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Direct and indirect technical guide for the early detection and management of fungal plant diseases

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ABSTRACT

Fungal plant diseases are a major threat to plants and vegetation worldwide. Recent technological advancements in biotechnological tools and techniques have made it possible to identify and manage fungal plant diseases at an early stage. These techniques include direct methods, such as ELISA, immunofluorescence, PCR, flow cytometry, and in-situ hybridization, as well as indirect methods, such as fluorescence imaging, hyperspectral techniques, thermography, biosensors, nanotechnology, and nano-enthused biosensors. Early detection of fungal plant diseases can help to prevent major losses to plantations. This is because early detection allows for the implementation of control measures, such as the use of fungicides or resistant varieties. Early detection can also help to minimize the spread of the disease to other plants. The techniques discussed in this review provide a valuable resource for researchers and farmers who are working to prevent and manage fungal plant diseases. These techniques can help to ensure food security and protect our valuable plant resources.

1. Introduction

Plant diseases caused by pathogenic fungi have been a chronic problem in agriculture for ages. To minimize the damage caused by diseases in crops during cultivation, yield, and post-harvest dispensation, and to make the most of productivity and certify cultivated sustainability, unconventional detection and prevention methods are need of the hour. The environment is perpetually altering, and the introductions of bellicose species as well as the effects of climate change, have substantial implications for of developing plant diseases and existing epidemics. To understand the origins of pathogenesis and successfully manage the diseases under changing environmental conditions, a comprehensive approach is required. The field of plant pathology is interdisciplinary and draws from various fields, such as epidemiology, microbial ecology, genetics and plant physiology, to understand the causes and dynamics of plant diseases. Plant pathogens such as bacteria, fungi, viruses and oomycetes play a crucial part in controlling plant populations and managing their impact on managed systems such as forests and agricultural ecosystems is crucial for preserving yields (Mainwaring et al., 2023). However, traditional approaches to studying plant diseases have been reductionist and focused on individual interactions between microbes and plants relatively than considering the composite of biological exchanges amid hosts, microbial groups, and the environment (Jeger et al., 2014; Bever et al., 2015;

Abbreviations: AP, Alkaline Phosphatase; CFDA, Carboxy Fluorescein Diacetate; COX 1, Cytochrome Oxidase Subunit; ELISA, Enzyme-Linked Immunosorbent Assay; FCM, Flow CytoMetry; FISH, Fluorescence In Situ Hybridization; GC–MS, Gas Chromatography-Mass Spectrometry; HRP, Horseradish Peroxidase; IF, Immuno-Fluorescence; ISH, In-Situ Hybridization; ITS, Internal Transcribed Spacer Region; LSU, large Sub-Unit; MIR, Mid-InfraRed; MWF, Metal Working Fluid; NIR, Near-InfraRed; PCR, Polymerase Chain Reaction; PI, Propidium Iodide; POCT, Point-Of-Care Testing; PRIn, Photo-Chemical Reflectivity Index; RAPD, Random Amplification of Polymorphic DNA; RGB, Red Green Blue; rRNA, Ribosomal RNA; RT-PCR, Reverse-Transcription PCR; SCAR, Sequence Characterised Amplified Region; qPCR, quantitative PCR; SERS, Surface Enhanced Raman Scattering; SSU, Small Sub-Unit; SWIR, ShortWave InfraRed; TEF-1α, Translation Elongation Factor-1 alpha; VOCs, Volatile Organic Compounds.

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Fodor et al., 2020; Roberts et al., 2020).

Recent technical advances, such as OMICS technologies, have enabled a more all-inclusive consideration of the devices underlying plant pathogenesis. These technologies allow for the analysis of microbial and plant features along the phenotype-genotype spectrum and have led to inventions in sympathetic plant defence, sleuthing plant strain, and supervision of disease with oppressive soils. Furthermore, multi-omics approaches hold promise for understanding how microbial groups think and work on how the environment changes, especially in light of increasing abiotic and biotic stressors that affect plant health (Bhadauria, 2016; Crandall et al., 2020). In addition, with the use of techniques such as comet assay, the extent of DNA damage can be quantified over a specific timeline (Agnihotri and Seth 2016; Gupta and Seth 2019; Kumar et al. 2023). The emergence of omics approaches has enabled the study of microbial multiplicity and "plant-microbe" relations across a wide range of ecological populations and spatiotemporal scales. A "multi-omics incline can give a comprehensive picture of microbial-plant interactions and allow us to develop prediction representations of microorganisms and plants will react to strain below changing ecological conditions. The advent of multi-omics tactics to plant ailment ecology is relevant given the rapidly changing environment (Sharma et al., 2020; Diwan et al., 2022). Climate change and ecological invasions, i.e., 'non-native species' can alter the conformation and ecology of surroundings, leading to the emergence of invasive microbial pathogens and soil-borne pathogens that can cause widespread damage to plant populations. OMICS technologies such as metabolomics, genomics, metagenomics, volatile omics and spectra omics have already been used to study plant disease ecology and are likely to lead to further breakthroughs in the years to come (Santini et al., 2015; Mourou et al., 2023). The promising techniques with multi-OMICS approaches have been displayed in Fig. 1.

2. Direct methods

Direct detection of diseases involves a highly effective method for identifying highly expressed antigens. This technique involves conjugating a primary antibody to an enzyme such as horseradish peroxidase (HRP) or alkaline phosphatase (AP) or a fluoro-chrome for enhanced detection. It is a convenient and cost-effective approach that allows for the use of different antibodies from the same host in a single phase. In the plant industry, direct detection of diseases involves both serological and molecular methods that can be used for high-throughput studies, especially when a large number of samples need to be analyzed. By directly detecting the disease-causing pathogens such as fungi, viruses, and bacteria, this method accurately identifies the pathogen/disease, which is crucial for effective disease management.

As a summary of all the analytical techniques which have been discussed in this review, the following table enlists various plant diseases and the recent techniques utilized to detect them for further action-taking (Table 1).

2.1. ELISA (Enzyme-linked immunosorbent assay)

The enzyme-linked immunosorbent assay (ELISA) technique is a molecular method used for disease identification based on antibodies and color change in the assay. ELISA is a widely used immunological technique for detecting diseases. This technique utilizes antibodies conjugated to an enzyme that specifically binds to target epitopes (antigens) from viruses, bacteria, and fungi. The interaction between the substrate and immobilized enzyme produces a color change that allows for visual detection. The use of specific monoclonal and recombinant antibodies, which are commercially available, can greatly improve the performance of ELISA (Clark et al., 1977; Zhe et al., 2017; Ulrich et al., 2020). The fundamental principle of ELISA is based on the interaction between antigens and antibodies. This technique uses specific antibodies to bind or associate with their target antigens. This approach has been extensively used for the detection of plant viruses since its first description by Clark and Adams in 1977.

2.2. Immunofluorescence method identifier

Immunofluorescence (IF) is a fluorescence microscopy-based technique commonly used to analyse microbiological samples. It is also applicable for detecting pathogen infections in plant tissues. The process involves fixing thin tissue sections of plant samples onto microscope slides. To visualize the distribution of target molecules, a fluorescent dye is conjugated with specific antibodies (Yang et al., 2023a). One notable application of IF is the detection of onion crop infection by the fungus *Botrytis cinerea* (Ward et al., 2004). By utilizing specific antibodies conjugated with fluorescent dyes, researchers can observe and analyze the presence and distribution of the pathogen within the onion tissues. In the case of *Solanum dulcamara* detection, which causes crown rot in potatoes, IF has been combined with other techniques, such as FISH (Fluorescence In Situ Hybridization). This combination allows for the

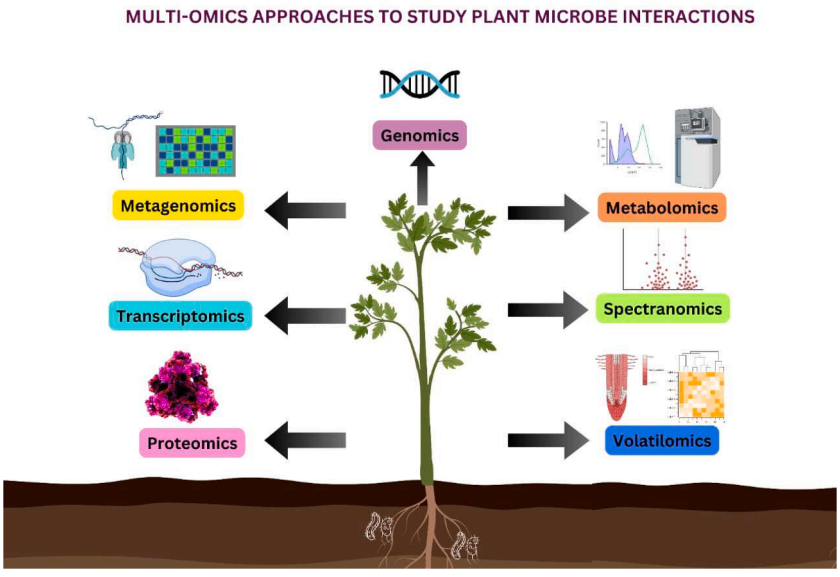

Fig. 1. Multi-OMICS approaches to detect fungal plant disease at an early stage.

Table 1
Latest techniques utilized to analyse sever plant diseases.

Disease	Plant	Causative Agent	Symptoms	Detection technique	References
Soybean Rust	Soybean	*Phakopsora pachyrhizi*	Tan or reddish-brown lesions	Biosensors	Twizeyimana et al. (2023)
Black mold	Tomato	*Alternaria alternata*	Pale leaf spots	ELISA	Nehela et al. (2023)
Seed Rots	Melon	*Fusarium* spp.	Fail to germinate	PCR	Aydi et al. (2023)
Necria canker	Apple	*Nectria galligena*	Infected branches and twigs	Flow Cytometry	Araujo et al. (2022)
Dothiorella canker	Avocado	*Neofusicoccum* spp.	Dries to a brown	PCR	Fiorenza et al. (2023)
Powdery mildew	Cherry	*Podosphaera clandestine*	White patches	Fluorescence Imaging	Sujatha et al. (2022)
Downy mildew	Spinach	*Peronospora farinosa*	Yellow angular spots	Fluorescence Imaging	Fondevilla et al. (2023)
Pierce's disease	Grape	*Xylella fastidiosa*	Die in concentric zones	PCR	Saunders et al. (2022)
Shot hole disease	Peach	*Wilsonomyces carpophilus*	Purplish hole	Thermography	Farooq et al. (2023)
Fusarium crown and foot rot	Pumpkin	*Fusarium solani*	Water-soaked lesions	Hyperspectral Techniques	Sritongam et al. (2022)

simultaneous visualization of specific nucleic acid sequences (using FISH) and the target molecules (using IF), providing a more comprehensive understanding of the infection.

Limitations of the FISH method also include fungal and substrate inherent autofluorescence, insufficient permeability of cell walls, non-specific binding of probes, and low ribosome contents. A common challenge encountered in fluorescence-based techniques, including IF, is photobleaching. Photobleaching refers to the fading or loss of fluorescence signal over time due to the damaging effects of light exposure. This can lead to false-negative results and decreased sensitivity. To mitigate the effects of photobleaching several strategies can be employed. These include reducing the intensity and duration of light exposure during imaging, increasing the concentration of fluorophores used in the staining process, and utilizing more robust fluorophores that are less susceptible to photobleaching. By carefully optimizing these parameters, researchers can minimize the impact of photobleaching and improve the reliability of IF results (Mancini et al., 2016; Lee et al., 2020; Luchi et al., 2020).

To address this issue a new method was developed that combines immunofluorescence with propidium iodide staining to perceive viable *P. pachyrhizi* urediniospores. This technique uses antibodies that react specifically to *P. pachyrhizi* and other *Phakopsora* spp. then not supplementary communal soybean pathogens or rust fungi (Fabiszewski et al., 2010; Krivitsky et al., 2021). Two vital stain methods were utilized to assess the spore viability: one used (carboxy fluorescein diacetate) CFDA and (propidium iodide) PI, and the other used fluorescent vital dye FUN1 (2-chloro-4-[2,3-dihydro-3-methyl-(benzo-1, 3-thiazol-2-yl)-methylidene]−1-phenyl quinolinium iodide). The CFDA-PI method identified viable spores as green-stained and non-viable spores as red-stained (Hartman et al., 1991; 1999; 2011). Meanwhile, the FUN 1 method induced cylindrical intravacuolar assemblies within metabolically vigorous urediniospores, causation them to fluoresce bright reddish-orange. In contrast, lifeless spores had a faint, subtle fluorescence. This new process has the probable to be applied in forecasting soybean erosion by specifically detecting viable urediniospores. It is rapid, reliable, and can help minimize unnecessary management measures and costs (Balouiri et al., 2016; Kolek et al., 2016).

2.3. Polymerase chain reaction

Initially used for the specific detection of bacterial and viral diseases, PCR has become a widely used method for the detection of plant pathogens as well (Luchi et al., 2020; Hariharan and Prasannath, 2021). In addition to the basic PCR technology, advanced methods such as reverse-transcription PCR (RT-PCR) have been utilized for high-sensitivity plant pathogen identification. Multiplex PCR allows for the simultaneous detection of different DNA or RNA in a single reaction. Real-time PCR platforms have also been utilized for on-site, rapid diagnosis of plant diseases caused by bacterial, fungal, and viral nucleic acids (Iwasaki et al., 2022). Despite its high sensitivity and specificity, PCR has limitations, such as a lack of operational robustness due to the efficacy of DNA extraction, the presence of inhibitors in the sample assay, polymerase activity, PCR buffer, and concentration of deoxynucleoside triphosphate.

Additionally, designing primers for initiating DNA replication can limit the practical applicability of PCR for field sampling of diseases (Mourou et al., 2023). Real-time PCR is now regarded as the furthermost accurate approach for detection plant pathogens. This technique employs a luminous signal that intensifications in proportion to the number of amplicons created and the number of aims present in the sample, allowing for accurate and high-throughput quantification of target pathogen DNA in a variety of environmental trials, as well as host tissues, water, air, and soil (Abdullah et al., 2018). This has created new avenues for study into diagnostics, inoculum verge levels, epidemiology, and "host-pathogen interactions". The technique of Real-time PCR provides a wide range of practical applications in plant disease detection. It not only identifies and detects the occurrence or absence of the aim pathogen in a sample, but it also quantifies the quantity present, giving a foundation for disease management choices. Other important use areas include determining pathogen vitality, detecting multiplexing, and monitoring fungicide resistance. Overall, real-time PCR knowledge offer increasing chances and play a substantial part in improved understanding of the subtleties of plant pathogenic microorganisms, allowing for better disease management.

Early detection of seed-borne fungal diseases is critical since they may not show obvious symptoms. The spread of these infections may be stopped by diagnosing the seeds, minimising economic losses, and lowering the need for fungicides, which decreases costs and reduces the entry of dangerous compounds into the environment. Conventional procedures for identifying these infections require incubation and grow-out, which are time-consuming and require specific expertise and may be insensitive to low levels of seed infection (Hua et al., 2011). Traditional PCR, nested PCR, multiplex PCR, real-time PCR, and "magnetic-capture hybridization" PCR are more efficient, with excellent sensitivity and specificity. Magnetic-Capture Hybridization PCR (MCH-PCR) is a technique that combines DNA isolation, purification and amplification. It involves the following steps: **Hybridization**: Single-stranded DNA (ssDNA) probes on magnetic beads bind to target DNA sequences in a sample. **Capture**: Magnetic beads with bound target DNA are separated from the sample using a magnetic field. **Purification:** The captured DNA is washed to remove non-target DNA and contaminants. **PCR Amplification:** The purified target DNA is then amplified using PCR to produce sufficient quantities for analysis. However, there are limits to molecular approaches, such as the difficulty in discriminating between living and dead pathogens and challenges in getting eminence DNA templates due to inhibitors of PCR in seeds (Patel et al., 2022). Adapted PCR procedures, such as loop-mediated isothermal intensification and non-destructive testing methods, have been developed to circumvent these constraints. Loop-mediated isothermal augmentation and, for the upcoming generation, sequencing has shown significant promise in nucleic acid scrutiny, and their use in the future may be expanded to enhance the identification of fungal infections in seeds.

Multiplex and real-time PCR assays were advanced for uncovering Rhizoctonia solani, Macrophomina phaseolina, Ascochyta rabiei, Alternaria alternata, A. tenuissima, Fusarium oxysporum f. sp. ciceris, Sclerotium (Athelia) rolfsii, Sclerotinia sclerotiorum, Pseudocercospora cruenta and Cercospora canescens causing various diseases in pulse crops Twenty-two sets of primers from various genomic regions such as cytochrome oxidase subunit (COX 1), internal transcribed spacer region (ITS), translation elongation factor-1 alpha (TEF-1α), large subunit (LSU), small subunit (SSU) and β-tubulin as well as two SCAR primers from RAPD profile were designed. The developed markers proved species-specific and validated against other fungal plant pathogens associated with pulses for cross-reactivity. The markers proved highly sensitive during conventional and qPCR analysis. Duplex PCR assays for R. solani and M. phaseolina, C. canescens and P. cruenta; A. alternata and A. tenuissima; and a quadruplex PCR assay for A. rabiei, S. sclerotiorum, S. rolfsii and F. oxysporum f. sp. ciceris were developed and validated for simultaneous detection of these pathogens in a single reaction. The assays developed in the present study could detect and identify major fungal plant pathogens causing disease in pulse crops (Aslam et al., 2017; Ciampi et al., 2020).

2.4. Flow cytometry

The phospholipid vesicles are recognized by phospholipid bilayer-containing proteins, whereas the inner cytosol is characterized as per the metabolites percentages. The research exploits the extracellular vesicles because of their enhanced diagnostic and therapeutic potential by utilizing flow cytometry for detection and analysis (Valkonen et al., 2017). Flow cytometry (FCM) is an optical technique that uses lasers for cell counting, sorting, and detection of biomarkers and proteins. It allows for rapid identification of cells as they pass through an electronic detection apparatus in a liquid stream, with the ability to measure multiple parameters simultaneously. FCM utilizes an incident laser beam and measures the scattering and fluorescence of the beam reflected from the sample. In this process, light hits a particle and then changes direction. This includes reflection and refraction. In the context of flow cytometry, a laser beam is focused upon a stream in which particles are suspended. When a particle traverses through the laser beam, light is scattered in all directions by the illuminated particle. In conventional flow cytometry, the light scatter is collected perpendicular to the illumination sources (Welsh et al., 2020). While primarily used for studying cell cycle kinetics and antibiotic susceptibility, as well as enumerating bacteria, differentiating viable from non-viable bacteria, and characterizing bacterial DNA and fungal spores, it is a relatively new technique for plant disease detection (Agnihotri and Seth, 2020; Talhinhas et al., 2021).

The cultivation of tomatoes is a significant aspect of agriculture in Algeria, but it is often threatened by early blight disease caused by *Alternaria alternata*. The usage of organic pesticides to protect tomato plants is common but raises concerns about environmental pollution and potential health risks. Researchers aimed to identify the most potential bio-controller negotiators from arid soil to find an alternative to chemical products. After isolating *A. alternata* from infested tomato plants, 35 bacterial insulate were gained from arid soil in southern Algeria, and tierce of them inhibited the development of *A. alternata*. The furthermost effective insulate, E1B3, exhibited a 75 percentage of inhibition proportion and was identified as *Bacillus mojavensis* through molecular analysis. This straining process does not form or produce chitinase but produces protease, lipase and lipopeptides. The researchers conducted flow cytometric analysis and found that the interaction flanked by *A. alternata* and *B. mojavensis* was antagonistic. This study is the first to investigate the interaction between *A. alternata* and *B. mojavensis*, and the findings suggest that *B. mojavensis* could be used as a bio-pesticide in the management of tomato harvests (Milet et al., 2016; Yang et al., 2023b).

Flow cytometry is an effective way to assess the viability of fungal conidia in metalworking fluids. Metalworking fluid (MWF) fungi contamination is an issue in automated processing facilities because it can clog machinery and certain species may be pathogenic. A flow cytometric approach was devised using *F. solani* as a model organism to correctly determine conidial viability in MWF (Kennedy et al., 2000). This procedure was tested by combining live and dead conidia in various amounts and examining the results with flow cytometry. FCM, microscopic analysis, and plating assays were used to evaluate the fungicidal efficacy of two commercial MWFs. FCM differentiated between living and dead conidia as early as 5 h after MWF exposure, whereas the microscopic technique identified conidial viability considerably later and with less accuracy. Microscopic and FCM studies corresponded well after 24 h. The flow cytometric approach has good sensitivity and allows for evaluating the fungicidal characteristics of two commercial MWFs. Significantly, the FCM results on the survivability of *F. solani* conidia at early time points agreed well with fungal biomass measurements determined by qPCR 7 days after the experiment began. As a result, FCM can be a useful method for assessing fungal vitality in MWF and for managing fungal contamination in automated processing plants (Vanhauteghem et al., 2017; 2019; Passman et al., 2020).

2.5. In-situ hybridization

The In-situ Hybridization (ISH) procedure is an effective method for examining the interactions between rust fungus and their hosts. The pathogenic rust fungi evolve through several life phases in the host plants, and it is critical to distinguish between fungal and plant tissue when researching these interactions. The ISH methodology reported here has been validated for use with *Chrysanthemum morifolium* infected with *Puccinia horiana, Gladiolus hortulanus* infected with *Uromyces transversalis*, Glycine max disease-ridden with *P. pachyrhizi*, and uninfected greenery tissue samples (Bamaga et al., 2003; Morales et al., 2022). This approach differentiates clearly amid rust fungus and their particular host plant tissues. It may be used for pathogens from different rust fungal genera through no contextual staining of plant tissue. The adoption of this approach for studying plant infective fungi in paraffin-entrenched slices of congregation plant tissue is advocated (Ellison et al., 2016).

Fluorescence in-situ hybridization (FISH) is a molecular detection technique used for bacterial detection in combination with microscopy and hybridization of DNA probes and target genes from plant samples. FISH can detect plant pathogen infections by recognizing pathogen-specific ribosomal RNA (rRNA) sequences (Kemp et al., 2003; Hijri et al., 2005). FISH is used to detect bacterial pathogens and fungi, viruses, and other such pathogens that infect the plant. The high affinity and specificity of DNA probes provide high single-cell sensitivity in FISH, enabling the detection of culturable microorganisms that cause plant diseases. FISH can also be used to detect yet-to-be-cultured (unculturable) organisms to investigate complex microbial communities. However, the practical limit of detection lies in the range of around 10^3 CFU/mL (Kliot et al., 2014; Salgado-Salazar et al., 2018).

2.6. Gas chromatography

A completely different non-optical indirect method for plant disease detection involves the profiling of the volatile chemical signature of the infected plants. As per their research, Fang et al. (2013) stated that the pathogen infections of plants could result in the release of specific volatile organic compounds (VOCs) that are highly indicative of the type of stress experienced by plants. To tackle fungicide resistance in fungal plant diseases new chemicals with distinct mechanisms of action must be developed. In this investigation, metabolic fingerprinting based on GC–MS (Gas chromatography-mass spectrometry) was utilised to determine the mechanisms of action of fungicides (Capote et al., 2012). *Botrytis cinerea*, a common vegetable and floral pathogen, was subjected to 13 distinct known mechanisms of action and one unknown mode of

action. GC–MS was used to investigate mycelial extracts and a hierarchical clustering model was developed to differentiate and categorise antifungal chemicals based on their modes of action (Chilvers et al., 2012). Fungicide mode of action biomarkers were also identified, and the novel fungicide SYP-14,288 was discovered to have the same mode of action as fluazinam (Dean et al., 2012; Yamaoka, 2014). This work creates a comprehensive data-base of metabolic trepidations caused by various mode-of-action inhibitors and emphasises the value of metabolic fingerprinting for establishing modes of action, which can assist in the creation and optimisation of novel fungicides.

GCMS has also been well-utilized to study the anti-fungal activity and detection of *Chenopodium album* leaf and root extract against certain phytopathogenic fungi. This study looked at the antifungal potential of aqueous extracts from *Chenopodium album* leaves and roots against five phytopathogenic fungi namely, *Alternaria alternata, Macrophomina* sp., *Colletotrichum gloeosporioides, Botrytis cinerea, and Sclerotium rolfesii* (Yilmaz et al., 2019). A study conducted by Ali et al. (2017) states that the extracts were examined at four different concentrations, and substantial decreases in fungal mycelial growth were detected. The existence of 6 chemicals in the extracts was shown by GC–MS analysis, including 2(3H)-furanone, dihydro-4,4-dimethyl, 9-octadecenoic acid (Z)- methyl ester, and hexadecanoic acid methyl ester. Water extracts successfully managed and controlled phytopathogenic fungi, implying their potential application as a broad-spectrum antifungal drug.

As discussed in the above section, the direct methods for early detection of fungal diseases in plants can be broadly classified under immunology-based and polymerase chain reaction-based methods. Whereas the indirect methods are divided under stress-based disease detection and biomarker-based detection techniques, as depicted in Fig. 2.

3. Indirect method

Indirect detection is advantageous for studying poorly expressed antigens since it involves using secondary reagents that amplify the signal. This approach is particularly beneficial for detecting antigens that are difficult to detect using direct detection methods. Indirect methods have also been used in plant stress profiling and plant volatile profiling for identifying biotic and abiotic stresses and pathogenic diseases in crops. Recent advancements in plant health monitoring have led to the development of new optical sensors capable of detecting biotic and abiotic stresses in plants. These sensors provide detailed information based on different electromagnetic spectra, enabling accurate prediction of the plant's health status. The development of such advanced tools and techniques is crucial for ensuring efficient crop management and improving overall crop yields (Mahlein et al., 2012).

3.1. Fluorescence imaging

The fluorescence imaging technique is used to detect pathogen infections by analyzing changes in chlorophyll fluorescence on plant leaves. It is a fluorescence microscopy technique used to analyze microbiological samples and detect pathogen infections in plant tissues. Plant samples are fixed on microscope slides as thin sections. Detection involves using antibodies conjugated with fluorescent dyes to visualize the distribution of target molecules within the sample. Also been utilized to detect infections in onion crops caused by the fungus *B. cinerea*. In this process, thin tissue sections of the infected onion plants are fixed onto microscope slides. Specific antibodies targeting *B. cinerea* are conjugated with fluorescent dyes, allowing visualization of the fungal infection under a fluorescence microscope. This technique helps in identifying and analyzing the distribution of the fungus within the plant tissues. The technique measures the fluorescence of chlorophyll as a function of

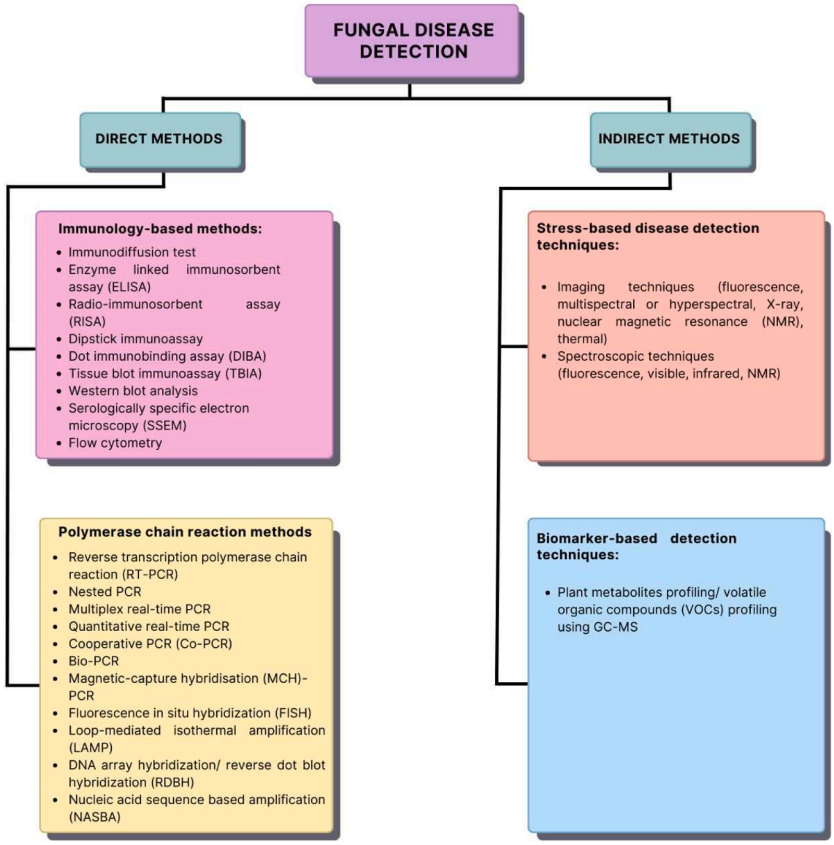

Fig. 2. Classification of techniques to detect fungal plant diseases at an early stage.

incident light and changes in fluorescence parameters can be used to identify pathogen infections based on changes in the photosynthetic apparatus and photosynthetic electron transport reactions. This method has been used to analyze temporal and spatial variations of chlorophyll fluorescence to detect leaf rust and powdery mildew infections in wheat leaves at 470 nm. Although fluorescence imaging provides sensitive detection of abnormalities in photosynthesis, its practical application in a field setting is limited (Firdous, 2018).

Chlorophyll fluorescence imaging is a valuable process for non-destructively and non-invasively assessing the effect of fungi on the metabolism of congregation plants involved in photosynthetic processes. High-throughput phenomics screening may be possible with this method, but its use necessitates a grasp of the biology of the plant-fungal interaction as well as the selection of suitable experimental conditions and methods. To demonstrate the potential of chlorophyll fluorescence imaging in this context the retorts of plants to various plant-fungal pathogens was investigated (Perez-Bueno et al., 2016; Fan et al., 2017). This included identifying heterogeneity in photosynthetic performance within the infected leaf and providing insights into the under-lying machineries. Nevertheless, there are other drawbacks and difficulties connected with by means of chlorophyll fluorescence imagery in high-throughput screens (Jones et al., 2016).

The live cell imaging method allows for the visualization of pathogen-induced cell death in rice cells. This is achieved through the use of FDA (fluorescein diacetate) and PI staining, which discriminates between live and dead cells by staining the cytoplasm and nuclei, respectively (Thomas and Franco, 2021; Ye et al., 2019). Also, the technique identifies previously unknown fluorescein patterns in mechanically injured cells, such as increased cytoplasmic area and intensity and confinement of stronger signals in afflicted cells. The hemi-biotrophic relationship, in which freshly invading cells die as the fungus grows into surrounding cells, was revealed by simultaneous imaging of fluorescently-tagged *M. oryzae* and FDA labelling. In rice, *M. oryzae* (strain CKF1996) and other host-pathogen interactions, this approach may be used to compare host cell death related to disease resistance and susceptibility. A live-cell imaging method using confocal microscopy provides insights into cell death dynamics in rice (*Oryza sativa*). This method involves mechanically damaging or invading rice sheath cells with fluorescently-tagged *Magnaporthe oryzae* and using fluorescent dyes fluorescein diacetate (FDA) and propidium iodide (PI). FDA stains the cytoplasm of live cells, visualizing the vacuole, while PI stains the nuclei of dead cells (Mengiste et al., 2012; Jones et al., 2021; Yu et al., 2022). According to time-lapse imaging, FDA staining patterns shift from conventional cytoplasmic localisation to unique patterns in dying cells with closed plasmodesmata and shrinking or ruptured vacuoles to loss of fluorescence in dead cells (Mur et al., 2008; Dickman et al., 2013).

3.2. Hyperspectral techniques

Hyperspectral imaging is a technique that can provide valuable information about plant health by analyzing reflectance across a wide range of the electromagnetic spectrum (350–2500 nm). It is becoming increasingly popular for plant phenotyping and crop disease identification in large-scale agriculture due to its robustness and rapid analysis of imaging data. Hyperspectral imaging has become a powerful tool for early plant disease detection, capable of identifying diseases from tissue to canopy levels. By capturing detailed spectral data across a wide range of wavelengths, these techniques can detect physiological changes in plants before symptoms are visible, differentiate between various diseases, and monitor crop health over large areas, aiding in precise and sustainable agricultural management. Hyperspectral imaging cameras allow for the collection of data in three dimensions (X, Y, and Z), providing detailed and accurate information about plant health across large geographic areas. The technique has been used for plant disease detection by measuring changes in reflectance resulting from biophysical and biochemical characteristic changes upon infection. Hyperspectral imaging has successfully identified infections of *Magnaporthe grisea* in rice, *Phytophthora infestans* in tomatoes and *Venturia inaequalis* in apple trees. However, the practical application of hyperspectral imaging for plant disease detection in a field setting is still limited (Terentev et al., 2022; Wan et al., 2022).

Image analysis techniques, such as hyperspectral imaging, can extract information from digital pictures, enabling automatic image processing to provide a dataset of required measurements (Horbach et al., 2011; Singh et al., 2020). Hyperspectral photography gathers high-fidelity colour reflectivity evidence throughout a broad assortment of the light spectrum, well elsewhere that of humanoid eyesight, and has been shown to improve accuracy. Because of dropping technological prices, this technique may be utilised for classifying and identifying primary phases of plant foliar ailment and strain, and it has become financially accessible to a wide range of users. Machine learning approaches may be employed for high-throughput phenotyping, as well as for researching the literature on stress recognition, categorisation, quantification, and prediction utilising various sensors (Hatsugai et al., 2004). Overall, hyperspectral imaging and other image analysis tools can potentially change crop management and plant health by reducing pesticide and herbicide use, benefiting the environment, eco-system amenities, grower finances, and the end consumer.

Hyperspectral photographs generally comprise hundreds of consecutive narrow wavelength bands over an ethereal assortment beyond the visible spectrum of light. These bands are much narrower than the bands used in multispectral imaging, typically ranging from 1 to 10 nanometers in width. Hyperspectral cameras capture images that provide detailed spectral information about the objects being imaged, allowing for the identification of materials based on their spectral signatures (Liu et al., 2023). The spectral range of hyperspectral imaging can be customized for different applications. In the context of plant and crop sciences, the spectral range of interest often includes the visible and near-infrared (NIR) range, as well as portions of the mid-infrared (MIR) and shortwave infrared (SWIR) ranges. These regions of the spectrum are useful for characterizing plant health, detecting stress responses, and identifying specific plant species or chemical compounds. Overall, hyperspectral imaging provides a powerful tool for the non-destructive and non-invasive analysis of plants and crops (Femenias et al., 2022; Wan et al., 2022).

By providing detailed spectral information about the objects being imaged, hyperspectral imaging can enable more accurate and precise characterization of plant health and growth, as well as more effective monitoring of crop conditions and disease outbreaks. However, the analysis of hyperspectral data requires specialized expertise and can be computationally intensive, so over haul essential be taken to ensure that the data is properly collected and analyzed for meaningful interpretation. The article discusses using hyperspectral imaging for crop monitoring, particularly concerning healthy and diseased plant classification, initial recognition of stress, and disease severity (Mahlein et al., 2015). The hyperspectral technique offers an impenetrable, information-rich colour data-set that may capture changes in water content in the extended range as well as changes in leaf pigmentation and mesophyll cell structure in the perceptible and near-infrared regions (400–1300 nm) (1300–2500 nm). According to the article, mild drought stress may not be noticeable, but severe dehydration can change the mesophyll structure of the leaf and its near-infrared reflectance. The study also discusses additional imaging methods for identifying biotic and abiotic stress in plants, along with a description of each technique's level of precision (Singh et al., 2016).

In order to detect and assess the level of non-native grey pine eruption rust infection in south-western white pine saplings from various seed-source families, this study used hyperspectral imaging. During the course of 16 image collecting dates, a sustenance trajectory mechanism was able to perceive infection with an accurateness of 87 %, missing only 4 % of infected seedlings. Seedlings were also categorised into a

"growth vigour" grouping with a 79.7 % accuracy rate, and the classification accuracy was substantially connected with the mortality rate within a family (Mahlein et al., 2017). The normalised photo-chemical reflectivity index (PRIn) was ranked top for contagion detection and had the highest cataloguing (83.6 %) accurateness pigment level after the most useful characteristics were found using a novel search technique (Fong et al., 2008; Haagsma et al., 2020). According to this study, hyperspectral imaging may be used to locate disease-resistant trees in advance of potential disease threats (Nascimento et al., 2005; Rajabi et al., 2012; Nguyen et al., 2021). Moreover, employing indices like PRIn may result in creating multispectral cameras that are less costly and more data-efficient (Peng et al., 2022).

3.3. Thermography

The physical environment can frequently be unfavourable for crop output and plant growth as a result of conditions such as water or nutrient scarcity, severe temperatures, illness, and insect damage. Infrared thermography can detect pathogens like *Aspergillus carbonarius* in grapes by measuring temperature differences on infected surfaces. This method, non-destructive and efficient, has shown promise in early disease detection in agriculture, particularly for monitoring the growth of harmful fungi and mycotoxin production. According to climate change projections, the frequency of these severe occurrences would rise, changing the biodiversity of plants and reducing food output. TIR (Thermal) imaging is often combined with other measurements to screen plants for stress responses and diagnose diseases before symptoms are visible. It can also be used for screening stomatal mutants during crop breeding. Since stomata react quickly to external challenges, stomatal control is essential for plant survival, adaptability, and growth (Trumbore et al., 2015; Pineda et al., 2021).

Thermal imaging is a tool for studying plant-environment interactions and genotypic variation in stress tolerance. It has been used effectively in a variety of plant species to estimate or quantify transpiration. However, measurement accuracy may be impacted by environmental fluctuation. Recent years have seen significant advances in the development of imaging-based approaches for detecting stress in crop fields, with thermography emerging as a valuable tool in agriculture. Leaf temperature, in particular, is an important indicator of plant physiological status and can be used to detect both biotic and abiotic stressors. Agriculture can become more automated, precise, and sustainable by combining thermography with other imaging sensors and data-mining techniques. However, to accurately interpret thermal data, corrections must be made for environmental and measurement conditions (Fei et al., 2019). This appraisal affords an indication of the current state of thermography in detecting biotic stress, discusses important abiotic stress factors that affect measurements, and addresses practical considerations for implementing this technique at the field scale (Kuska et al., 2015).

Thermography has gained attention for non-destructive monitoring of the physiological status of plants (Erich-Christian, 2020). Researchers have applied this technique to assess spatial temperature heterogeneity in table grapes infected with the filamentous fungus *Aspergillus carbonarius*, which causes sour rot of grapes and produces ochratoxin-A, a harmful mycotoxin. Ochratoxin-A is known for its nephrotoxic, hepatotoxic, teratogenic, and immunosuppressive properties, posing significant health risks to humans. To evaluate the temperature differences associated with *A. carbonarius* infection in table grapes, researchers calculated the average temperature of the grape surface as well as the maximum temperature difference between infected and non-infected areas. The results showed that the average temperature of grapes during fungal mycelium development was significantly lower than that of healthy grapes. Additionally, the maximum temperature difference increased as the fungal colonization progressed, while the healthy grapes exhibited a constant temperature difference (Mastrodimos et al., 2019). To distinguish between healthy and infected areas of the berries, researchers used estimated shape factors derived from fitting the temperature data of thermal images to the Weibull distribution. This approach enabled the identification of infected berry areas, even in the early stages of *A. carbonarius* infection. In summary, thermography, specifically infrared thermography, shows promise as a sensitive method for detecting early changes in plant transpiration and identifying pathogen activities within plant tissues. In the case of *A. carbonarius* infection in table grapes, thermography was able to detect temperature variations and differentiate healthy from infected areas, providing valuable insights for early detection and management of fungal pathogens in crops (Raza et al., 2015; Al-Doski et al., 2016; Baylis, 2017; Liu et al., 2020).

3.4. Biosensor

Biosensor's concept was first addressed by Clark and Lyons around 1962 when they developed an oxidase enzyme electrode for glucose detection. Biosensors have been widely used for the detection of plant diseases. Affinity biosensors use antibodies or aptamers that are specific to the pathogen or its components, allowing for highly specific detection. Enzymatic electrochemical biosensors, conversely, detect the activity of enzymes produced by the pathogen or the plant in response to infection. The amperometric biosensor provides a rapid and accurate method for diagnosing fungal infections, addressing the limitations of current techniques. This technology can improve patient outcomes by enabling timely treatment. These biosensors offer several advantages over traditional methods, including real-time monitoring, high sensitivity, specificity, and portability. However, some limitations still need to be addressed, such as their cost, stability, and reproducibility. Nonetheless, biosensors hold great promise for rapidly and accurately detecting plant diseases in the field (Vu et al., 2020; Al-Hindi et al., 2022).

Due to the limits of conventional detection techniques, there is considerable interest in creating biosensing devices that can identify pathogens early and precisely. This involves modifying nanoparticle-based biosensors that were first created for the diagnosis of human disease for the recognition of plant pathogens (Alchanatis et al., 2010).

3.5. Nano-biotechnological implications

Recent advances in nanotechnology have made it possible to prepare various nanoparticles and nanostructures for biosensing applications. Nanoparticles possess unique electronic and optical properties and can be synthesized using different materials, making them attractive for sensor development (Shivashakarappa et al., 2020). *Penicillium aurantiogriseum, Penicillium citrinum*, and *Penicillium waksmanii* have been used to synthesize copper nanoparticles, showcasing the potential of fungal diversity in nanoparticle synthesis. The high surface area, electronic conductivity, and plasmonic properties of nanomaterials improve the limit of detection and overall performance of biosensors. Various nanostructures have been evaluated as platforms for immobilizing bio-recognition elements to construct biosensors, including nano-chips made of microarrays containing fluorescent oligo probes for detecting single nucleotide changes in bacteria and viruses. Fluorescent silica nanoparticles combined with antibodies have also been studied as probes for detecting plant pathogens such as *Xanthomonas axonopodis* pv. *vesicatoria* causes bacterial spot diseases in Solanaceae plants (Chitra et al., 2013; Awad-Allah et al., 2021; Du et al., 2022).

Viruses are small parasites that can infect various hosts, including bacteria, plants, animals, and humans, and can significantly impact their physiological behaviour. Biosensors are devices premeditated to perceive and quantify biochemical particles, such as DNA sequences, antibodies, enzymes, and proteins, and consist of a bioreceptor, transducer, and detector. The emergence of nanotechnology has allowed for the development of novel biosensors known as nano-biosensors, which have shown exciting potential for improving biosensing capabilities. These devices use nanomaterials to enhance sensitivity, specificity, and

selectivity and can be used for the recognition of a wide assortment of viruses besides other biomolecules. Overall, biosensors and nano-biosensors offer fast and efficient technologies for another study developed a plasmonic gold nanoparticle-based method for diagnosing *Aspergillus* fungal infections. It generated colored solutions with distinct tones by measuring the shape change of gold nanoparticles and HIV-related diseases such as cardiovascular and rheumatoid arthritis. The unique properties of nanomaterials enable the construction of nano-biosensors with high sensitivity and reproducibility, allowing for faster and more accurate detection. Different techniques, including electrochemical and optical biosensing and point-of-care diagnostics, have been employed for the detection of various diseases using nano biosensors (Adriaenssens et al., 2012). For instance, electrochemical biosensors utilize the electrical properties of nanomaterials to detect and quantify the target molecule, while optical biosensors use light to measure the concentration of the analyte (Arora et al., 2018; 2019). The potential of nano-biosensors in disease diagnosis and treatment has made them a promising area of research in biosensing.

Nanotechnology has enabled the development of biosensors for disease detection, and various nanomaterials have been explored for this purpose (Shaw and Honeychurch, 2022). Quantum dots, with their unique optical properties, have been used in biosensors based on the fluorescence resonance energy transfer (FRET) mechanism (Zhang et al., 2023). Plant protection is feasible using nanotechnology tools such as microneedle patches, nanopore sequencing, nano barcoding, nano biosensors, quantum dots, nano diagnostic kits, metal nanoparticles, microRNA (miRNA)-based nanodiagnosis, and array-based nanosensors for plant pathogen diagnosis. Other novel materials, such as gold nanoparticles, have also been studied for their high electroactivity and conductivity, which allow for electron transfer and improved sensitivity in biosensor construction. Some researchers for plant disease detection have developed nanomaterial-based electrochemical sensors. Nanotechnology provides a promising platform for developing biosensors with high sensitivity and low detection limits (Umasankar and Ramasamy, 2014; Sharma et al., 2021; Kumar and Arora, 2020).

Irrefutable, environmental, and quality-regulator applications are just a few areas where nano-inspired biosensors are becoming more and more crucial. As well as monitoring abiotic strain, metabolic contented, microRNAs, phytohormones, (genetically modified) GM plants, transcriptional and genetically encoded biosensors, and plant infections caused by fungal, viral, and bacterial pathogens, a number of nano-inspired biosensors have been developed in recent years. These biosensors were created using a variety of nanomaterial characteristics, including molecularly engraved polymers, micro-fluidics, plasmonic nano-sensors, (surface-enhanced Raman scattering) SERS, chemiluminescence, fluorescence, quartz crystal microbalance, and progressive electrochemical measurements, in combination with adaptable nano-materials or nanocomposites (Berensmeier et al., 2006; Asal et al., 2018). These technologies have made it possible to create plant biosensors inspired by nanotechnology that provide hitherto unseen levels of performance and sensitivity, enabling the detection of ultra-trace concentrations of target analytes both in vitro and in vivo.

In recent years, microfluidics-based three-electrode potentiostat sensing platforms have gained significant interest in sustainable food safety research. These platforms offer high selectivity and sensitivity for pathogen detection. Researchers have made notable advancements in signal enrichment techniques, measurement devices, and portable tools, which can be utilized for food safety investigations. These devices need to have simple working conditions, automation, and miniaturization to meet the critical requirements of on-site pathogen detection in food safety. The integration of point-of-care testing (POCT) with microfluidic technology and electrochemical biosensors is necessary to address the urgent need for on-site pathogen detection in food safety. This integration allows for rapid and efficient detection of pathogens at food production or consumption sites. By combining the advantages of microfluidics, electrochemical biosensors, and POCT, a comprehensive solution for ensuring food safety can be achieved. Overall, developing rapid, portable, and cost-effective technologies for pathogen detection in food is crucial to preventing foodborne illnesses and protecting public health. Integrating microfluidics, electrochemical biosensors, and POCT holds great potential for advancing food safety investigations and providing real-time, on-site detection of pathogens (Bruijns et al., 2016; Kulkarni and Goel, 2022).

Despite the recent surge of interest in nano-inspired plant biosensors, relatively few research findings remain available (Bilkiss et al., 2019). However, there is great potential for developing these biosensors, particularly in agriculture, where they can be used to improve crop productivity and mitigate the undesirable effect of plant diseases and abiotic stress features. Nano-inspired bio-sensors shouldn't constrain the development of plant biosensors for applications of non-plant. To develop the area of plant biosensors, novel and creative methods, including transcriptomic biosensors, genetically encoded biosensors, and chimaera biosensing machinery, should be investigated. Overall, nano-inspired plant biosensors have a lot of promise to transform agriculture and elevate living standards through a variety of uses (Addy et al., 2012; Shivashakarappa et al., 2022).

Table 2 below summarizes various advantages and disadvantages associated with direct as well as indirect methods to diagnose fungal plant diseases.

4. Conclusions and future research direction

Fungal plant diseases significantly threaten global agriculture, jeopardizing food production and supply. Early detection and management of fungal plant diseases are critical for ensuring global food security and sustainable agriculture. By continuously advancing and integrating detection technologies, researchers can make significant strides in mitigating the impact of these diseases on crop yields and safeguard them.

Future research in fungal plant disease detection and management should prioritize several key areas to enhance current methodologies and address emerging challenges. Integrating multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics,

Table 2
Advantages and disadvantages of diagnostic method.

Techniques	Advantages	Disadvantages
PCR	High sensitivity, rapid, automated, and can detect uncultured microbes	Affected by PCR inhibitors, abundance of false-positive and false-negative results
Microarray	High-throughput technology, enables detection of multiple pathogens, allows detection of specific serotype	Difficult to distinguish between viable and nonviable cells, requires trained personnel, needs oligonucleotide probes, and labeling of target genes, low-signal intensity due to improper content of targeted DNA and probe can lead to inaccurate analysis
Immunodiagnostic	Sensitive, specific, rapid, and culture independent analysis could be done	Costly
ELISA	Can handle large number of samples and give precise results, time saving	Pre-enrichment is needed in order to produce the cell surface antigens, highly trained personnel required, proper labeling of antibodies or antigens is needed
Flow cytometry	Simultaneous detection and quantification of multiple pathogens in a reliable way	High cost, limited knowledge regarding the potential of this technique, Immunofluorescence, Target distribution can be visualized Photobleaching (fading)

will provide a comprehensive understanding of plant-pathogen interactions, identifying novel biomarkers for early detection and deeper insights into fungal pathogenesis and plant defense mechanisms. Advancements in bio-sensor technology, particularly portable and cost-effective bio-sensors for field use, are critical. Research should focus on improving the robustness, sensitivity, and specificity of biosensors, leveraging nanotechnology to enhance their performance. The integration of artificial intelligence (AI) and machine learning (ML) can revolutionize data analysis from hyperspectral imaging, thermography, and other indirect methods, enabling predictive modelling and decision support systems for disease management.

Developing field-deployable diagnostic tools, such as portable PCR devices and handheld biosensors, will facilitate timely intervention and empower farmers with minimal training. Understanding the impacts of climate change on pathogen virulence and plant susceptibility is essential for developing climate-resilient detection methods and management strategies. Exploring the plant microbiome's role in disease resistance can lead to microbiome-based preventive measures. Novel antifungal compounds with distinct mechanisms of action should be identified to combat fungicide resistance. Collaborative, interdisciplinary research efforts, supported by socio-economic studies, will ensure the adoption of advanced technologies, fostering resilient agricultural systems and sustainable crop production.

Credit authorship contribution statement

Gargi Sharma has contributed in exploring literature and writing. Vagish Dwibedi, Chandra Shekhar Seth, Simranjeet Singh and Joginder Singh conceptualized, and acquired the entire concept and guided the authors for writing this review. Praveen C Ramamurthy, Vagish Dwibedi, Chandra Shekhar Seth, Simranjeet Singh and Joginder Singh, have made the contributions including drafting and data verification under the leadership of Dr. Pooja Bhadrecha and Prof. Joginder Singh (Corresponding authors). All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Compliance with Ethical Requirements

This article does not contain any studies with animals performed by any of the authors.

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