required for an Assay	Time before results
Conventional methods	10^7–10^8 cfu/ml	72 h	72 h
Nucleic acid-based methods	10^3–10^4 cfu/ml	1-3 h	6-12 h
Immunological methods	10^5–10^6 cfu/ml	1-3 h	6-12 h
Nanotechnology-based methods	1 fmol/l	30 min-1 h	30 min-1 h

Table 1 shows nanotechnology-based methods offer significant advantages over conventional techniques for detection of plant pathogens. They exhibit superior sensitivity, requiring a much lower concentration of pathogens (1 fmol/l) to be detected. Additionally, nanotechnology-based assays are significantly faster, reducing the time needed for analysis and providing results in a fraction of the time (30 min- 1h) compared to conventional methods (Shivashakarappa *et al*., 2022).

CERTAIN NANOTECHNOLOGICAL TOOLS FOR THE DETECTION OF PLANT PATHOGENS

1. Nano biosensors: Nano sensors with immobilized bioreceptor probes that are selective for target analyte molecules are termed as nano biosensors. It has been developed to detect a wide range of plant pathogens (Bacteria, Fungi, Nematodes, Viroid's and Viruses). The combination of biotechnological and nanotechnological approaches in bio-sensors can be used to construct equipment with increased sensitivity, allowing an earlier response to ecological changes and disease prevalence. Ideal characteristics of nano biosensors are it should be cheap, portable, stable when stored under regular conditions, signals received from it should be reproducible, and the interaction with analyte should be extremely specific (Rai *et al*., 2012).

Components of Nano Biosensor

- It has three main components
    I. **Bioreceptor**: They are generally biological materials (antibodies, enzymes, microorganisms, nucleic acids, cell organelles, tissues etc. that interact and accept

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the chemical signal from the analyte to be detected, and then transfer it to the transducer.

II. **Transducer:** It works as an interface that measures the signal from the bioreceptor and transforms it into a quantifiable electrical signal in the detector.

III. **Detector:** It is last component that processes the signals to amplify and analyze them.

Table 2: Various biosensors for the detection of fungal plant pathogens

Biosensor	Pathogen	Detection Limit	Reference
Electrochemical	*Phytophthora spp.*	0.1 pg/µL	Zhan *et al*. (2018)
Immunosensor	*Pseudocercospora fijiensis*	1.7 µg/mL	Luna-Moreno *et al*. (2019)
Immunosensor	*Pectobacterium atrosepticum*	10^3 CFU/mL	Hashemi Tameh *et al*. (2020)
Impedimetric	*Sclerotinia sclerotiorum*	7.8×10^4 ascospores/mL	Shoute *et al*. (2018)
Optical	*Aspergillus niger*	NA	Gambhir *et al*. (2018)

Yao *et al.* (2009) evaluated the conjugation of silica nanoparticles with the secondary antibody of goat anti-rabbit immunoglobulin G (IgG) and detected *Xanthomonas axonopodis* pv. *vesicatoria* that causes bacterial spot disease in *Solanaceae* plant. Nano biosensor has significant baseline shift of -337 compared to ELISA which has an absorbance of 0.09 at lower concentration of 1µg/ml underscores the superior sensitivity and efficiency of the nano biosensor for detecting *Polymyxa betae* compared to traditional ELISA techniques (Safarpour *et al.,* 2012). Lin *et al*. (2014) compared Gold nanorod Fiber Optic Particle Plasmon Resonance (AuNR-FOPPR) and ELISA for the detection of *Cymbidium mosaic virus* and *Odontoglossum ringspot virus* in orchids. Out of 20 samples, samples 17 and 19 have lower concentrations of 0.81 and 0.40 respectively, showing ability to detect viral pathogens by AuNR-FOPPR even at lower concentrations. The anodic signal measured for the non-target organism like *Colletotrichum gloeosporioides*, *Fusarium equiseti*, *Lasiodiplodia theobromae*, are not significantly different from the blank while the anodic signal measured for *Phytophthora palmivora* is significantly different from the blank, suggesting that the nano biosensor detection is selective towards *P. palmivora* DNA (Franco *et al*., 2019).

2. Quantum dots: Quantum dots (QDs) are semiconductor nanocrystals that have shown tremendous potential as optical nanosensors. The quantum dots have a narrow defined tunable emission peak, longer fluorescence lifetime, resistance to photobleaching and 10–100 times higher molar extinction coefficient. These properties of quantum dots allow multi-color quantum dots to

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be excited from one source by common fluorescent dyes without emission signal overlap and results in brighter probes compared to conventional fluorophores (Zhao and Zeng, 2015). QDs, with their exceptional and advantageous photophysical properties, have been successfully employed as biosensors for plant imaging and disease detection (Wang *et al.,* 2016). Rad *et al.* (2012) enhanced the specificity of a nano biosensor using quantum dots (QDs) for the detection of lime plants infected with phytoplasma (P. *aurantifolia*) by showing cent percent specificity as well as detection limit of 5 *ca*. *P*. *aurantifolia*/µl and acceptable stability that used for detection of witches' broom disease of lime. The electrochemical ELISA using functionalized CdSe quantum dots could detect the *Banana bunchy top virus* in plant sap up to dilution of 1:25 as compared to 1:10 of conventional ELISA, capable to detect pathogen at lower concentrations (Majumder *et al*., 2020).

Table 3: Detection of plant viruses by QD-based biosensors

Virus	Interface	Conjugate	LOD*	Reference
Cauliflower mosaic virus	PbS nanoparticle	23-mer derived from CaMV 35S	4.38×10^{-12} mol/l	Sun *et al*., 2008
Citrus tristeza virus	InP	Antibody to CTV coat protein	2 nM for antibody	Moreau *et al*., 2012
	CdTe	CTV-CP antibody	220 ng/ml	Shojaei *et al*., 2016b
	AuNPs/QD	AuNPs-CTV CP/QDs-CTV-CP antibody	130 ng/ml	Shojaei *et al*., 2016a
	CdTe	CTV-CP antibody	198 ng/ml	Safarnejad *et al*., 2017
Grapevine virus A	ZnO films	Grapevine virus A type proteins	1 pg/ml-10 ng/ml	Tereshchenko *et al*., 2017

3. Microneedle patches: Patches about 5 millimeters wide, covered with hundreds of tiny, cone-shaped microneedles. The needles are crucial for extracting DNA from plant cells. It is a rapid process for DNA extraction that can be completed within minutes, allowing for quick detection of pathogens, minimal sample preparation, non-destructive and sensitive as it can detect low levels of pathogen DNA, even in early stages of infection are the main advantages of Microneedle

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patches. Microneedle patch designed to penetrate the leaf's adaxial epidermis, the upper protective layer, and access the mesophyll tissue beneath. This tissue contains both healthy mesophyll cells and those infected with pathogens. The microneedles efficiently collect cellular material, including DNA, from healthy and infected mesophyll regions without causing extensive damage to the plant. Infected mesophyll is particularly targeted for the extraction of pathogen DNA.

Phytophthora infestans, the pathogen causing late blight in potatoes and tomatoes, can be efficiently and precisely identified using a polymer-based microneedle patch approach (Paul *et al*., 2019). The overall sensitivity (true positive) and specificity (true negative) of the MN RNA extraction method for the detection of *Tomato spotted wilt virus* in tomato in laboratory-inoculated leaves were 97.5% and 100%, respectively (Paul *et al*., 2021).

4. Nanopore sequencing platform

Nanopore sequencing presents two primary challenges: (1) identifying the nucleotides as the DNA strand moves through the nanopore, and (2) regulating the speed at which the strand passes through the nanopore (Clarke *et al*., 2009). A protein nanopore and an enzyme are engineered to manage the movement of a single DNA strand. A direct electronic analysis is performed as the DNA passes through the nanopore. The protein nanopore is embedded in a polymer bilayer membrane positioned over a microwell. Each microwell contains a sensor chip that detects ionic current changes as the single molecule travels through the nanopore. The MinION nanopore sequencing device, developed by Oxford Nanopore Technologies, enabled real-time sequencing of long DNA or RNA reads. The MinION platform is compact, portable, and capable of long-read sequencing, making it a powerful tool for applications like genomic research, pathogen detection, and clinical diagnostics.

The effectiveness of nanopore sequencing in detecting plant infections, including tomato yellow leaf curl virus and watermelon chlorotic stunt virus, was recently assessed through DNA or RNA sequencing of virus-infected plant tissues. The entire process is completed in under two hours, with results comparable to traditional diagnostic techniques (Chalupowicz *et al*., 2019). Marcolungo *et al*. (2022) developed a novel all-in-one diagnostic technology utilizing nanopore sequencing and evaluated it for the simultaneous identification and characterization of pathogens, specifically targeting *Xylella fastidiosa*. Faino *et al*. (2021) found the detection of *Xylella fastidiosa* and suggested that in sample 9, the presence of multiple species, represented by a variety of colors, suggests contamination or reduced specificity of XF detection.

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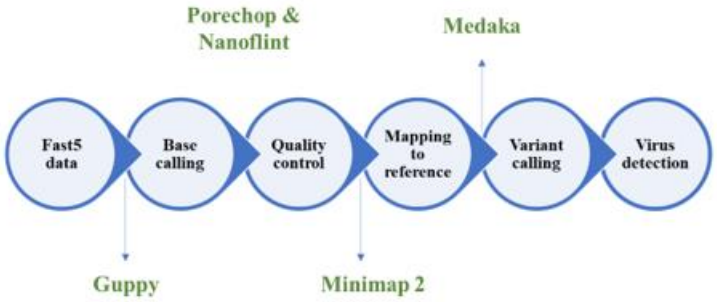

Fig. 1: Outline of potential stages in the workflow for nanopore sequencing analysis for plant virus detection

Sun *et al*. (2022) gave workflow for plant virus detection. In this, the guppy tool is used to convert the raw signals into readable nucleotide sequences. This data undergoes quality control, where tools like Porechop and NanoFilt are used to trim adapters and filter low-quality reads. The cleaned data is then mapped to a reference genome using Minimap2, aligning the sequences for comparison. Medaka is used for sequence polishing to improve the accuracy of the alignment. Variant calling is performed to identify any mutations, and the virus is identified based on the detected variants.

5. Nano barcode assay

Advances in nanotechnology have led to the development of a novel method called "bio-barcoding," designed for the ultra-sensitive detection of proteins and DNA without relying on enzymes. Unlike traditional ELISA-based assays, which depend on target and sample density, protein barcode assays are more complex, sensitive, and versatile. Compared to conventional techniques like ELISA and qPCR, the nanoparticle-based bio-barcode test offers heightened sensitivity for pathogen detection. Barcode assay uses two probes.

1. Magnetic microbeads (MMB), which carry an antibody or DNA as a biological probe are designed for target recognition.
2. Au-NPs (Gold nanoparticles) containing polyclonal antibodies or an oligonucleotide (Bio-barcode).

Amini *et al*. (2017) evaluated the selectivity of the proposed probes and barcode DNA for the detection of target DNA from various bacterial species. They found that *Pseudomonas aeruginosa* exhibits the highest fluorescence intensity (approximately 600 ng/ml), significantly surpassing other species such as *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysentery* and *Vibrio* cholera, highlighting the high specificity of the probes and barcode DNA for *Pseudomonas aeruginosa* detection. A barcode lateral flow assay for detecting *Potato virus x* in the buffer solution in potato indicating 10 µg/mL to 3.9 ng/mL PVX concentrations. They found that PVX

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concentration decreases from strips 6 to 13, the intensity of the test lines diminishes, demonstrating the assay's sensitivity and its ability to detect PVX down to very low concentrations (around 3.9 ng/mL) (Panferov *et al*., 2017).

Risks Associated with Nanotechnology Affecting Plant Pathogens

1. **Impact on Beneficial Microorganisms**: Nanoparticles used for pathogen detection may inadvertently affect beneficial soil microorganisms that play a crucial role in plant health. Disruption of these communities can lead to increased susceptibility of plants to diseases.
2. **Ecosystem Disruption**: The introduction of nanoparticles into agricultural ecosystems can alter interactions between plants, pathogens, and other organisms. For example, if nanoparticles affect the behavior of pollinators or natural pest predators, this could indirectly influence plant health and disease dynamics.
3. **Persistence in the Environment**: Some nanoparticles may persist in the soil or water, leading to long-term exposure for plants and soil organisms. This persistence can result in chronic toxicity or bioaccumulation, potentially affecting plant health and pathogen resistance.

Nanoparticle Toxicity

1. **Phytotoxic Effects**: Nanoparticles may exhibit phytotoxicity, causing damage to plant tissues. This can weaken plants, making them more susceptible to pathogens. For instance, if a nanoparticle interferes with a plant's immune response, it could facilitate pathogen invasion.
2. **Toxicity to Pathogens**: Nanoparticles are designed to target and kill pathogens, there is a risk that they could also harm beneficial microbes that help suppress diseases. This could lead to an imbalance in the microbial community, potentially resulting in increased disease incidence.
3. **Human Health Concerns**: Nanoparticles are used in agricultural practices and enter the food supply, there may be concerns about their potential toxicity to humans. This is particularly relevant if the nanoparticles affect the plants' ability to resist pathogens, leading to increased pesticide use or other interventions that could have health implications.

CONCLUSION

Nanotechnology presents a transformative approach to plant pathogen detection, offering unprecedented sensitivity, precision and versatility through advanced tools and techniques. In contrast to conventional methods which require more testing time and have few limitations,

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Nanotechnology tools offer significant advances through rapid and highly sensitive pathogens probes that can be used as a speedy approach for the detection of various plant pathogens. Tools like Nano biosensor, Quantum dots, Micro-needle patches, Nanopore sequencing platform and Nano barcode assay detect pathogens at extremely low concentrations and in complex environments. By integrating these tools into agricultural practices, nanotechnology paves the way for smarter, more sustainable crop management, ultimately improving food security and resilience against plant diseases.

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