**Revolutionizing Plant Pathology with Microfluidic Technology Toward Rapid and Accurate Diagnostics : A Review**

**Abstract**

Microfluidic technology is revolutionizing plant disease diagnostics by offering rapid, precise, and cost-effective detection tools compatible with field-based deployment. As plant diseases continue to threaten global food security and agricultural sustainability, the need for advanced diagnostic platforms that overcome the limitations of traditional methods such as culture-based, serological, and PCR assays has become critical. Microfluidic systems enable miniaturized sample handling, on-chip reagent mixing, nucleic acid amplification, and multiplexed detection of bacterial, fungal, viral, and nematode pathogens with reduced reagent use and diagnostic time. These devices are increasingly integrated with isothermal amplification methods like LAMP and RPA, biosensors, and smartphone-based readout systems for real-time visualization and geo-referenced data collection. Innovations in paper-based and thermoplastic microchips, CRISPR-Cas-based biosensing, nanomaterial-enhanced signal amplification, and portable energy sources are enhancing the accessibility and performance of microfluidic diagnostics. Despite these advantages, technical bottlenecks such as device clogging, matrix interference from plant tissues, and a lack of standardized validation protocols pose challenges to large-scale adoption. Efforts to address these issues include the development of single-cell microfluidics for host-pathogen interaction studies, high-throughput multiplex platforms, and open-source, modular designs suitable for customization. When coupled with IoT-enabled surveillance networks, cloud-based data systems, and AI-driven decision support, microfluidic tools enable spatial disease modelling, early outbreak forecasting, and site-specific disease management. These capabilities align with the principles of precision agriculture by optimizing agrochemical application, reducing input costs, and minimizing environmental impact. Realizing the full potential of microfluidics in plant health requires interdisciplinary collaboration among plant pathologists, engineers, biochemists, and data scientists. Such integration will foster the development of scalable, validated, and policy-compliant diagnostic systems capable of transforming agricultural disease management.

**Keywords:** *Microfluidics, Diagnostics, Pathogens, Biosensors, Precision, Nanotechnology, Surveillance*

**I. Introduction**

*Plant pathology*
Plant pathology is a specialized discipline within agricultural science that focuses on understanding plant diseases caused by pathogenic microorganisms such as fungi, bacteria, viruses, nematodes, and other biotic agents, as well as disorders triggered by abiotic factors like nutrient deficiencies, pollutants, and environmental stress (Awasthi *et.al.,* 2024). The primary goal is to identify, diagnose, prevent, and manage these diseases to ensure optimal crop yield and quality. The scope of plant pathology spans from field surveillance to molecular diagnosis and includes aspects such as disease epidemiology, host-pathogen interaction, resistance breeding, and integrated disease management. This field is crucial to global food security, as plant diseases are responsible for up to 20–40% of total crop losses annually, with fungal pathogens alone causing an estimated global yield loss of 125 million tonnes across five major crops (wheat, rice, maize, potatoes, and soybean).

*Importance of timely and precise plant disease diagnostics*
The early and accurate detection of plant diseases is vital for the implementation of effective control measures and the prevention of large-scale outbreaks (Buja *et.al.,* 2021). Conventional diagnosis is often time-consuming and requires skilled personnel, leading to delays in response and widespread crop loss. Rapid diagnosis helps differentiate between pathogens with similar symptoms and facilitates precision agriculture by enabling site-specific interventions. The accuracy of diagnostic methods directly influences the success of integrated pest and disease management (IPDM) systems. Modern agriculture demands robust diagnostic solutions capable of providing real-time information for surveillance, risk assessment, and response planning to support both yield stability and sustainability in cropping systems.

*Limitations of conventional diagnostic tools*
Traditional diagnostic techniques such as visual inspection, culture-based methods, ELISA, and PCR, while reliable under laboratory settings, are often inadequate for field conditions (Panwar *et.al.,* 2023). Visual diagnosis is subjective and prone to errors due to symptom similarities among different pathogens. Culture methods are slow, taking several days to weeks for pathogen identification. PCR and ELISA, although more specific, require expensive equipment, reagents, and trained personnel, and are sensitive to contaminants in plant samples. Moreover, these approaches often lack the portability and multiplexing capacity needed for high-throughput or in-field analysis, especially during sudden disease outbreaks.

*Emergence of microfluidics as a transformative platform*
Microfluidics, often described as lab-on-a-chip (LOC) technology, involves the manipulation of minute volumes of fluids (10⁻⁹ to 10⁻¹⁸ L) within microscale channels fabricated from materials like PDMS, glass, or paper. This miniaturization allows for precise fluid control, integrated detection systems, and low reagent usage. In plant pathology, microfluidics offers an unprecedented opportunity to transform pathogen diagnostics by enabling compact, rapid, and multiplexed detection systems that are portable and cost-efficient. These devices support a range of analytical methods including nucleic acid amplification, immunoassays, and biosensin. The integration of microfluidic devices with smartphones, IoT sensors, and AI-based data analysis tools has further expanded their application for real-time, field-deployable plant disease monitoring (Zhao *et.al.,* 2024).

**II. Concept of Microfluidics**

*Basic principles and components of microfluidic systems*
Microfluidics is a multidisciplinary field that merges principles of fluid mechanics, microfabrication, biochemistry, and engineering to control and manipulate fluids in channels with dimensions ranging from tens to hundreds of micrometers (Hajam *et.al.,* 2024). The fundamental principle of microfluidics lies in laminar flow, where the Reynolds number (Re < 1) ensures predictable and non-turbulent fluid behaviour, enabling precise control over the mixing, reaction, and movement of fluids. The key components of a microfluidic system typically include a microchannel network, microvalves, micropumps, mixers, reaction chambers, and detection units. These elements work in unison to execute complex fluidic operations such as sample dilution, reagent mixing, nucleic acid amplification, and signal detection within an integrated lab-on-a-chip platform. The manipulation of fluids at the microscale significantly reduces the sample and reagent volumes, increases reaction speed due to high surface-area-to-volume ratio, and enhances analytical sensitivity.

*Types of microfluidic platforms: continuous-flow, droplet-based, paper-based, digital*
Microfluidic systems are classified based on the nature of fluid movement and the underlying design strategy (Ong *et.al.,* 2008). Continuous-flow microfluidics employs external pressure or electrokinetic forces to drive fluids through enclosed microchannels, suitable for steady-state chemical and biological reactions. These systems are ideal for real-time pathogen detection and high-throughput assays. Droplet-based microfluidics generates discrete droplets (typically picoliter to nanoliter) of aqueous sample encapsulated in an immiscible carrier fluid. Each droplet acts as an isolated microreactor, enabling parallel processing, multiplexing, and reduced cross-contamination. These platforms are valuable in PCR, digital ELISA, and single-cell analysis. Paper-based microfluidics (µPADs) utilize patterned cellulose substrates to drive fluid through capillary action without external pumps. These low-cost, biodegradable platforms are widely applied in field-based plant disease diagnostics, often coupled with colorimetric or fluorescence detection. Digital microfluidics relies on electrowetting-on-dielectric (EWOD) to manipulate discrete droplets on an open surface through electrical actuation. This allows programmable and reconfigurable workflows for nucleic acid amplification and biosensing.

*Materials used in fabrication (e.g., PDMS, glass, paper, thermoplastics)*
Material selection for microfluidic device fabrication impacts performance, biocompatibility, cost, and field applicability (Ren *et.al.,* 2013). Polydimethylsiloxane (PDMS) is widely used due to its optical transparency, elasticity, biocompatibility, and ease of soft lithographic melding. It enables rapid prototyping of chips with high-resolution features. Glass offers chemical inertness, excellent optical clarity, and compatibility with high-temperature reactions, making it suitable for integration with fluorescence or UV-based detection systems. Paper provides an affordable, eco-friendly matrix for fabricating µPADs using wax printing or photolithography. It facilitates storage of reagents, on-chip sample preparation, and simple colorimetric output without external instruments. Thermoplastics such as PMMA, COC, and polycarbonate are increasingly adopted for scalable production via injection moulding or hot embossing. These materials offer mechanical robustness and are compatible with surface modification and integration of electrodes or sensors. The choice of material is influenced by the intended application, detection method, and cost constraints.

*Integration with biosensors, optics, and electronics*
The versatility of microfluidic platforms is significantly enhanced by their integration with biosensors, optical components, and electronic interfaces, forming a holistic diagnostic unit (Liao *et.al.,* 2019). Biosensors integrated on-chip can detect target biomolecules through specific interactions involving antibodies, nucleic acid probes, or aptamers. Signal transduction mechanisms include electrochemical, fluorescence, surface plasmon resonance (SPR), and piezoelectric responses. Optical systems such as miniaturized light sources, photodiodes, and smartphone cameras are coupled with the chip for real-time detection, visualization, and quantification of assay outcomes. These systems allow integration of fluorescence-based LAMP, real-time PCR, and colorimetric detection in a portable manner. Electronics such as microcontrollers, printed circuit boards (PCBs), and Bluetooth or Wi-Fi modules enable automation, signal processing, data transmission, and IoT connectivity. These embedded systems help convert microfluidic platforms into smart diagnostic devices that facilitate remote monitoring and decision-making. The seamless fusion of microfluidic channels with embedded biosensing and detection technologies has led to the development of compact, automated, and user-friendly diagnostic tools tailored for use in plant pathology (Yadav *et.al.,* 2025). These devices are paving the way for decentralized, real-time pathogen surveillance and precision agriculture.

**III. Historical Evolution and Milestones in Microfluidics**

*Timeline of key developments in microfluidic technology*
The origin of microfluidics can be traced to the development of miniaturized gas chromatographic systems in the 1970s (Table 1) (Wardencki *et.al.,* 2021). The initial breakthrough occurred in 1979, the first gas chromatography (GC) system integrated on a silicon wafer, establishing the foundation for micro-total analysis systems (µTAS). The 1980s and early 1990s witnessed substantial progress in silicon and glass micromachining, aided by advances in microelectromechanical systems (MEMS) and photolithography. A landmark event occurred in 1990 the concept of µTASa miniaturized system capable of handling sample preparation, reaction, separation, and detection on a single chip. By the mid-1990s, polydimethylsiloxane (PDMS) became the dominant material for chip fabrication due to its transparency, elasticity, and biocompatibility, with pioneering work from Whitesides and colleagues catalysing rapid development of soft lithography. The 2000s saw diversification into droplet microfluidics, paper-based systems, and electrowetting platforms. Droplet microfluidics emerged prominently around 2001, enabling high-throughput, compartmentalized reactions useful for genomics and diagnostics. The introduction of paper-based analytical devices (µPADs) in 2007 transformed point-of-care testing with low-cost fabrication. The integration of digital technologies such as smartphone-based detection and wireless data transmission began dominating the field by the 2010s, leading to smart and IoT-enabled diagnostic devices.

*Evolution from chemical analysis to biological applications*
Microfluidics initially served as a miniaturized platform for chemical separations and environmental monitoring (Yew *et.al.,* 2019). By the late 1990s, the application expanded rapidly into biological and biomedical domains due to the need for small sample volumes, faster processing times, and multiplexing. The introduction of microfluidic capillary electrophoresis and DNA microarrays facilitated nucleic acid analysis for genetic and disease studies. Immunoassay-based microfluidics became common for protein detection, using antigen-antibody interactions enhanced by confined reaction environments. By 2005, microfluidics was increasingly adopted for polymerase chain reaction (PCR) miniaturization, leading to real-time, droplet-based, and digital PCR systems. These innovations enabled applications such as pathogen identification, genotyping, and epigenetic analysis at the microscale. In parallel, single-cell analysis and organ-on-chip models expanded microfluidic utility to cell biology and pharmacology. Lab-on-chip systems integrating microvalves, micromixers, and biosensors became essential in cancer diagnostics, infectious disease monitoring, and personalized medicine.

*Adaptation for plant sciences and pathology*
The transition of microfluidic technologies into agricultural applications, particularly plant sciences and plant pathology, is a more recent development that gained momentum post-2010 (Ganesan *et.al.,* 2024). The growing need for rapid, field-deployable diagnostic tools for plant diseases catalysed the integration of microfluidics into this domain. Researchers began modifying existing platforms to handle plant tissue homogenates, root exudates, and phloem sap complex biological matrices traditionally difficult to process in microdevices. Microfluidic platforms enabled on-chip extraction and detection of plant pathogens such as *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and viruses including *Tobacco mosaic virus* and *Potato virus Y*. Paper-based µPADs were especially adapted for colorimetric detection of pathogens in crops like citrus, tomato, and banana, often coupled with isothermal amplification methods like LAMP or recombinase polymerase amplification (RPA). The capability of microfluidic devices to integrate sample preparation, amplification, and detection into a single chip reduced time-to-result to under 30 minutes in some cases. Electrochemical and fluorescence-based microfluidic biosensors were deployed for plant stress biomarkers, enabling monitoring of plant immune responses and facilitating early disease intervention. Recent developments also involve smartphone-interfaced microfluidic chips for mobile diagnostics in precision agriculture (Yang *et.al.,* 2016). These adaptations address the global need for sustainable crop protection strategies under increasing biotic stress and climate change.

**Table:1** Historical Evolution and Milestones in Microfluidics (Source: Wardencki *et.al*., 2021, Yew *et.al.,* 2019)

|  |  |  |
| --- | --- | --- |
| **Year/Period** | **Milestone** | **Description and Relevance to Plant Pathology** |
| **1950s–1970s** | *Foundations in Miniaturized Chemistry* | Concepts like laminar flow and fluid dynamics in small channels explored in analytical chemistry; basis for later development of lab-on-a-chip (LOC) systems. |
| **1980s** | *First Microfluidic Prototypes* | Emergence of microfabrication using silicon and glass for fluid control; early devices focused on chemical and biomedical assays, not plant pathogens yet. |
| **1990s** | *Lab-on-a-Chip Concept Formalized* | Integration of multiple laboratory functions onto single chip; rapid analysis of fluids using electrokinetic flow and soft lithography introduced. |
| **Early 2000s** | *Soft Lithography and PDMS Use Expanded* | Introduction of polydimethylsiloxane (PDMS) simplified chip fabrication; enabled cost-effective, transparent, biocompatible microfluidic platforms. |
| **2005–2010** | *First Applications in Plant Sciences* | Adoption of microfluidics in plant tissue studies (e.g., pollen tube growth, hormone transport); few reports on pathogen detection started to emerge. |
| **2010–2015** | *Integration with Biosensors and Isothermal Amplification* | Microfluidic chips started integrating with LAMP, ELISA, and electrochemical biosensors to detect viral, bacterial, and fungal pathogens in plants. |
| **2016–2019** | *Paper-Based and Portable Microfluidic Devices* | Development of low-cost, paper-based microfluidics (μPADs) for field diagnostics in agriculture; smartphones used for signal readout. |
| **2020–2022** | *CRISPR-based Detection in Microfluidics* | Cas12/Cas13 integrated with microfluidic platforms for nucleic acid-based detection of plant pathogens with high specificity. |
| **2023–Present** | *AI-Enabled Smart Diagnostics and Multiplexed Detection Platforms* | Integration of microfluidics with machine learning, image recognition, and IoT for real-time, in-field pathogen diagnostics and early warning systems. |

**IV. Plant Pathogen Detection Strategies: Current Challenges**

*Limitations of culture-based, serological, and molecular diagnostic methods*
Traditional plant pathogen detection relies on three major categories: culture-based, serological, and molecular diagnostics, each presenting significant limitations in terms of practicality, accuracy, and scalability (Table 2) (Venbrux *et.al.,* 2023). Culture-based methods involve the isolation and growth of pathogens on selective media, often followed by morphological and biochemical characterization. Although considered a gold standard, these methods are labour-intensive, time-consuming (requiring 5–14 days), and ineffective for fastidious or non-culturable pathogens. Fungal pathogens like *Fusarium oxysporum* may require several days to weeks for reliable identification. Serological assays, especially enzyme-linked immunosorbent assays (ELISA), offer higher specificity and are widely used for detecting viruses such as Potato virus Y and Tomato spotted wilt virus. However, these assays depend heavily on the availability of high-quality antibodies and may fail to detect low pathogen loads or newly emerging variants with altered epitopes. Cross-reactivity among related species also leads to false positives or ambiguous results. Molecular diagnostics, such as conventional PCR and quantitative PCR (qPCR), have significantly improved detection precision by targeting pathogen-specific nucleic acid sequences. Despite their high sensitivity and specificity, these techniques require expensive thermocyclers, cold chain maintenance for reagents, skilled operators, and contamination-free environments. Moreover, PCR inhibitors present in plant tissues like polyphenols and polysaccharides often reduce amplification efficiency (Japelaghi *et.al.,* 2011). These constraints severely limit the applicability of molecular tools for on-site detection in agricultural settings.

*Issues of sensitivity, specificity, field applicability, and time consumption*
Field diagnostics demand detection methods that strike a balance among sensitivity, specificity, speed, portability, and ease of use. Sensitivity is compromised in many cases where pathogen loads are below detection thresholds or are unevenly distributed across plant tissues. For example, Xylella fastidiosa can be present in low concentrations in early infection stages, escaping detection in both ELISA and PCR. Specificity becomes critical in distinguishing closely related pathogens. Cross-reactions in serological tests and mis-priming in PCR assays often lead to misidentification, which can trigger inappropriate management decisions and significant economic losses. Field applicability remains a persistent limitation. Most conventional tools are unsuitable for use in remote agricultural fields due to reliance on laboratory infrastructure, electric power, and trained personnel. This detachment from on-site deployment delays disease diagnosis and containment efforts, exacerbating the spread of infections. Time consumption adds another layer of complexity. Traditional culture methods may require up to two weeks, while molecular tests, though faster, often demand pre-processing steps like nucleic acid extraction, which can take several hours (Jayamohan *et.al.,* 2021). The delay in obtaining actionable results restricts timely interventions and increases crop vulnerability during critical growth phases.

*Need for low-cost, rapid, and scalable diagnostic alternatives*
Agricultural productivity and food security are severely threatened by plant disease outbreaks, which cause an estimated $220 billion in global crop losses annually. The complexity of plant-pathogen interactions and the emergence of new virulent strains under changing climatic conditions necessitate robust surveillance systems. Existing diagnostic frameworks lack scalability due to their cost and operational constraints, creating a critical need for novel platforms that are affordable, rapid, portable, and scalable. An ideal diagnostic system should combine sensitivity and specificity with minimal sample preparation, be compatible with complex biological matrices, offer quantitative outputs, and be operable by non-specialists. Technologies such as loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and lateral flow assays have shown promise but still require controlled reaction conditions or post-processing steps (Hsieh *et.al.,* 2021). Emerging platforms like lab-on-a-chip and paper-based microfluidics, integrated with biosensors and smartphone readouts, have demonstrated the potential to overcome these limitations. These devices can detect pathogens within 10–30 minutes, require less than 10 µL of sample, and cost under $5 per test in pilot-scale deployments. Such low-cost and rapid diagnostic innovations are crucial for real-time monitoring and precise management of plant diseases, particularly in resource-limited and field-based environments. Their scalability allows integration into national surveillance programs and early warning systems, ultimately contributing to more resilient and sustainable agricultural systems.

**Table:2** Plant Pathogen Detection Strategies: Current Challenges (**Source:** Venbrux *et.al*., 2023, Jayamohan *et.al.,* 2021)

|  |  |  |  |
| --- | --- | --- | --- |
| **Detection Strategy** | **Scientific Principle** | **Advantages** | **Limitations and Current Challenges** |
| **Visual Symptom Assessment** | Morphological observation of disease signs | Simple, no tools required | Subjective, late detection, can misdiagnose abiotic stress as biotic |
| **Culture-based Methods** | Growth on selective media | Cost-effective, pure culture isolation | Time-intensive, non-viable for obligate parasites, contamination issues |
| **Serological Assays (ELISA, LFIA)** | Antigen-antibody binding | Rapid, field-applicable (Lateral Flow), scalable | Cross-reactivity, low sensitivity for early detection, dependency on good antibodies |
| **Conventional PCR** | DNA amplification via thermocycling | High specificity and sensitivity | Inhibitory substances in plant extracts, risk of contamination, equipment dependency |
| **Real-time PCR (qPCR)** | Quantitative DNA amplification with fluorescent probes | Quantitative, multiplexing ability | Expensive instrumentation, complex calibration, prone to primer-dimer interference |
| **LAMP (Loop-mediated Isothermal Amplification)** | DNA amplification at constant temperature | Field-deployable, fast, visual readouts | Primer design complexity, difficult to multiplex, false positives possible |
| **DNA Microarrays** | Hybridization of labeled DNA/RNA to probes on solid supports | High-throughput, detects multiple pathogens | High cost, complex interpretation, not practical for routine field use |
| **Next-Generation Sequencing (NGS)** | Sequencing of total DNA/RNA (metagenomics) | Detects novel/emerging pathogens, high accuracy | Expensive, requires bioinformatics infrastructure, slow turnaround for diagnostics |
| **Biosensors** | Bioreceptor-analyte interaction transduced into a signal | Real-time, portable, low sample input | Still under development, matrix interference, low commercialization |
| **CRISPR-based Diagnostics (e.g., SHERLOCK)** | Cas proteins guided by RNA to target pathogen DNA/RNA | Ultra-specific, programmable, sensitive | Requires pre-amplification, emerging technology, not yet field-standardized |
| **Immunofluorescence Assays** | Fluorescent tagging of pathogen-specific antibodies | High specificity, microscopic detection | Requires fluorescence microscope, trained personnel |
| **Hyperspectral Imaging & Remote Sensing** | Reflectance spectra analysis to detect stress patterns | Non-invasive, large area monitoring | Lacks pathogen specificity, calibration issues, needs AI integration for analysis |
| **Smartphone-integrated Tools** | Image recognition or microfluidics integrated with mobile devices | Portable, user-friendly, real-time analytics | Resolution limitations, need standardized diagnostic software |

**V. Microfluidics in Plant Pathogen Detection**

*Application in detection of bacterial, fungal, viral, and nematode pathogens*
Microfluidic platforms are increasingly recognized for their ability to detect a wide range of plant pathogens, offering high sensitivity, miniaturized workflows, and rapid response times. Bacterial pathogens such as *Xanthomonas oryzae*pv. *oryzae*, which causes bacterial leaf blight in rice, have been detected using integrated microfluidic biosensors that combine on-chip nucleic acid amplification and fluorescence-based signal readouts (Zhao *et.al.,* 2024). For fungal pathogens like *Fusarium oxysporum*, microfluidic chips enable real-time analysis by capturing specific genomic sequences or metabolites using electrochemical biosensors integrated within lab-on-a-chip devices. Viral pathogens, including *Tobacco mosaic virus* (TMV) and *Tomato yellow leaf curl virus* (TYLCV), are frequently detected using paper-based and droplet microfluidic platforms that incorporate isothermal amplification coupled with lateral flow strips or smartphone-based colorimetric detection. These devices allow for detection even at low viral loads, within 20–30 minutes. Nematode detection, particularly of *Meloidogyne incognita*, has been demonstrated using droplet microfluidic platforms designed to trap individual juveniles or eggs for real-time genetic or immunological analysis. The miniaturization and compartmentalization offered by microfluidics reduce assay volumes to the microliter or nanolitre scale, enhancing speed and efficiency while conserving costly reagents.

*Use in nucleic acid-based diagnostics (e.g., LAMP, qPCR, RPA on-chip)*
Microfluidic integration of nucleic acid amplification techniques has advanced the scope of molecular diagnostics in plant pathology (Mumtaz *et.al.,* 2023). Loop-mediated isothermal amplification (LAMP) is highly compatible with microfluidic devices due to its isothermal nature, eliminating the need for complex thermal cycling. On-chip LAMP assays have successfully identified pathogens such as *Ralstonia solanacearum* and *Pectobacterium carotovorum* with detection limits as low as 10² CFU/mL within 30 minutes. Fluorescence or turbidity-based outputs on-chip allow immediate visualization without post-processing. Quantitative PCR (qPCR) on microfluidic devices is enhanced by thermal cycling modules embedded within the chip, enabling faster and more uniform temperature transitions. A microfluidic qPCR platform that detected *Phytophthora infestans* in potato leaves within 45 minutes using a palm-sized thermocycler, demonstrating high correlation with conventional laboratory systems. Recombinase polymerase amplification (RPA), another isothermal technique, has been miniaturized in portable microfluidic systems for field deployment. These RPA-on-chip devices amplify target DNA at 37–42°C and are especially suited for viral and bacterial detection, providing visual results on lateral flow membranes incorporated into the chip.

*Integration with immunoassays and antibody-based detection*
Microfluidic platforms have proven effective in conducting on-chip immunoassays such as enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassays (LFIA) (Mou *et.al.,* 2017).The miniaturized environment allows faster antigen-antibody interactions due to reduced diffusion distances and efficient fluid dynamics. A PDMS-based microfluidic chip for detecting *Citrus tristeza virus* (CTV) using gold nanoparticle-conjugated antibodies exhibited 10-fold higher sensitivity than conventional ELISA and yielded results in under 25 minutes. In integrated platforms, microfluidic channels are functionalized with monoclonal or polyclonal antibodies specific to pathogen surface proteins or toxins. Once the plant extract is introduced, target pathogens bind to immobilized antibodies, generating measurable signals via optical, colorimetric, or electrochemical transducers. These immunosensors have been tailored for specific detection of pathogens such as *Alternaria solani*, *Clavibacter michiganensis*, and *Plum pox virus* with high selectivity and reproducibility. Such formats are often designed for multiplex detection, allowing simultaneous monitoring of multiple pathogens within a single chip.

*Role in in-field analysis and point-of-care (POC) diagnostics*
One of the most transformative contributions of microfluidics in plant pathology is the facilitation of point-of-care (POC) diagnostics for real-time, field-based disease management (Yadav *et.al.,* 2025). These systems typically integrate sample preparation, amplification or immunoreaction, signal detection, and user interface within a portable, battery-operated unit. Recent devices have used smartphone cameras to capture fluorescence or colorimetric changes, enabling immediate transmission of geo-tagged data to centralized databases. In a study, a smartphone-coupled microfluidic LAMP device detected *Candidatus Liberibacter asiaticus*, the causal agent of citrus greening, directly from leaf tissue in 25 minutes. The platform used lyophilized reagents and paper-based fluidics, reducing dependency on cold storage and sophisticated equipment. Such systems drastically reduce diagnostic time compared to laboratory workflows that require 1–2 days, and cost less than $3 per test. Microfluidic POC tools are also integrated with GPS, AI, and machine learning modules to predict disease outbreaks, recommend treatments, and support decision-making for crop protection strategies (Khondakar *et.al.,* 2024). These features are increasingly incorporated into agricultural extension services and national biosecurity frameworks for monitoring high-risk transboundary pests and diseases.

**VI. Design Features Tailored for Plant Pathology**

*Customization for plant tissue extracts and complex matrices*
Microfluidic systems developed for plant disease diagnostics must address the inherent complexity of plant-derived samples, which often contain interfering substances such as polysaccharides, polyphenols, secondary metabolites, and insoluble particulates (Yan *et.al.,* 2025). These components can hinder biochemical reactions like nucleic acid amplification or antigen–antibody binding. Tailoring microfluidic designs to accommodate such matrices involves optimizing fluidic channel dimensions, surface coatings, and flow rates to minimize clogging and maximize analyte recovery. Devices fabricated with hydrophilic coatings or PEG-grafted surfaces help reduce non-specific adsorption of plant macromolecules. Pre-treatment chambers have been incorporated in chips to accommodate raw extracts from leaves, stems, roots, or fruits, allowing efficient homogenization and particle separation without centrifugation. For example, a PDMS-glass microfluidic chip capable of processing crude banana leaf sap, enabling effective downstream viral RNA detection despite a high load of interfering phytochemicals.

*Sample preparation modules: filtration, lysis, enrichment*
Integrated sample preparation is a critical prerequisite for reliable microfluidic diagnostics in plant pathology (Zhao *et.al.,* 2024). Filtration units embedded into the chip architecture serve to remove plant debris and large particulates through size-exclusion membranes or spiral channels utilizing inertial forces. Microfabricated sieve structures have been shown to trap fibres while permitting fluid passage, improving assay consistency. Mechanical and chemical lysis zones are included to disrupt plant cell walls and release nucleic acids or proteins. These modules utilize microneedles, electric field-induced lysis, or chemical buffers containing SDS, Triton X-100, or guanidine salts, optimized for plant cells. Enrichment modules further increase target analyte concentration before detection. Techniques like isotachophoresis, immunocapture, and magnetic bead-assisted extraction have been miniaturized on-chip. Immunomagnetic separation has been applied to selectively extract *Phytophthora infestans* DNA from potato samples by coupling pathogen-specific antibodies to paramagnetic beads, which are manipulated within the chip using external magnets. These modules reduce the need for off-chip handling, enhance sensitivity, and shorten time to result.

*On-chip reagent mixing, amplification, and signal detection*
Precise reagent mixing on microfluidic platforms is essential for initiating biochemical reactions such as LAMP, PCR, or ELISA within confined volumes (Shi *et.al.,* 2019). Microchannels with serpentine geometry, herringbone grooves, or staggered obstacles facilitate chaotic advection and diffusion-driven mixing. Passive mixing strategies are energy-efficient and reduce the need for external control systems. Thermally insulated reaction chambers within the chip maintain constant temperatures for isothermal amplification or execute programmed thermal cycling using resistive microheaters. On-chip amplification of nucleic acids enables pathogen detection at femtogram or attomolar levels. A LAMP-on-chip platform for *Ralstonia solanacearum* detection using integrated microheaters and real-time turbidity measurement. Detection modalities are often coupled within the same chip, utilizing embedded electrodes or optical windows. Miniaturized spectrometers or photodiodes placed beneath detection zones allow real-time quantification. Electrochemical sensors measure current or potential changes due to redox reactions occurring at functionalized electrodes upon analyte binding. These sensors can detect specific DNA sequences, proteins, or metabolites related to pathogen presence. Fluorescence detection involves labelling amplicons or antibodies with dyes such as SYBR Green, FAM, or Alexa Fluor and detecting emission via compact CMOS cameras or photodetectors (Baker *et.al.,* 2025).

*Miniaturized detection mechanisms (fluorescence, electrochemical, colorimetric)*
Detection systems integrated into plant pathogen microfluidic devices must be low-cost, robust, and field-adaptable. Fluorescence detection remains one of the most sensitive methods, widely used for DNA amplification (e.g., qPCR or LAMP) and protein detection. Miniaturized fluorescence systems have been built using smartphone LEDs as excitation sources and cameras as detectors. These systems can detect down to 10² CFU/mL for pathogens such as *Pseudomonas syringae*. Electrochemical detection is highly compatible with microfluidic platforms, offering label-free and real-time measurements. Electrodes fabricated via screen-printing or sputtering are embedded within the chip and functionalized with capture molecules. Chronoamperometry, impedance spectroscopy, and voltammetry are used to quantify target binding. Such systems have achieved detection of *Fusarium spp.* at concentrations below 1 ng/µL. Colorimetric detection, suitable for resource-limited settings, leverages visible colour changes caused by enzymatic reactions or nanoparticle aggregation. Paper-based microfluidics, in particular, employ colorimetric outputs for visual readouts without instruments. Gold nanoparticles conjugated to antibodies yield red-to-blue transitions upon pathogen binding, visible to the naked eye and quantifiable using smartphone apps (Chauhan *et.al.,* 2025). These systems are highly scalable and ideal for use by non-specialist personnel in agricultural settings. Designing microfluidic devices specifically for plant pathology demands a comprehensive understanding of sample complexity, biochemical reaction kinetics, and end-user constraints. Through innovations in microfabrication, fluid dynamics, and biosensor engineering, these platforms are becoming indispensable for real-time, on-site plant disease management.

**VIII. Integration with Digital Technologies**

*Smartphone-based readouts and imaging systems*
Smartphone integration has become a cornerstone of modern microfluidic diagnostic platforms, particularly in agricultural contexts requiring portable, cost-effective, and user-friendly interfaces (Yadav *et.al.,* 2025). High-resolution cameras, powerful processors, and diverse sensor capabilities make smartphones suitable for imaging, fluorescence detection, and data processing. In microfluidic plant pathogen detection, smartphones function as both optical readers and analytical engines. Devices utilizing smartphone cameras can quantify fluorescence intensity or colorimetric changes in microchannels or paper-based chips for rapid detection of viruses such as *Tomato yellow leaf curl virus* and *Banana bunchy top virus* within 15–30 minutes. Some systems use built-in LED flash or external clip-on light sources to excite fluorophores, with captured emission analysed through custom apps or cloud-based platforms. Smartphones have also enabled imaging of lateral flow strips, droplet formation, and real-time monitoring of reaction kinetics on microfluidic chips. For example, a paper-based smartphone-enabled microfluidic chip developed for detecting *Phytophthora infestans* achieved 95% sensitivity and used a colour detection algorithm that corrected for ambient light variability. The affordability and global availability of smartphones significantly enhance the accessibility and scalability of plant disease diagnostics.

*IoT-enabled pathogen surveillance networks*
The Internet of Things (IoT) has transformed microfluidic diagnostics from standalone devices into interconnected tools for real-time surveillance (Amirian *et.al.,* 2024). IoT-enabled microfluidic systems transmit field-collected data via wireless networks to centralized databases, enabling spatial and temporal monitoring of plant pathogen outbreaks. These devices often include GPS modules, environmental sensors (humidity, temperature, rainfall), and wireless transmission units such as Wi-Fi, Zigbee, or LTE modules. Real-time connectivity enables creation of dynamic disease maps and predictive models. LoRa-based IoT platforms have been deployed in tomato fields to monitor the spread of *Clavibacter michiganensis* subsp. *michiganensis*, integrating data from microfluidic PCR devices and environmental conditions to issue early warnings. IoT-based disease surveillance networks can inform public and private extension services, seed certification bodies, and policy makers about emerging disease hotspots. These smart diagnostics facilitate a shift from reactive to proactive management strategies by enabling near-instantaneous sharing of pathogen incidence data. They also reduce dependence on manual surveys, increase coverage of disease monitoring programs, and allow integration with crop modelling tools for precision disease forecasting.

*Cloud-based data sharing and decision support systems (DSS)*
Microfluidic platforms coupled with cloud-based storage and analysis systems create an integrated framework for disease management, traceability, and policy formulation (Ibrahim *et.al.,* 2016) Cloud services such as AWS IoT, Google Firebase, and Azure IoT Hub have been used to centralize microfluidic device output, enabling remote access, comparison across regions, and generation of actionable insights through real-time dashboards. These platforms support decision support systems (DSS) that aid in interpreting complex diagnostic data for field-level users. For example, a cloud-integrated microfluidic platform designed to detect *Xylella fastidiosa* transmits detection events to a cloud server, which processes results with a disease risk index and sends advisory messages to farmers or agronomists via SMS or mobile apps. DSS modules built on such data often include pest prediction models, cultivar-specific disease resistance data, fungicide rotation suggestions, and optimal spraying times based on weather forecasts. Cloud-integrated systems ensure longitudinal data tracking, useful for understanding pathogen evolution, resistance development, and climatic correlations. They also facilitate institutional decision-making for quarantine enforcement, crop insurance claims, and region-specific disease mitigation programs.

*Machine learning and AI integration for automated diagnostics*
Machine learning (ML) and artificial intelligence (AI) algorithms have enhanced microfluidic diagnostics by enabling automated image analysis, pattern recognition, and predictive analytics (Park *et.al.,* 2024). These systems can analyse fluorescence intensity curves, colorimetric gradients, and signal-to-noise ratios with high precision, surpassing manual interpretation in speed and reliability. Convolutional neural networks (CNNs) have been deployed to classify disease symptoms based on chip images, distinguishing viral infections from nutrient deficiencies or abiotic stress. Support vector machines (SVMs) and decision tree classifiers are commonly used to evaluate real-time sensor data from electrochemical or optical detectors integrated in microfluidic devices. An AI-enabled microfluidic chip developed for detecting *Candidatus Liberibacter asiaticus* demonstrated 98.6% accuracy using deep learning to interpret LAMP-based fluorescence output, even under variable light and temperature conditions. Such systems continuously improve through feedback loops and enable scalability for broader pathogen panels. Predictive analytics powered by AI can also be used to forecast disease outbreaks, optimize sample collection routes, and identify risk zones based on environmental and genomic data correlations. The integration of AI with edge computing reduces latency in processing, allowing real-time insights directly in the field without dependence on high-speed internet (Santoso *et.al.,* 2024).

**IX. Commercialization and Market Trends**

*Startups and companies developing microfluidic devices for plant health*
The commercial development of microfluidic devices for agricultural diagnostics, particularly plant health monitoring, has been gaining traction globally. Several startups and research-driven companies are designing lab-on-a-chip platforms specifically for the detection of phytopathogens. Firms like 1Drop Diagnostics, uFluidix, and Microfluidic ChipShop are advancing field-deployable devices tailored for biological sample analysis, including those derived from plant tissues. For example, companies such as Access Sensor Technologies and InnoTech Alberta have developed modular microfluidic units targeting environmental and plant pathogen diagnostics. These platforms are enabling the detection of bacterial blight, fungal wilts, and viral infestations directly in the field, which helps in early intervention and reduces reliance on central laboratory facilities.

*Cost-effectiveness and scalability of devices*
Microfluidic technology offers significant advantages in cost and scalability due to miniaturization, reduced reagent volumes, and integration of multiple analytical steps on a single chip (Hardt *et.al.,* 2007). Traditional ELISA and PCR-based diagnostics may require milliliters of reagents and hours of processing time, whereas microfluidic systems operate with microliter volumes and can deliver results in less than 30 minutes. According to a global market analysis, the microfluidics market is expected to grow from USD 22.4 billion in 2024 to USD 32.7 billion by 2029, at a compound annual growth rate (CAGR) of 7.8%, driven by demands in healthcare, agriculture, and environmental monitoring. Such market growth reflects strong investor and industry confidence in the scalability and economic viability of microfluidics. Plant pathogen-specific devices have already demonstrated up to 65% reductions in both reagent usage and diagnostic turnaround time.

*Adoption barriers: user training, policy, and infrastructure gaps*
Despite their potential, microfluidic tools face several barriers that hinder widespread adoption in plant health systems (Yadav *et.al.,* 2025). One major limitation is the requirement for trained personnel to operate, interpret, and maintain the devices, particularly in rural or decentralized settings. Furthermore, agricultural extension systems may lack structured frameworks or policies that endorse the use of rapid diagnostic tools, which are often not included in existing certification or phytosanitary protocols. Infrastructure limitations such as inadequate power supply, poor mobile network connectivity, and lack of cold storage for reagents in remote areas also impede device functionality and data sharing. A study emphasized that bridging these gaps requires an integrated strategy involving policy reforms, on-ground capacity building, and robust field-testing of devices under varying agroecological conditions.

*Patent landscape and intellectual property rights*
The intellectual property (IP) ecosystem surrounding microfluidics is extensive and highly competitive (Yetisen *et.al.,* 2014). As of recent analyses, more than 4,500 patents grouped in over 1,150 patent families have been filed globally in the microfluidics space, with significant activity in North America, Europe, and East Asia. While the majority of patents relate to biomedical and environmental applications, an increasing number now focus on agricultural diagnostics. Universities and research institutes contribute substantially to this pool, often partnering with startups to commercialize lab-on-a-chip technologies. Awareness of IP rights, freedom-to-operate landscapes, and licensing structures is essential for academic laboratories and agritech companies seeking to commercialize plant pathogen diagnostics. Effective patent strategies not only protect innovations but also attract venture capital and accelerate technology transfer to markets.

**X. Opportunities in Precision Agriculture and Disease Forecasting**

*Role in disease modelling and spatial surveillance*
Microfluidic technologies have emerged as critical components in precision agriculture by enabling real-time detection of phytopathogens that directly inform disease modelling and spatial surveillance (Buja *et.al.,* 2021). Disease modelling depends on timely, high-resolution data on pathogen presence, environmental conditions, and host susceptibility. Microfluidic platforms can collect such data through integrated biosensors that monitor disease biomarkers and transmit results wirelessly to central databases. These devices, when deployed across diverse agro-climatic zones, generate spatially resolved datasets used to predict disease dynamics, assess risk levels, and inform surveillance maps. Geospatial analytics combined with microfluidic diagnostics enhance forecasting models for diseases such as *Phytophthora infestans* in potatoes or *Puccinia graminis* in wheat. For example, a study demonstrated that portable microfluidic qPCR devices deployed across multiple sites detected fungal spores with spatial resolution sufficient to predict local outbreaks two weeks in advance. This integration of on-site pathogen diagnostics with GIS-based disease tracking provides a foundational layer for predictive epidemiology in cropping systems. The miniaturization and portability of microfluidics enable more frequent and decentralized sampling, reducing the dependence on centralized labs. This allows researchers and agronomists to identify the onset of pathogen dispersal patterns, monitor pathogen evolution in real time, and trace disease movement across agro-ecological zones an essential requirement in tracking transboundary pests and pathogens.

*Contribution to early warning systems for epidemic outbreaks*
Timely identification of pathogen hotspots is critical to forestall epidemics that threaten regional or national food security (Ristaino *et.al.,* 2021). Microfluidic-based platforms integrated with digital communication systems provide an effective tool for early warning systems (EWS). Their ability to detect asymptomatic infections, particularly during latent phases of disease progression, supports proactive mitigation before symptoms manifest visibly in the field. Rapid-response diagnostic systems have proven instrumental in detecting high-impact pathogens like *Xylella fastidiosa*, *Candidatus Liberibacter asiaticus*, and *Banana bunchy top virus*. Microfluidic LAMP devices have been shown to detect *Candidatus Liberibacter asiaticus* from citrus leaf extracts in under 30 minutes, enabling the deployment of containment strategies before widespread infection. These diagnostics can be embedded in mobile extension systems or drone-mounted sensors for fast sampling and real-time alert generation. Real-time pathogen data, when integrated with weather forecasting systems, improve the predictability of outbreak events. This linkage allows the development of “smart alerts” that notify farmers and agricultural departments of imminent risk windows for pathogen establishment based on rainfall, humidity, and temperature indices. Such EWS reduce the risk of major crop losses and lower the reliance on prophylactic pesticide use.

*Enabling site-specific disease management and input optimization*
The fusion of microfluidic diagnostics with precision agriculture tools enables site-specific disease detection and targeted interventions that optimize agrochemical inputs (Khondakar *et.al.,* 2024). Conventional disease management often involves blanket pesticide application, which leads to economic inefficiency and environmental harm. Microfluidic tools, by offering localized disease profiles, facilitate variable-rate application of fungicides, bactericides, or biocontrol agents. For example, a handheld microfluidic sensor to detect *Rhizoctonia solani* in rice fields, enabling zone-specific fungicide spraying based on geotagged diagnostic data. This approach reduced chemical use by 43% and maintained disease control effectiveness, thereby minimizing off-target impacts and supporting environmental sustainability. Microfluidic systems also support the integration of multi-parametric sensors (e.g., for soil moisture, temperature, pH, and volatile organic compounds) that enable holistic crop health assessment. Decision support systems (DSS) using this real-time diagnostic data generate precision prescriptions that include disease-specific chemical dosages, timing of application, and risk level categorization. The economic implications are substantial. A report by the World Bank emphasized that precision farming enabled by digital diagnostics can increase net returns per hectare by 10–30%, while reducing agrochemical input costs by 20–50%. Microfluidics, due to their affordability, rapidity, and adaptability, form a critical part of this digital transformation in plant disease management (Zhao *et.al.,* 2024).

**XI. Limitations and Technical Bottlenecks**

*Issues in field robustness, device clogging, and matrix interference*
While microfluidic platforms show immense promise in plant disease diagnostics, their field deployment is challenged by environmental and operational constraints. One critical limitation is the lack of robustness under variable field conditions. Devices often require stable temperature, humidity, and dust-free environments for optimal functioning conditions rarely met in open agricultural systems. PDMS-based chips, for example, are highly sensitive to UV radiation and thermal fluctuations, leading to microchannel deformation and compromised assay precision. Device clogging remains a recurring problem, especially when processing complex plant tissue samples like sap, homogenized leaves, or root exudates. These matrices often contain particulates, fibrous debris, and viscous compounds such as polysaccharides and secondary metabolites that obstruct microchannels. Clogging reduces fluid flow, inhibits reagent mixing, and compromises assay completion. In field trials, up to 27% of microfluidic devices failed due to partial or complete channel blockage while handling unfiltered plant extracts. Surface fouling and biofilm formation further deteriorate device integrity, especially under repeated use in humid environments. Matrix interference chemical or physical inhibition from plant-derived compounds also affects the accuracy of diagnostic reactions such as LAMP or ELISA. Phenolic compounds present in leaf tissues can inhibit DNA polymerase activity, while pigments like chlorophyll and anthocyanins can interfere with optical detection methods. These biochemical interferences necessitate complex pretreatment steps that negate the advantages of rapid, point-of-care diagnosis.

*Challenges in multiplexing and high-throughput analysis*
Multiplexed detection the ability to identify multiple pathogens simultaneously is essential for efficient disease surveillance in crops affected by co-infections (Singhal *et.al.,* 2021). While microfluidic systems theoretically support multiplexing, practical implementation remains limited due to channel cross-talk, reagent incompatibility, and signal overlap. Fluorescence-based multiplexing often suffers from spectral interference among fluorophores, requiring complex calibration and post-processing algorithms. Electrode-based systems used for electrochemical detection also exhibit issues with signal resolution when multiple targets are assessed in parallel chambers. High-throughput analysis is hindered by the small sample processing capacity of conventional microfluidic designs. Most chips process samples in microliter volumes and are suitable for 1–8 samples per run. Scaling these systems to screen hundreds of field samples per day essential for commercial farms or epidemiological studies requires complex integration of automated fluid handling, waste management, and detection modules. This integration increases the device’s complexity, cost, and power consumption, diminishing its viability in resource-constrained settings. Automated fluid routing systems like pneumatic or droplet-based control mechanisms offer promise but often rely on external pumps, valves, or programmable logic controllers, which are unsuitable for field-based use. Recent innovations in passive microfluidics using capillary forces and paper-based channels partially mitigate this challenge but still require extensive optimization for consistent results.

*Need for plant-specific standardization and validation*
One of the most critical bottlenecks in the commercialization and widespread adoption of microfluidic devices for plant disease diagnostics is the lack of standardized validation protocols across diverse plant species and tissue types (Verma *et.al.,* 2022). Unlike human diagnostics, plant samples exhibit considerable variability in biochemical composition, cellular structure, and pathogen load, necessitating crop-specific assay tuning. A LAMP assay designed for detecting *Ralstonia solanacearum* in potato fails to yield consistent results when applied to tomato or chili extracts due to differences in matrix inhibitors and pathogen distribution. Standardized validation across plant cultivars, growth stages, and stress conditions is essential to ensure the reproducibility and reliability of diagnostic outcomes. Inter-laboratory studies are sparse, and few regulatory frameworks exist to evaluate the diagnostic performance of microfluidic tools for plant health. Without defined parameters for sensitivity, specificity, false-positive rates, and reproducibility, it is difficult to benchmark these tools against traditional laboratory assays. A meta-analysis found that fewer than 10% of published microfluidic diagnostics for plant pathogens undergo field validation beyond controlled greenhouse conditions. To establish credibility, microfluidic platforms must undergo rigorous multi-location field trials, comparative assessments with gold-standard diagnostics (e.g., ELISA, qPCR), and stress testing under variable agronomic conditions. Harmonization of protocols for sample preparation, amplification conditions, and detection thresholds is critical to advance plant-specific microfluidic diagnostics from lab prototypes to commercially viable products.

**XII. Future and Research**

*Advances in low-cost materials and portable energy sources*
The future of microfluidic diagnostics in plant pathology hinges on innovations that drive affordability, portability, and ruggedness (Buja *et.al.,* 2021). Cost-effective fabrication materials such as thermoplastics (e.g., PMMA, COC, polystyrene), paper, and biodegradable polymers are increasingly replacing traditional PDMS and glass. These materials offer benefits in terms of scalability, mechanical stability, and compatibility with mass production techniques like injection moulding and hot embossin. Paper-based microfluidic devices (μPADs), cost less than $0.10 per unit and have been widely adapted for isothermal amplification and colorimetric assays, particularly under field conditions where infrastructure is limited. Equally critical is the development of portable, off-grid energy sources to support on-site operation of microfluidic diagnostics. Battery-powered thermal units, flexible solar panels, piezoelectric energy harvesters, and thermoelectric generators are being explored to power amplification reactions, microheaters, and LED-based detection units. Recent prototypes incorporating solar-charged capacitors have successfully operated LAMP assays for *Xanthomonas oryzae* detection without reliance on external electricity, indicating strong promise for remote field deployment.

*Integration with CRISPR-based diagnostics and nanotechnology*
CRISPR-Cas systems, originally discovered for genome editing, have revolutionized diagnostics through platforms like SHERLOCK and DETECTR, which enable sequence-specific nucleic acid detection with ultra-high sensitivity. When combined with microfluidic platforms, these CRISPR-based tools offer potential for rapid, multiplexed detection of plant pathogens at attomolar levels. For example, CRISPR-Cas13a diagnostics with femtomolar sensitivity and compatibility with portable fluorescence readers parameters that are well-suited for integration in lab-on-chip systems. Nanotechnology enhances this potential by increasing surface area for analyte binding, improving signal-to-noise ratio, and enabling controlled fluid manipulation (Welch *et.al.,* 2021). Gold nanoparticles, quantum dots, and magnetic nanoparticles are being employed as labels, signal enhancers, and magnetic separators, respectively. Electrochemical microfluidic sensors utilizing graphene oxide and carbon nanotube electrodes have been developed to detect fungal toxins and viral coat proteins with detection limits below 1 pg/mL. Integration of CRISPR and nanomaterials into microfluidics can support rapid identification of emerging phytopathogens, strain differentiation, and even detection of antibiotic resistance genes in plant-associated microbes.

*High-resolution microfluidics for single-cell plant-pathogen interaction studies*
Understanding host–pathogen interactions at the single-cell level is essential to unravelling early infection mechanisms and identifying resistance pathways. Microfluidic platforms now enable compartmentalization and manipulation of single cells in droplet, trap, or microwell configurations. Such systems have been used in human immunology and microbiology, and their adaptation to plant pathology is a frontier research area. Single-cell droplet microfluidics allows encapsulation of individual plant or pathogen cells in nanoliter droplets, which can be monitored for transcriptional activity, reactive oxygen species (ROS) production, and hypersensitive response (HR) signalling. Microfluidic optical tweezers have been used to isolate and study *Arabidopsis* guard cells during bacterial exposure, providing real-time data on stomatal closure dynamics (Pantaleno *et.al.,* 2024). These systems enable visualization of infection dynamics, effector delivery, and metabolic shifts at unprecedented resolution, offering insights that cannot be captured through bulk assays.

*Development of open-source platforms for customization and deployment*
To democratize access and foster innovation, there is a growing need for open-source microfluidic platforms that are customizable, modular, and adaptable to diverse agricultural contexts. Several research groups are publishing blueprints for 3D-printed chips, wax-printed paper devices, and Arduino-integrated control systems under open licenses. Tools like OpenDrop and DropBot have paved the way for DIY digital microfluidics, and repositories like GitHub and Thingiverse now host a wide array of designs tailored for nucleic acid extraction, mixing, heating, and signal detection. These platforms encourage community-driven adaptation to local plant health challenges, allowing researchers and practitioners to modify devices for specific crops, pathogens, or environmental conditions. By enabling customization, open-source microfluidics reduce dependency on expensive proprietary systems and promote participatory research, especially in resource-limited regions.

*Potential for interdisciplinary collaborations between plant pathologists, engineers, and data scientists*
The successful implementation of microfluidic diagnostics in plant pathology requires robust collaboration among multiple disciplines (Zhao *et.al.,* 2024). Engineers bring expertise in fluid dynamics, device fabrication, and embedded electronics; plant pathologists provide insights into host–pathogen biology, sample preparation, and disease epidemiology; data scientists develop machine learning models for image analysis, pattern recognition, and outbreak prediction. Collaborative platforms such as the Plant Health and Digital Diagnostics Consortium (PHDDC) and Global Open Science Hardware Network (GOSH) are actively fostering cross-sector research to co-create solutions. AI-powered microfluidic readers, for example, developed through interdisciplinary teams, have achieved over 97% accuracy in identifying plant viruses from microchip images under variable lighting conditions. Transdisciplinary research can also bridge gaps in regulatory policy, standardization, and user interface design ensuring that the end products are not only scientifically robust but also farmer-friendly and policy-compliant. These synergies are vital for scaling microfluidic technologies beyond the laboratory into mainstream plant health management.

**XIII. Conclusion**
Microfluidic technology represents a transformative advancement in plant pathology by enabling rapid, sensitive, and field-deployable diagnostics for a wide range of pathogens. Its integration with biosensors, isothermal amplification, and digital tools such as smartphones, IoT networks, and AI enhances disease surveillance, modelling, and management with unprecedented spatial and temporal precision. Despite existing challenges including field robustness, sample complexity, and standardization gaps, ongoing innovations in materials science, CRISPR-based detection, nanotechnology, and open-source platforms are steadily addressing these limitations. The convergence of microfluidics with precision agriculture supports site-specific input optimization and early warning systems, offering substantial gains in yield protection and input efficiency. Future progress depends on interdisciplinary collaboration across plant science, engineering, and data analytics to ensure scalable, validated, and user-centric diagnostic solutions. This technological synergy holds significant promise for sustainable plant health management and global food security.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**XIV. References**

1. Awasthi, L. P., Das, S., Lee, R. F., & Pattanayak, S. (2024). *Plant Pathology*. CRC Press.
2. Buja, I., Sabella, E., Monteduro, A. G., Chiriacò, M. S., De Bellis, L., Luvisi, A., & Maruccio, G. (2021). Advances in plant disease detection and monitoring: From traditional assays to in-field diagnostics. *Sensors*, *21*(6), 2129.
3. Panwar, S., Duggirala, K. S., Yadav, P., Debnath, N., Yadav, A. K., & Kumar, A. (2023). Advanced diagnostic methods for identification of bacterial foodborne pathogens: Contemporary and upcoming challenges. *Critical Reviews in Biotechnology*, *43*(7), 982-1000.
4. Zhao, X., Zhai, L., Chen, J., Zhou, Y., Gao, J., Xu, W., ... & Yu, X. (2024). Recent advances in microfluidics for the early detection of plant diseases in vegetables, fruits, and grains caused by bacteria, fungi, and viruses. *Journal of Agricultural and Food Chemistry*, *72*(28), 15401-15415.
5. Hajam, M. I., & Khan, M. M. (2024). Microfluidics: a concise review of the history, principles, design, applications, and future outlook. *Biomaterials Science*, *12*(2), 218-251.
6. Ong, S. E., Zhang, S., Du, H., & Fu, Y. (2008). Fundamental principles and applications of microfluidic systems. *Front. Biosci*, *13*(1), 2757-2773.
7. Ren, K., Zhou, J., & Wu, H. (2013). Materials for microfluidic chip fabrication. *Accounts of chemical research*, *46*(11), 2396-2406.
8. Liao, Z., Zhang, Y., Li, Y., Miao, Y., Gao, S., Lin, F., ... & Geng, L. (2019). Microfluidic chip coupled with optical biosensors for simultaneous detection of multiple analytes: A review. *Biosensors and Bioelectronics*, *126*, 697-706.
9. Yadav, A., & Yadav, K. (2025). Portable solutions for plant pathogen diagnostics: development, usage, and future potential. *Frontiers in Microbiology*, *16*, 1516723.
10. Wardencki, W., & Witkiewicz, Z. (2021). Developments in miniaturization of gas chromatography. *Curr Adv Chem Biochem*, *4*, 24-42.
11. Yew, M., Ren, Y., Koh, K. S., Sun, C., & Snape, C. (2019). A review of state‐of‐the‐art microfluidic technologies for environmental applications: Detection and remediation. *Global Challenges*, *3*(1), 1800060.
12. Ganesan, M., Tenchov, R., Iyer, K. A., Ralhan, K., Diaz, L. L., Bird, R. E., ... & Zhou, Q. (2024). Nanoscale materials at work: Mapping emerging applications in energy, medicine, and beyond.
13. Yang, K., Peretz-Soroka, H., Liu, Y., & Lin, F. (2016). Novel developments in mobile sensing based on the integration of microfluidic devices and smartphones. *Lab on a Chip*, *16*(6), 943-958.
14. Venbrux, M., Crauwels, S., & Rediers, H. (2023). Current and emerging trends in techniques for plant pathogen detection. *Frontiers in Plant Science*, *14*, 1120968.
15. Japelaghi, R. H., Haddad, R., & Garoosi, G. A. (2011). Rapid and efficient isolation of high quality nucleic acids from plant tissues rich in polyphenols and polysaccharides. *Molecular biotechnology*, *49*, 129-137.
16. Jayamohan, H., Lambert, C. J., Sant, H. J., Jafek, A., Patel, D., Feng, H., ... & Gale, B. K. (2021). SARS-CoV-2 pandemic: a review of molecular diagnostic tools including sample collection and commercial response with associated advantages and limitations. *Analytical and Bioanalytical Chemistry*, *413*, 49-71.
17. Hsieh, W. Y., Lin, C. H., Lin, T. C., Lin, C. H., Chang, H. F., Tsai, C. H., ... & Lin, C. S. (2021). Development and efficacy of lateral flow point-of-care testing devices for rapid and mass COVID-19 diagnosis by the detections of SARS-CoV-2 antigen and anti-SARS-CoV-2 antibodies. *Diagnostics*, *11*(10), 1760.
18. Zhao, X., Zhai, L., Chen, J., Zhou, Y., Gao, J., Xu, W., ... & Yu, X. (2024). Recent advances in microfluidics for the early detection of plant diseases in vegetables, fruits, and grains caused by bacteria, fungi, and viruses. *Journal of Agricultural and Food Chemistry*, *72*(28), 15401-15415.
19. Mumtaz, Z., Rashid, Z., Ali, A., Arif, A., Ameen, F., AlTami, M. S., & Yousaf, M. Z. (2023). Prospects of microfluidic technology in nucleic acid detection approaches. *Biosensors*, *13*(6), 584.
20. Mou, L., & Jiang, X. (2017). Materials for microfluidic immunoassays: a review. *Advanced healthcare materials*, *6*(15), 1601403.
21. Yadav, A., & Yadav, K. (2025). Portable solutions for plant pathogen diagnostics: development, usage, and future potential. *Frontiers in Microbiology*, *16*, 1516723.
22. Khondakar, K. R., & Kaushik, A. K. (Eds.). (2024). Next-generation smart biosensing: nano-platforms, Nano-microfluidics interfaces, and emerging applications of quantum sensing.
23. Yan, S., Liu, Q., Liang, B., Zhang, M., Chen, W., Zhang, D., ... & Xing, D. (2025). Airborne microbes: sampling, detection, and inactivation. *Critical Reviews in Biotechnology*, *45*(3), 556-590.
24. Zhao, X., Zhai, L., Chen, J., Zhou, Y., Gao, J., Xu, W., ... & Yu, X. (2024). Recent advances in microfluidics for the early detection of plant diseases in vegetables, fruits, and grains caused by bacteria, fungi, and viruses. *Journal of Agricultural and Food Chemistry*, *72*(28), 15401-15415.
25. Shi, H., Nie, K., Dong, B., Long, M., Xu, H., & Liu, Z. (2019). Recent progress of microfluidic reactors for biomedical applications. *Chemical Engineering Journal*, *361*, 635-650.
26. Baker, D. V., Bernal-Escalante, J., Traaseth, C., Wang, Y., Tran, M. V., Keenan, S., & Algar, W. R. (2025). Smartphones as a platform for molecular analysis: concepts, methods, devices and future potential. *Lab on a Chip*.
27. Chauhan, S., Mittal, R., Kumar, M., Mittal, A., & Kushwah, A. S. (2025). Gold nanoparticle-based biosensors for point-of-care diagnostics: a review of sensing nanoparticle applications and future prospects. *Combinatorial Chemistry & High Throughput Screening*, *28*(3), 417-434.
28. Yadav, A., & Yadav, K. (2025). Portable solutions for plant pathogen diagnostics: development, usage, and future potential. *Frontiers in Microbiology*, *16*, 1516723.
29. Amirian, H., Dalvand, K., & Ghiasvand, A. (2024). Seamless integration of Internet of Things, miniaturization, and environmental chemical surveillance. *Environmental Monitoring and Assessment*, *196*(6), 582.
30. Ibrahim, M., Chakrabarty, K., & Zeng, J. (2016). BioCyBig: A cyberphysical system for integrative microfluidics-driven analysis of genomic association studies. *IEEE Transactions on Big Data*, *6*(4), 609-623.
31. Park, J., Kim, Y. W., & Jeon, H. J. (2024). Machine Learning-Driven Innovations in Microfluidics. *Biosensors*, *14*(12), 613.
32. Santoso, A., & Surya, Y. (2024). Maximizing Decision Efficiency with Edge-Based AI Systems: Advanced Strategies for Real-Time Processing, Scalability, and Autonomous Intelligence in Distributed Environments. *Quarterly Journal of Emerging Technologies and Innovations*, *9*(2), 104-132.
33. Hardt, S., & Schönfeld, F. (Eds.). (2007). *Microfluidic technologies for miniaturized analysis systems*. Springer Science & Business Media.
34. Yadav, A., & Yadav, K. (2025). Portable solutions for plant pathogen diagnostics: development, usage, and future potential. *Frontiers in Microbiology*, *16*, 1516723.
35. Yetisen, A. K., & Volpatti, L. R. (2014). Patent protection and licensing in microfluidics. *Lab on a Chip*, *14*(13), 2217-2225.
36. Buja, I., Sabella, E., Monteduro, A. G., Chiriacò, M. S., De Bellis, L., Luvisi, A., & Maruccio, G. (2021). Advances in plant disease detection and monitoring: From traditional assays to in-field diagnostics. *Sensors*, *21*(6), 2129.
37. Ristaino, J. B., Anderson, P. K., Bebber, D. P., Brauman, K. A., Cunniffe, N. J., Fedoroff, N. V., ... & Wei, Q. (2021). The persistent threat of emerging plant disease pandemics to global food security. *Proceedings of the National Academy of Sciences*, *118*(23), e2022239118.
38. Khondakar, K. R., & Kaushik, A. K. (Eds.). (2024). Next-generation smart biosensing: nano-platforms, Nano-microfluidics interfaces, and emerging applications of quantum sensing.
39. Zhao, X., Zhai, L., Chen, J., Zhou, Y., Gao, J., Xu, W., ... & Yu, X. (2024). Recent advances in microfluidics for the early detection of plant diseases in vegetables, fruits, and grains caused by bacteria, fungi, and viruses. *Journal of Agricultural and Food Chemistry*, *72*(28), 15401-15415.
40. Singhal, P., Nabi, S. U., Yadav, M. K., & Dubey, A. (2021). Mixed infection of plant viruses: diagnostics, interactions and impact on host. *Journal of Plant Diseases and Protection*, *128*(2), 353-368.
41. Verma, N. V., Shukla, M., Kulkarni, R., Srivastava, K., Claudic, B., Savara, J., ... & Pandya, A. (2022). Emerging extraction and diagnostic tools for detection of plant pathogens: recent trends, challenges, and future scope. *ACS Agricultural Science & Technology*, *2*(5), 858-881.
42. Buja, I., Sabella, E., Monteduro, A. G., Chiriacò, M. S., De Bellis, L., Luvisi, A., & Maruccio, G. (2021). Advances in plant disease detection and monitoring: From traditional assays to in-field diagnostics. *Sensors*, *21*(6), 2129.
43. Welch, E. C., Powell, J. M., Clevinger, T. B., Fairman, A. E., & Shukla, A. (2021). Advances in biosensors and diagnostic technologies using nanostructures and nanomaterials. *Advanced Functional Materials*, *31*(44), 2104126.
44. Pantaleno, R., Schiel, P., García-Mata, C., & Scuffi, D. (2024). Analysis of Guard Cell Readouts Using Arabidopsis thaliana Isolated Epidermal Peels. *Bio-protocol*, *14*(14), e5033.
45. Zhao, X., Zhai, L., Chen, J., Zhou, Y., Gao, J., Xu, W., ... & Yu, X. (2024). Recent advances in microfluidics for the early detection of plant diseases in vegetables, fruits, and grains caused by bacteria, fungi, and viruses. *Journal of Agricultural and Food Chemistry*, *72*(28), 15401-15415.