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Mass Spectrometry in Crop Pathogens Diagnosis: A Comprehensive Review

Kalaichelvi Kalaignan[1], Jeya Rani Maria Michael[2], Vinothini Selvaraj[3], Shanmugapackiam Subbaiah[3], Ehab Abdellah Abdelaziz Salama[4], Murali Sankar Perumal[3]

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ABSTRACT

Crop pathogens pose a significant threat to global food security, causing substantial economic losses and food safety concerns. The identification and quantification of compounds produced by these pathogens are crucial for understanding their diagnosis by biochemistry, virulence mechanisms and predict for disease control. Initially, diagnosed by mass spectrometry such as GCMS and LCMS are powerful analytical techniques used for the identification and quantification of compounds in various fields, including environmental science, pharmaceuticals and food analysis. This study aims to explore the application of mass spectrometry for the diagnosis of crop pathogens, focusing on the identification and quantification of volatile organic compounds (VOCs) and secondary metabolites produced by fungal and bacterial pathogens. The study will also evaluate the advantages and disadvantages of GCMS and LCMS for crop pathogen analysis, including sensitivity, resolution, selectivity, range of applications, sample preparation requirements, cost and data interpretation challenges. The results will provide valuable insights into the potential of GCMS and LCMS for crop pathogen diagnosis and highlight areas for further research and development in this field.

Key words: Agriculture, *B. cinerea*, *F. graminearum*, GC-MS, LC-MS, *Xanthomonas* spp.

Plant pathogens are microorganisms that cause diseases in plants, leading to significant economic losses and food security concerns. The identification and characterization of plant pathogens are crucial for understanding their biology, virulence mechanisms and potential for disease control (Singh *et al*., 2023). Traditional methods for plant pathogen identification rely on morphological and cultural characteristics, which can be subjective and time-consuming (Venbrux *et al*., 2023). In recent years, analytical techniques such as gas chromatography-mass spectrometry (GCMS) and liquid chromatography-mass spectrometry (LCMS) have emerged as powerful tools for the identification and characterization of plant pathogens (Zhu *et al*., 2021). GCMS and LCMS are analytical techniques that combine chromatography with mass spectrometry to separate, identify and quantify compounds in complex mixtures (Unuh *et al*., 2019). GCMS is commonly used for the analysis of volatile organic compounds (VOCs), while LCMS is used for the analysis of non-volatile compounds, including secondary metabolites (Cannavacciuolo *et al*., 2023). In the context of plant pathogen analysis, GCMS and LCMS have been used to identify and quantify VOCs and secondary metabolites produced by fungal and bacterial pathogens (Pang *et al*., 2021). These techniques have several advantages over traditional methods, including high sensitivity, selectivity and resolution, as well as the ability to provide structural information about identified compounds (Amirav *et al*., 2020). However, there are also some challenges associated with GCMS and LCMS for plant pathogen analysis, including sample preparation requirements, cost and data interpretation challenges (Yang *et al*., 2023). This review will provide an overview of the principles and applications of mass spectrometry for plant pathogen diagnosis, highlighting their advantages and disadvantages in this field.

[1]Department of Agronomy, Krishi Vigyan Kendra, Virdhachalam-606 001, Tamil Nadu, India.
[2]Department of Agronomy, Pushkaram College of Agriculture Sciences, Pudukkottai-622 303, Tamil Nadu, India.
[3]Department of Plant Pathology, Pushkaram College of Agriculture Sciences, Pudukkottai-622 303, Tamil Nadu, India.
[4]Department of Agricultural Botany (Genetics), Saba Basha (Faculty of Agriculture), Alexandria University, Alexandria-21531, Egypt.

Corresponding Author: Murali Sankar Perumal, Department of Plant Pathology, Pushkaram College of Agriculture Sciences, Pudukkottai-622 303, Tamil Nadu, India.
Email: muralisankar58@gmail.com

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Principles

In mass spectrometry both techniques (GCMS and LCMS) are applicable to identify and characterize plant pathogens by accuracy and timely dependent nature. It consists of basic principles of sample preparation, involving separation techniques, mass spectrometry, data interpretation, standardization and merging or collaboration with fine

results (Chong *et al*., 2018). Further, these are described below.

Sample preparation

Proper sample preparation is essential for obtaining accurate results. For GCMS, samples should be dried and derivatized to make volatile compounds more stable and detectable. For LCMS, samples can be directly injected into the system without derivatization (Malcangi *et al*., 2022).

Separation techniques

GCMS and LCMS both use separation techniques to separate the complex mixtures of compounds found in plant pathogens. Additionally, they were used in various fields such as pharmaceuticals, environmental analysis and food science (Nagana Gowda and Djukovic, 2014). GCMS uses gas chromatography to separate compounds based on their volatility and polarity, while LCMS uses liquid chromatography to separate compounds based on their solubility and interactions with the stationary phase (Iwasaki *et al*., 2012). In gas chromatography (GC) is an analytical technique used to separate, identify and quantify different chemical compounds in a mixture. The technique involves passing a gas (usually helium or nitrogen) through a column containing a stationary phase (such as silica or carbon) and a mobile phase (such as hydrogen or helium) (Song *et al*., 2023). The compounds in the mixture interact differently with the stationary and mobile phases, causing them to travel at different rates through the column. This results in each compound eluting (exiting the column) at a different time, allowing for their separation and identification. GC is commonly used in various fields such as environmental analysis, pharmaceuticals, food science and petroleum refining (Coskun, 2016). It is particularly useful for separating and identifying volatile organic compounds (VOCs) and other gasses. The technique can also be coupled with other analytical methods such as mass spectrometry (GC-MS) for enhanced identification and quantification of compounds (Fig 1) (Kunze-Szikszay *et al*., 2021). Liquid chromatography (LC) is another analytical technique used for separating, identifying and quantifying different chemical compounds in a mixture. Unlike gas chromatography (GC), which uses a gas as the mobile phase, LC uses a liquid as the mobile phase. The LC system consists of a pump, an injector, a column filled with a stationary phase (such as silica or polymeric materials) and a detector (Hussan Ali, 2022). The sample is injected into the system and the liquid mobile phase is pumped through the column at a controlled flow rate. The compounds in the mixture interact differently with the stationary and mobile phases, causing them to travel at different rates through the column. This results in each compound eluting (exiting the column) at a different time, allowing for their separation and identification (Patel *et al*., 2022). LC is commonly used in various fields such as pharmaceuticals, environmental analysis and food science. It is particularly useful for separating and identifying non-volatile compounds that cannot be analyzed by GC. The technique can also be coupled with other analytical methods such as mass spectrometry (LC-MS) for enhanced identification and quantification of compounds (Fig 1) (Sun *et al*., 2023).

Mass spectrometry

Both GCMS and LCMS use mass spectrometry to identify the separated compounds based on their mass-to-charge

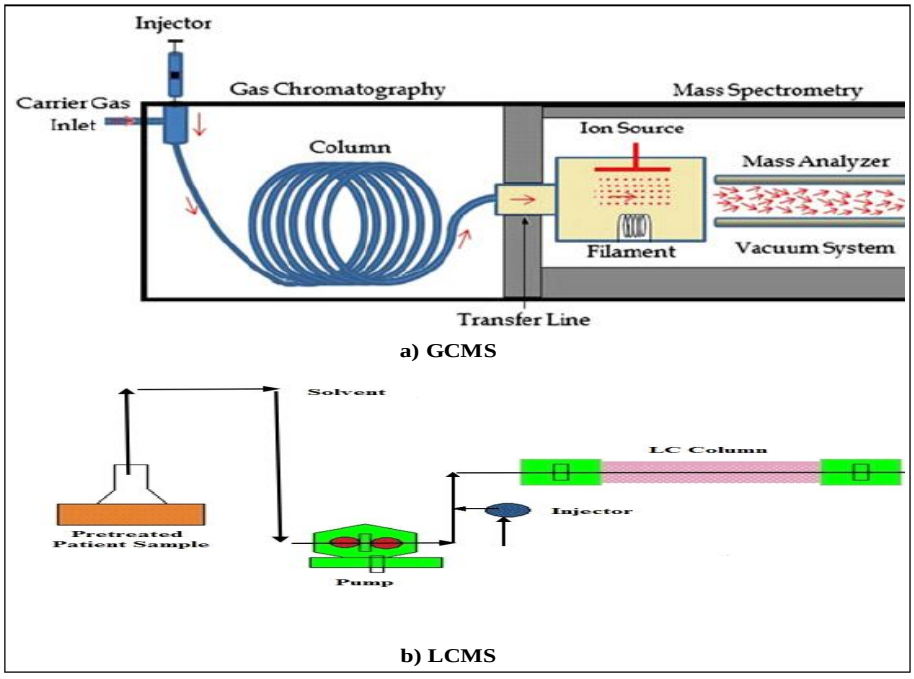

Fig 1: Diagrammatic representation of GCMS and LCMS for analysis of microbes and plants.

ratio. GCMS typically uses electron ionization (EI) to fragment the molecules, while LCMS can use various ionization techniques such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) (Pitt, 2009).

Data interpretation

The large amounts of data generated by GCMS and LCMS require sophisticated software for interpretation and analysis. This can include peak identification, compound quantification and statistical analysis of the results (Fiehn, 2016).

Standardization and collaboration

To ensure accurate and reliable results, it is essential to use standard reference compounds for calibration and quantification. This can involve spiking samples with known concentrations of reference compounds or using external standards for quantification (Visconti *et al*., 2023). The identification and characterization of plant pathogens using GCMS and LCMS often require collaboration between different disciplines such as microbiology, chemistry and bioinformatics to interpret the complex data generated by these techniques (Franco-Duarte *et al*., 2019).

Applications

Nowadays, analysis and identification of these approaches are broadly used in several fungal and bacterial pathogens and their associated compounds (Fang *et al*., 2023). Here are some examples such as *Fusarium graminearum* is a fungal pathogen that causes *Fusarium* head blight (FHB) in cereal crops. GCMS analysis of headspace samples from FHB-infected grains has identified several VOCs, including 2-pentanone, 2-heptanone and 2-nonanone, which are associated with the infection process (Drakopoulos *et al*., 2021). *Botrytis cinerea* is a fungal pathogen that causes gray mold disease in a wide range of vegetables and fruit crops. During analysis of headspace samples from *B. cinerea* infected grapes have identified several VOCs, including ethanol, acetaldehyde and hexanal, which are associated with the infection process (Soares *et al*., 2022). Eighteen different bio active compounds were produced by six isolates of A. niger during submerged conditions in phosphate solubilizing media such as PVK and NBRIP under in vitro conditions (Al-Zubaidi and Al-Taie, 2022). These VOCs can be used as biomarkers for early detection of gray mold disease and for monitoring the effectiveness of fungicides (Nawrocka *et al*., 2023). A widespread bacterial pathogen, *Pseudomonas syringae* that causes diseases in various crops, including tomatoes and peppers. A study of infected plants has identified several VOCs, including acetaldehyde, ethanol and hexanal, which are associated with the infection process (Yang *et al*., 2023). Such volatile organic compounds can be employed as biomarkers to detect *Pseudomonas* incidence in their early stages and to assess treatment efficacy (Bos *et al*., 2013; Kunze Szikszay *et al*., 2021). An oomycete pathogen *Phytophthora infestans* that causes late blight disease in potatoes and tomatoes. LCMS analysis of culture extracts from *P. infestans* has identified several secondary metabolites, including phytocassanes - A and B, which are involved in the pathogen's virulence strategy (Resjo *et al*., 2017). Leaves and roots parts of *Chenopodium album* extracts exhibited a seven different antifungal compounds like, 2(3H)-furanone, dihydro-4,4-dimethyl; 9-octadecenoic acid (Z), methyl ester; 9,12-octadecenoic acid (Z), methyl ester; 6-methylene bicyclo (3.2.0) hept-3-en-2-one., 1,2-benzene dicarboxylic acid, mono (2-ethylhexyl) ester and hexadecanoic acid and methyl ester against to phytopathogens such as *A. alternata, F. solani, P. aphanidermatum, R. solani* and *S. sclerotium* (Alkooranee *et al*., 2019). In bacteria, *Xanthomonas campestris causes* diseases in various crops, including rice and cruciferous vegetables. Culture extracts from *X. campestris* has identified several proteins, including *XopD* and *XopE*, which are involved in the pathogen's virulence strategy. These metabolites can be used as targets for developing new control strategies against late blight disease (Liu *et al*., 2022). On the other hand, it was utilized for characterization of biocontrol species of *Trichoderma, Beauveria, Clonostachys, Pseudomonas, Bacillus, Streptomyces, Saccharomyces* and mushrooms for novel production of antimicrobial metabolites and management of crop diseases at sustainable (Lahlali *et al*., 2022). Not only from microbes, host resistance analysis also examined through GCMS in green gram at more than 200 lines against bruchid beetle (Hema *et al*., 2024). Seed quality and viability of groundnut assess through principal component analysis by GCMS revealed that ethanol, 1-butanol, 1-hexanol, acetaldehyde, hexanal, Nonenal, 9,12,15-octadecatrionic acid, acetic acid and 3-methyl acetate were found to be highly negatively correlated with seed germination (Chinnasamy *et al*., 2024). Antimicrobial activity of cinnamon oil extracts against postharvest pathogenic bacteria by the exploitation of biocompounds such as cinnamaldehyde (75%) and linalool (63.7%) (Naik *et al*., 2021).

Advantages

Commonly, these techniques were highly used in several fields like agriculture, medicine and environmental safety. But they were specific in nature in working principle and applications with some limitations (Lee *et al*., 2021). GCMS is highly sensitive, allowing for the detection of trace amounts of compounds. This makes it a useful technique for analyzing complex matrices with low concentrations of analytes. It provides high resolution, which enables the separation and identification of complex mixtures of compounds particularly useful for separating isomeric compounds (Vaye *et al*., 2022). Especially, detection of specific compounds based on their mass-to-charge ratio, which reduces interference from other compounds in the sample with a wide range of fields (Wilschefski *et al*., 2019).

Limitations

During applications of GCMS, with certain limitations like interest of analysis in volatile compounds, requires broad sample preparation which can be time-consuming and labor-intensive. Mostly errors occurred in the handling and processing of samples additional with extended budget for interpretation of large dataset and adequate repository is mandatory (Wang *et al*., 2022). LCMS can limit its ability to detect trace amounts of analytes in complex matrices with least resolution during separation of compounds from complex mixtures due to their limited sensitivity of detection based on their mass-to-charge ratio (Franco-Duarte *et al*., 2019).

Challenges and future directions

Both techniques have become essential analytical techniques for the identification and quantification of compounds associated with plant pathogens. The following are some challenges and future directions for GCMS and LCMS in the analysis of plant pathogens. One of the major challenges in GCMS and LCMS analysis of plant pathogens is the complexity of biological samples, which can lead to interference from matrix components (Steiner *et al*., 2021). Advances in sample preparation techniques, such as solid-phase extraction, QuEChERS (quick, easy, cheap, effective, rugged and safe) and micro-extraction methods, can help to reduce matrix effects and improve the accuracy and sensitivity of GCMS and LCMS. These two approaches rely on separation techniques to separate complex mixtures of compounds (Lou *et al*., 2023). The development of new separation techniques, such as supercritical fluid chromatography (SFC), capillary electrochromatography (CE) and high-performance thin-layer chromatography (HPTLC), can provide additional separation capabilities for GCMS and LCMS analysis, particularly for non-volatile compounds that are difficult to separate using traditional GCMS (Amin *et al*., 2022).

The integration of GCMS and LCMS with other analytical techniques, such as nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) and Raman spectroscopy, can provide complementary information about the structure and properties of compounds associated with plant pathogens. This can help to improve the accuracy and specificity of GCMS and LCMS analysis by providing additional context about the chemical environment in which these compounds are produced (Valasi *et al*., 2021). A development of new mass spectrometry technologies, such as time-of-flight (TOF) MS, ion mobility spectrometry (IMS) and orbitrap MS, can provide improved sensitivity, resolution and selectivity for GCMS and LCMS analysis. These technologies can also enable the simultaneous detection of multiple analytes in complex matrices, which can reduce analysis time and improve throughput (Ma, 2022). Further, the expansion of GCMS and LCMS applications to new fields, such as environmental science, pharmaceuticals, food analysis and clinical research, can provide new insights into the biochemistry of plant pathogens and their interactions with host plants. This can also lead to the development of new control strategies for plant diseases based on a better understanding of the chemical signals produced by these pathogens (Putri *et al*., 2022).

CONCLUSION

Both are powerful analytical techniques that have revolutionized the analysis of plant pathogens by providing insights into the biochemistry of these pathogens and their interactions with host plants. However, these techniques also have some limitations and challenges that need to be addressed in order to fully realize their potential. Here are some conclusions regarding GCMS and LCMS, along with their advances (identification of novel compounds, understanding host-pathogen relationships and early disease detection); challenges and future directions (complexity of sample matrices, sensitivity and cost base), Integration with improved new techniques and make a repository in the analysis of plant pathogens given a better outcome in crop management at sustainable soon.

Conflict of interest

All authors declare that they have no conflict of interest.

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