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Smart Tools for Diagnosing and Managing Pest-Pathogen Complexes in Vegetables

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Vegetable crops suffer considerable losses due to various pests and diseases during the growing and post-harvest stages. Farmers employ different protective strategies to reduce this damage, beginning with seed and seedling treatments and continuing with regular crop monitoring. In many situations, synthetic pesticides are used, though integrated pest management (IPM) practices are increasingly adopted to lessen risks to human health and the environment. Recently, advanced "smart" technologies have been introduced to forecast, identify, and measure pathogen presence and contamination. This review highlights the range of practical tools and techniques currently available, from visual inspections to protein and DNA/RNA-based detection methods, as well as novel innovative strategies, emphasising user-friendly solutions that can assist growers in accurately diagnosing plant pathogens.

Keywords: Smart, hybrid, helping hands, effective, rapid

Introduction

Vegetables are a vital part of the human diet, providing essential vitamins, minerals, and nutrients, and are widely produced and traded globally. Like other cultivated crops, they interact with both beneficial and harmful microorganisms that influence yield and quality. These microbial communities are naturally present in fields, but the international trade of seeds, seedlings, and produce enables plant pathogens to move across borders and establish in new regions. Studies have shown that the diversity and abundance of phytopathogens in a given area often correlate with the volume of imported plant material. Consequently, the likelihood of pathogen introduction and spread between countries has been rising. To limit such invasions, national authorities have developed control systems based on scientific guidance and international regulations. Farmers also take measures to prevent local pathogens from infecting their fields and remain vigilant against potential threats migrating from nearby areas. Effective management of vegetable diseases requires preventive strategies that incorporate soil health practices, seed treatments, resistant crop varieties, and monitoring systems suited for conventional, biotechnological, or organic farming, all of which align with integrated pest management (IPM) principles. Detecting pathogens at the earliest stages of infection is crucial, as it enables timely and effective interventions. Therefore, reliable tools for disease diagnosis and pathogen identification are indispensable; without them, preventive measures and appropriate control strategies cannot be effectively implemented (Miller *et al.*, 2009).

A variety of methods and technologies have been developed to support the detection, monitoring, and investigation of plant pathogens in different vegetable crops. Some of these are well-established, validated, and routinely applied by accredited laboratories for official testing and regulatory purposes. Others, though still emerging, hold considerable promise for integration into "smart" farming systems enabling targeted and data-driven surveillance, planning, and monitoring of phytopathogens. With the growing emphasis on farm-level precision disease diagnosis, our objective was to review both fully validated protocols used in official control programs and experimental tools at varying stages of technological readiness (TRL), often tested in only a single laboratory, to assess their potential as future devices for pathogen detection (Ovesná *et al.,* 2023).

Symptomatic Diagnosis

In the past, visual assessment of symptoms was the only method available for diagnosing diseases in field crops. However, early-stage symptoms are often vague and difficult to distinguish, making this method heavily reliant on the expertise and experience of trained specialists. Since plant pathogens are microscopic, farmers without expert support can typically identify them only after visible damage occurs. Because specialists are not always accessible, alternative diagnostic methods have been developed and tested. Traditional techniques, such as culturing pathogens, remain useful but are slow, require specialized mycological knowledge, and depend on laboratory facilities. Additionally, these methods lack the sensitivity needed for detecting infections at early stages. Although advanced image analysis for crop disease detection was once limited, its effectiveness has improved when combined with digital tools, such as mobile devices or video systems linked to expert databases (e.g., VACDDS). To enhance accuracy, computational algorithms and diagnostic models have also been explored, with the success of such systems depending largely on the availability of extensive and reliable image databases. Despite these advances, many image-based diagnostic tools remain costly and are still underdeveloped for vegetables. Practical challenges, such as indistinct lesion margins, irregular leaf coloration, and variable lighting conditions, further complicate analysis. While current imaging methods can achieve high accuracy in controlled experiments, their reliability decreases under field conditions, creating an urgent need for more precise, crop-specific pathogen detection tools.

Serological tests

Serological assays are extensively applied in pathogen detection, relying on the diverse interactions between antibodies and pathogens. Among these, the enzyme immunoassay (EIA) is used to identify specific ligands, such as proteins, in liquid samples through antibodies that recognize the target molecule. A widely adopted variation of this method is the enzyme-linked immunosorbent assay (ELISA), which has become a standard tool in diagnostics. ELISA is employed not only in medical fields but also in agriculture, where it is particularly valuable for identifying plant viruses, fungal pathogens, and other viral agents. Typically, the test detects proteins unique to a species or genus, such as viral coat proteins, using polyclonal or monoclonal antibodies. Detection occurs through antigen, antibody binding, which produces measurable results, often visible as colour changes. To enhance sensitivity, signal amplification is frequently incorporated. While ELISA remains the most widely used approach, several alternative serological techniques have also been developed, documented, and successfully implemented in practice (Phatsaman *et al.*,2020)**.**

E-Senses

Unlike methods that rely on species- or strain-specific proteins (such as ELISA) or nucleic acids (like PCR or sequencing), alternative approaches focus on other measurable changes, including metabolites (eNose), visual cues like color shifts, structural modifications (eEyes), or variations in soluble compounds (eTongue), mimicking human sensory systems. Numerous studies have described the underlying principles of these technologies and outlined their current advancements. Among these, the eNose is one technique designed to detect

volatile compounds released by organisms, using portable gas chromatography-based instruments. This method targets unique metabolites generated both by pathogens and by the host plants during infection. Since an organism's volatile emissions vary depending on its physiological condition and developmental stage, these compounds provide valuable diagnostic cues. Extensive research has documented volatile profiles associated with plant infections, identified through chromatography and mass spectrometry analysis. With comprehensive libraries of infection-related volatile mixtures similar to metabolomics databases that are continually being expanded, farmers could eventually gain access to simple, field-ready tools for large-scale crop monitoring, including vegetables. Some studies have already demonstrated the practical application of eNose technology in detecting diseases in palm trees.

Biosensors

Biosensors represent one of the most effective methods for achieving rapid and low-cost diagnostics. Essentially, these devices detect biological interactions and convert them into signals that correspond to the concentration of the targeted analyte. Their functionality is based on the integration of biorecognition elements with transducers that translate biological changes into measurable outputs, such as electrical signals, which can be easily interpreted. Initially, biosensors were applied in point-of-care settings for the detection of human diseases. Modern biosensor development focuses on incorporating advanced materials and nanotechnology to enhance sensitivity, specificity, and biological compatibility, ultimately leading to the creation of next-generation diagnostic tools (Li *et al.*, 2010).

DNA barcoding

DNA barcoding is a quick and effective method for identifying species. It uses a short, specific, and consistent DNA sequence, much like a unique barcode, to catalogue different organisms. The process involves isolating and sequencing these specific DNA markers, which are conserved within a species but vary significantly between different species. These sequences are flanked by highly conserved regions, which allows for the design of specific PCR primers to amplify them. A commonly used marker for fungi is the ribosomal internal transcribed spacer (ITS) region, which has been officially designated as the fungal barcode.
After amplification, the DNA sequences are compared to established databases like BOLD, UNITE, and NCBI to identify the organism. This technique is used in various fields, from tracing goods to molecular diagnostics. It's particularly useful for regulatory bodies like the EPPO and national plant health authorities, which use it to identify harmful or quarantine microorganisms. A more advanced version of this technique, metabarcoding, uses high-throughput sequencing (NGS) to simultaneously identify multiple organisms or pathogens. This is especially useful for analysing samples from natural environments, where plants may be infected by several pathogens at once. For DNA barcoding to be used reliably in a professional setting, such as in control laboratories, validated protocols are essential to ensure the results are accurate and reproducible (Schoch *et al.*, 2012).

CRISPR for Pathogen Detection

CRISPR/Cas, a system most known for its role in genome editing, is also emerging as a powerful tool for detecting viruses and other pathogens in a wide range of materials, from human tissue to food and environmental samples. The "molecular scissors" proteins of the CRISPR/Cas system can identify and bind to specific DNA and RNA sequences, making them highly effective for diagnostics. Unlike the current "gold standard" of real-time PCR, which requires advanced equipment and skilled technicians, CRISPR-based tests are much more accessible. When combined with isothermal technologies, these assays are both sensitive and affordable. This has already been proven with the rapid, low-cost detection of human viruses like COVID-19. A key advantage is their speed and simplicity. A CRISPR/Cas test can be completed in less than 30 minutes at a single temperature, and the results can be visually read with a simple fluorescent dye. This makes them ideal for rapid,

on-site diagnostics in the field, including the detection of plant pathogens. Given these benefits, it is very likely that we will see the widespread use of such fast, easy-to-use tests for plant disease diagnosis soon (Zhai *et al.*, 2023).

Conclusion

Timely and accurate detection of plant pathogens remains fundamental to safeguarding vegetable production, maintaining yield, and ensuring food security. Although traditional approaches such as visual inspection and pathogen culturing have provided the basis for disease diagnosis, they are often slow, labour-intensive, and ineffective for detecting early infections. The development of advanced tools, ranging from serological assays and biosensors to e-sensing systems, DNA barcoding, and CRISPR-based diagnostics, has significantly improved the speed, sensitivity, and practicality of pathogen detection. Many of these innovations are now being incorporated into precision agriculture and integrated pest management programs, allowing for earlier interventions and more targeted control strategies. While certain technologies are already standardised and widely used, others are still in experimental stages and require validation before large-scale deployment. Moving forward, the integration of these emerging diagnostic methods into smart farming frameworks offers a promising pathway to strengthen biosecurity, minimise disease spread, and promote sustainable vegetable production systems.

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