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Unravelling advanced methods of detection of anthracnose disease in mango (*Mangifera indica* L.) and banana (*Musa* sp.)

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Abstract

Colletotrichum species cause anthracnose, which is one of the major post-harvest diseases in bananas and mangoes, resulting in huge losses to the fruit industry. This review examines the different detection technologies that have been used to identify and determine the infection of anthracnose in these fruits. We discuss traditional techniques like visual examination and culturing, as well as newer, more accurate technologies like molecular diagnostics, spectroscopy and sophisticated imaging procedures. Special attention is paid to such high-throughput methods such as polymerase chain reaction (PCR), computer-based methods and biomarkers, which are more sensitive and specific in the detection of *Colletotrichum* infections. Moreover, we emphasise the application of imaging systems, including hyperspectral and infrared spectroscopy, which make it possible to detect infections quickly and without destruction. The combination of these technologies has improved disease management strategies, offering a more precise and timely way of disease surveillance. On the whole, the purpose of the review is to give a general presentation of the technologies for detecting anthracnose, which will promote the development of disease management strategies for banana and mango crops in the future.

Keywords: *Colletotrichum*; molecular diagnostics; near-infrared spectroscopy; post-harvest disease; spectroscopy

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1. Introduction

Food security is a major problem in the world today and in the post-harvest operations, handling, storage and distribution of the perishable horticultural commodities; losses of 20–50 % of the crop are attributed to microbial spoilage, fungal diseases, insect infestation and physiological deterioration (1). Among these, fungal pathogens of the genus *Colletotrichum* are among the most serious causes of post-harvest diseases in tropical and subtropical fruit crops, resulting in substantial economic losses worldwide. Anthracnose is one of the most important diseases caused by these pathogens (2).

Mangifera indica L. is a member of the Anacardiaceae family and is a widely cultivated tropical and subtropical tree with highly sought-after edible fruit. The popularity of mango is due to its unique taste, aroma, texture and nutritional value, which make it the “king of fruits” (3). Mango is one of the most important fruit crops in India and is also widely cultivated in other countries, including China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria and the Philippines (4). Banana is among the most significant commercial crops and plays a significant role in ensuring food security worldwide. It belongs to the family Musaceae and the genus *Musa*. It is a large perennial herbaceous monocotyledon. Bananas are grown in over 150 countries, primarily in Asia, Latin America and Africa. India is followed by China, the Philippines, Ecuador and Brazil as the world's biggest banana-producing countries (5). In terms of food security, bananas are considered a source of energy, vitamins, minerals and protein (6).

Anthracnose is a disease affecting a wide variety of crops and is a serious threat in tropical and subtropical regions, resulting in significant economic losses both in the field and after harvest. It is caused by many species of *Colletotrichum* such as *Colletotrichum coccodes, C. fructicola, C. siamense, C. capsici,*

C. gloeosporioides, C. acutatum, C. dematium, C. panacicola, C. boninense and *C. godetiae*. *Colletotrichum gloeosporioides* has been proved as the most common cause of anthracnose (7). *Colletotrichum* spp. infects various parts of the mango plant such as leaves, flowers, inflorescences and fruits and can infect at any stage of mango fruit development (8). Anthracnose occurs in both the field and post-harvest banana tissues and manifests as lesions on immature fruit and leaves (9). Typically, infection starts with the appressorium being melanised and penetrating the host cuticle, then infection vesicles and primary hyphae are formed. It then produces secondary, destructive hyphae which cause death of the host cell. The hemibiotrophic infection strategy is the first phase of the infection which is biotrophic and the second phase of the infection is the necrotrophic phase corresponding to lesion development (10). High relative humidity (RH > 95 %), rain splash, irrigation water, insect dispersal, contaminated tools and handling activities promote disease development, which occurs best at warm temperatures (25–30 °C) and with prolonged periods of surface wetness (8).

Anthracnose has a characteristic quiescent stage, in which the pathogen causes infection in developing fruit in the field without showing any symptoms until ripening. This latent infection is the reason that matures green fruits may look healthy, but then quickly show visible symptoms. Pathogen reactivation is an important factor in post-harvest losses in climacteric fruits like mango and banana due to ripening-associated physiological changes. After reactivation, *Colletotrichum* switches to a necrotrophic mode of growth and spreads through the tissue, causing fruit quality losses (11). The mature mango fruit usually develops irregular dark brown lesions, while a heavy infection on young fruit may cause fruit mummification (8). The initial symptom in banana is small brown spots followed by larger, depressed lesions. In the later stages of disease, the mature lesions will bear orange to pink fruiting bodies (acervuli) with orange to pink conidia that are the main cause of future infection and spread (12). The comparative symptomatology presented in Table 1 provides an overview of anthracnose manifestations in banana and mango. To better understand the development and progression of the disease, the life cycle of *Colletotrichum* spp. is illustrated in Fig. 1.

The disease cycle begins with conidial germination on the fruit surface, followed by appressorium formation and penetration of host tissues. After an initial biotrophic (latent) phase, the pathogen switches to a necrotrophic phase, resulting in tissue necrosis and the development of characteristic anthracnose lesions. Under favourable conditions, acervuli are formed on infected tissues and release conidia, which serve as the primary source of secondary infections. The pathogen also survives on infected crop debris through saprophytic growth and may undergo sexual reproduction by forming perithecia that produce asci containing ascospores, thereby completing the disease cycle.

Based on the banana production value estimation in 2024, the global gross production value of banana is estimated at US$ 51.91 billion, while the mango, guava and mangosteen production value is estimated at US$ 44.60 billion. India is the biggest producer of mango, with an estimated area of 2.42 million hectares and production of 23.44 million tonnes in 2025–26 (First Advance Estimates). The national average productivity is 9.70 million tonnes ha^{-1}. The top five mango-producing states are: Uttar Pradesh (6.17 million tonnes), Andhra Pradesh (4.99 million tonnes), Karnataka (1.72 million tonnes), Bihar (1.59 million tonnes), Gujarat (1.27 million tonnes) and West Bengal (1.10 million tonnes). The highest area under mango is in Andhra Pradesh (398.91 thousand hectares), followed by Uttar Pradesh (325.72 thousand hectares), Odisha (230.96 thousand hectares), Gujarat (184.03 thousand hectares), Bihar (165.16 thousand hectares) and Maharashtra (151.79 thousand hectares). Punjab has the highest production level among the major mango-producing states (19.67 million tonnes ha^{-1}), followed by Uttar Pradesh (18.95 million tonnes ha^{-1}), Puducherry (15.99 million tonnes ha^{-1}), Madhya Pradesh (14.24 million tonnes ha^{-1}) and Assam (13.35 million tonnes ha^{-1}), which shows relatively higher production efficiency (21).

The estimated area under banana cultivation in India is 925.48 thousand hectares (2025–26 – First Advance Estimates) and its production is 39.62 million tonnes (2025–26 – First Advance Estimates), which makes it the largest producer in the world. The average productivity of the country is estimated at 42.81 million tonnes ha^{-1}. Maharashtra is the highest banana-producing state with 8.39 million tonnes, followed by Andhra Pradesh, Tamil Nadu, Uttar Pradesh and Gujarat respectively. Maharashtra is also ahead of other states in the area under banana cultivation, with 143.77 thousand hectares, followed by Tamil Nadu (116.54 thousand hectares), Andhra Pradesh (110.82 thousand hectares) and Uttar Pradesh (94.69 thousand hectares). The productivity of the major producing states ranges from 72.40 million tonnes ha^{-1} in Madhya Pradesh to 70.19 million tonnes ha^{-1} in Gujarat, indicating good cultivation practices and favourable agro-climatic conditions (21).

Numerous studies have documented the widespread occurrence and economic impact of anthracnose in major mango- and banana-producing regions. A summary of the reported disease incidence, severity and associated yield or post-harvest losses is presented in Table 2.

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Table 1. Comparative symptomatology of anthracnose in banana and mango caused by *Colletotrichum spp*.

Feature	Banana	Mango
Causal organism	*Colletotrichum musae* sp. complex (11).	*Colletotrichum gloeosporioides* sp. complex (11).
Infection stage	Post-harvest disease (Latent infection) (11).	Pre and post-harvest disease (13).
Symptoms	The typical symptoms of disease become evident at the ripening stage. The major symptoms of the anthracnose appear on fruit peel as brown to black spots, which become sunken lesions with salmon or orange-coloured acervuli. Additionally, wounding can severely damage the fruit if it develops during transport and reduces the quality of the bananas before they reach the ripening stage (14).	The fungus *C. gloeosporioides* can infect mangoes at various stages, particularly during the ripening process. The fungus leads to the development of sunken, dark brown to black rot patches on the fruit (15).
Conidia	The conidia are cylindrical and straight with rounded ends, often featuring one to two oil globules at the tips. On the pure culture plate, the conidia form small pink clusters, acervuli can be observed on the reverse side of the Petri dish (16). Conidia are elliptical or cylindrical and the average size is 13.14–5.86 µm (16).	*Colletotrichum* spp. produces hyaline cylindrical conidia with obtuse to slightly rounded ends (86), the conidia are hyaline with oil globules and the size ranges from 11.34–16.33 mm to 4.10–6.30 mm (85). The conidia are straight with rounded ends, straight with constriction and straight and tapered at one end (17).
Colony characters	Orange to pinkish-white, cottony mycelium with abundant yellow/ orange conidial masses, the lower surface is white to greyish orange colour (18).	White to grey-coloured colonies with a dark and grey conidial mass in the centre (19).
Appressoria	Single, medium to dark brown, smooth-walled, oval, elliptical or heart-shaped, with undulate or slightly lobate margin (18).	Unlobed or slightly lobed appressoria (20), irregular and lobulated irregular and slightly lobulated and rounded and smooth (17).

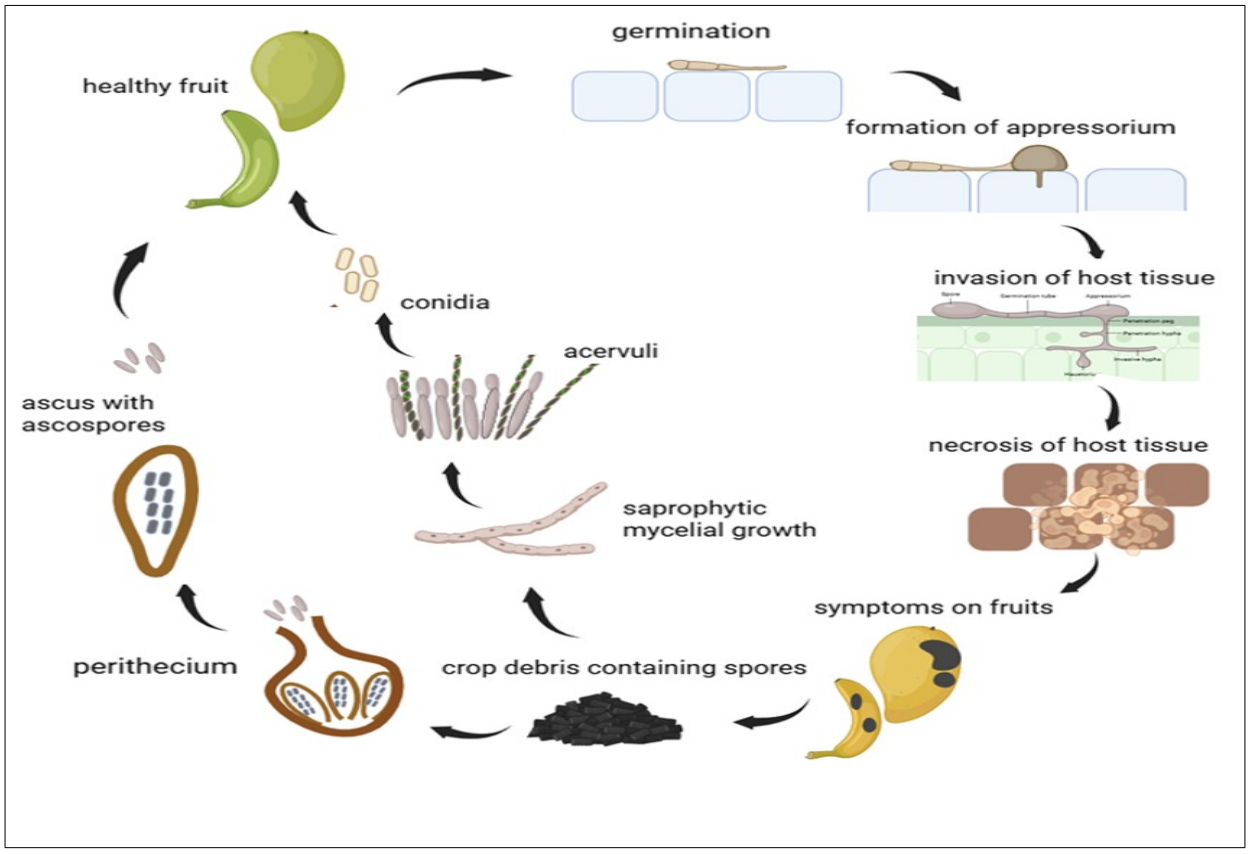

Fig. 1. Life cycle of *Colletotrichum* spp.

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Table 2. Regional incidence and postharvest losses associated with anthracnose in mango and banana

Crop	Country/region	Disease incidence / yield or postharvest loss	Reference
Mango	Côte d'Ivoire	Rainy-season orchard incidence up to 32.25 %; post-harvest incidence 90 %, severity 100 % at one locality	(13)
Mango	India	20–40 % pre-harvest incidence	(22)
Mango	Peru	55–80 % postharvest incidence	(23)
Banana	Karnataka, India	Disease intensity ranged from 10–38 %, highest in Grand Naine	(24)
Banana	Minas Gerais, Brazil	Highest disease intensity from November–March; lower from September–October and April–June, indicating seasonal disease peaks	(25)
Banana	Jimma, Ethiopia	Mean fungal disease incidence 32.9 %; severity 21.1 %; retailer incidence 54.2 %, severity 34.0 %; fruit damage 56.2 %	(26)

Loss of productivity in mango is generally reported as reduction in yield, fruit quality and post-harvest rejection. In reviews, mango anthracnose results in a 30–60 % annual loss and yield loss can be up to 100 % in orchards that are not managed, under highly favourable conditions (20). The present farm level evidence shows losses exceeding 50 % for growers in Samal, while losses of mango due to anthracnose caused by *C. gloesporioides* were estimated at around 261.154 tonnes in Colombia in 2019 (27). The most consistent indicator of productivity is the loss of marketable fruit, shelf life and fruit quality after harvest. The losses due to banana anthracnose are estimated at approximately 30–40 % of the marketable product or commercial value (28).

In wet or very humid conditions, mango fruit anthracnose incidence can be as high as 100 %, with losses potentially as high as 30–60 % or even 100 % if the disease is severe (11). Wound effects on virulence and cross-infection have been shown to contribute to the virulence of *Colletotrichum* on banana fruits, with losses of 30–40 % in marketable fruit (29).

Anthracnose management is challenging because infections often remain latent until fruit ripening or after harvest, delaying disease control and increasing post-harvest losses. Therefore, rapid, sensitive, accurate and non-destructive detection methods are essential for early diagnosis. Advanced techniques, including molecular diagnostics, biosensors, spectroscopy, hyperspectral imaging, near-infrared (NIR) spectroscopy, electronic noses (E-noses) and biomarker-based detection, have significantly improved early anthracnose detection in mango and banana. This review compares conventional and advanced detection methods, highlighting their principles, advantages, limitations and applications, while identifying current research gaps and future directions for developing reliable and field-deployable detection systems.

2. Materials and Methods

Literature search strategy and study selection

To identify empirical studies published in peer-reviewed journals on the detection of anthracnose disease in mango and banana, a systematic literature search was made according to the Preferred reporting items for systematic reviews and meta-analyses (PRISMA) 2020 guidelines (30). Relevant studies published between January 1997 and June 2026 were considered for inclusion. The literature search was conducted across four major databases (Scopus, ScienceDirect, Springer Nature and Google Scholar) and the final search update was completed in May 2026. The search strategy was designed using disease-related terms: "post-harvest disease", "anthracnose", "mango", "banana", "*Colletotrichum*" and "tropical fruits" with the detection method-specific terms: "molecular detection", "serological methods", "biomarkers", "electronic nose (E-nose)", "hyperspectral imaging", "near-infrared spectroscopy (NIRS)", "surface plasmon resonance (SPR)" and "spectroscopy", with Boolean operator linkage (AND/OR).

The first search returned a total of 524 records. Titles and abstracts were screened for the removal of duplicates (n = 94), with 430 remaining, 291 of which were excluded. Next, the 139 full-text articles were evaluated for eligibility and 54 were ruled out as they did not have a specific emphasis on post-harvest anthracnose or were not specifically associated with testing methods for detecting the disease. There were 85 studies included in the qualitative synthesis and included in the final dataset. A summary of the study selection process is provided in the PRISMA flow diagram, which illustrates how publications were identified, screened, assessed for eligibility and included in the review (Fig. 2). To ensure a systematic and transparent literature review, predefined inclusion and exclusion criteria were applied during the selection process and the criteria used for screening and selecting eligible publications are summarised in Table 3.

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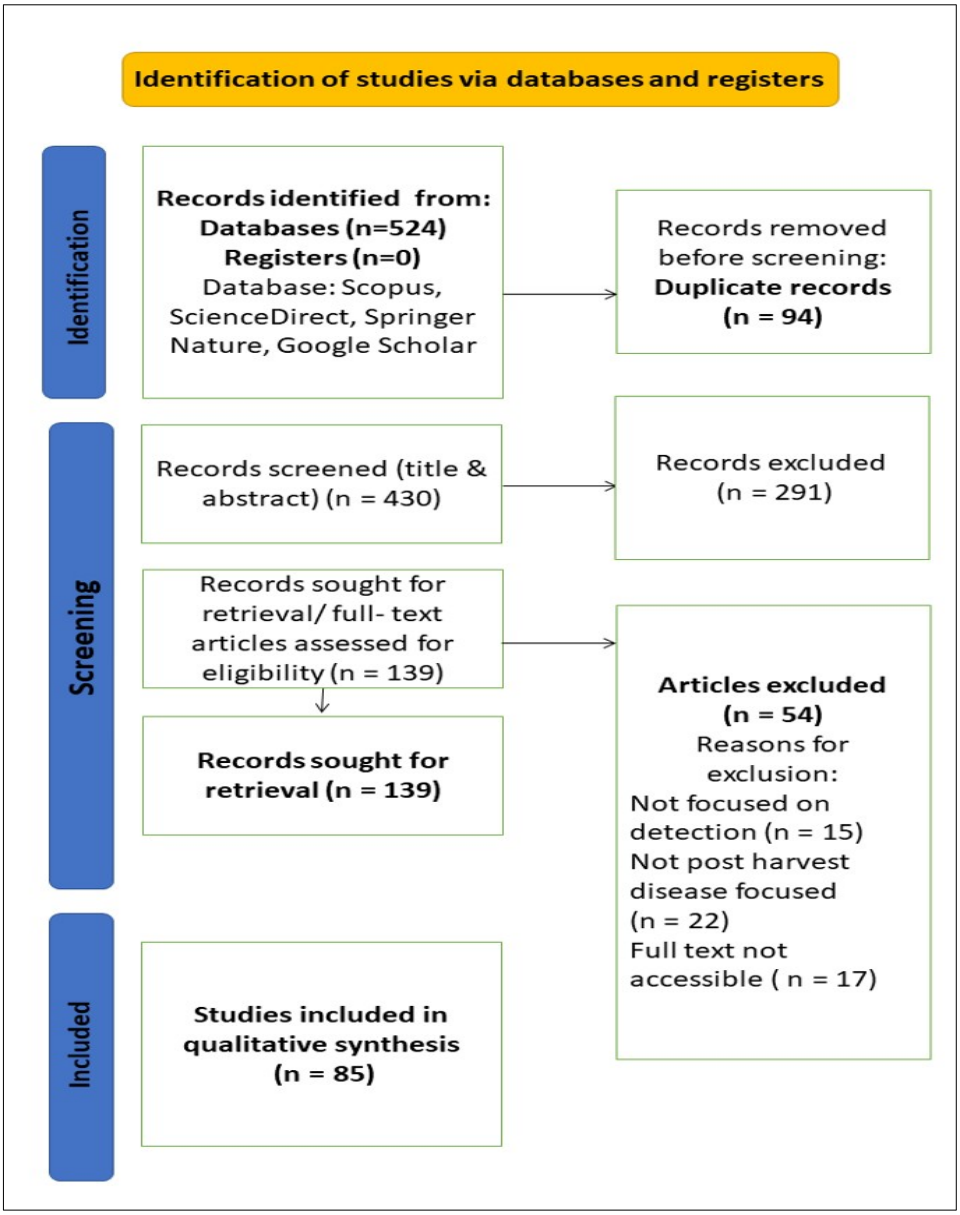

Fig. 2. PRISMA flow diagram.

Table 3. Inclusion and exclusion criteria used for the systematic literature search and study selection

Criteria	Inclusion	Exclusion
Time period	1997–2026	<1997
Subject area	Immunology, engineering, environmental science, multidisciplinary	Biochemistry, genetics and molecular biology, chemistry, medicine, physics and astronomy, chemical engineering, materials science, mathematics, social science, energy.
Document type	Article, review	Book chapter, note
Languages	English	Chinese, Spanish, Portuguese
Source type	Journal	Trade journal
Publication stage	Final	Press
Open access	All open access	Restricted access
Title and abstract	Existence of predefined keywords in the title, abstract, or keywords part of the paper	
Full text	Included relevant papers that discuss the predefined keywords	

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Conventional and advanced detection methods

The detection methods included in this review were classified into four major categories: conventional, serological, molecular and advanced diagnostic techniques (Table 4). Conventional methods comprise visual symptom assessment, fungal isolation and microscopic examination, whereas serological methods rely on antigen–antibody interactions for pathogen detection. Molecular techniques, including polymerase chain reaction (PCR), quantitative polymerase chain reaction (qPCR) and loop-mediated isothermal amplification (LAMP), enable rapid and sensitive detection of *Colletotrichum* spp. Recent advances in non-destructive imaging, spectroscopy, biosensors and artificial intelligence have further enhanced the accuracy and early detection of anthracnose. An overview of the diagnostic methods is presented in Fig. 3.

Traditional detection method

Principle: In the traditional approach to anthracnose detection in banana and mango, symptoms are initially observed in the fruit, leaves and other infected tissues which are typically dark brown to black, sometimes with orange or salmon conidial masses, but sometimes latent or quiescent until the fruit is ripe (11). After surface sterilisation, the suspected pathogen is cultured from the margins of the lesions or from the symptomatic tissues, usually on potato dextrose agar and subsequently purified by single spore isolation, hyphal tipping and/or sub-culturing to obtain a pure culture (29). Traditionally, the identification of *Colletotrichum* species is made on the basis of morpho-cultural characters like the growth rate and colony colour as well as on the basis of the microscopic examination of conidia, appressoria, acervuli and related structures; multiple studies however, mention that the use of morpho-cultural characters alone does not provide a reliable separation of closely related *Colletotrichum* species (40).

Traditionally, visual scales based on subjective assessments by trained evaluators have been used to measure disease severity (41). Certain characteristic features such as mycelial development, conidia size, colony colour, texture and whether setae are present or absent are used to identify mango anthracnose disease (19). The presence of latent infection is confirmed by soaking the fruits in paraquat solution or by isolating the fungus from surface-sterilised fruits (42). The anthracnose pathogen is identified using this method by examining its shape, growth and spore development. Following isolation, the pathogen is identified through microscopic examination of colony morphology, hyphal characteristics and conidial development. However, these methods have drawbacks; they harm plant tissue and require a lot of time. Because the chemicals are systemic, the pathogen might not appear normal on fruits treated with fungicides or paraquat (43).

Table 4. Sample preparation procedures for different detection methods used in the diagnosis of anthracnose in mango and banana

Sl. No.	Detection methods	Sample preparation
1.	**Traditional methods (visual observation, isolation, microscopy)**	Diseased mango or banana fruits showing anthracnose symptoms are selected. Small pieces are cut from the edge of healthy and infected tissue, surface sterilised and used directly for visual examination or fungal isolation (31).
2.	**Molecular methods (PCR/qPCR/LAMP)**	Fresh infected fruit tissue or fungal mycelium is collected under sterile conditions. DNA is extracted from the sample and purified to remove contaminants before amplification by PCR or related techniques (31).
3.	**Serological methods (ELISA, immunoassays)**	Infected fruit tissue is ground in an extraction buffer to release fungal proteins or antigens. The extract is filtered or centrifuged and then used for antibody-based detection (32).
4.	**Spectroscopic analysis**	Fruits are gently cleaned to remove dust and surface moisture before analysis. The intact fruit or a freshly cut surface is scanned without the need for chemical treatment (33).
5.	**Hyperspectral imaging**	Fruits are cleaned and positioned under controlled lighting. Images are captured without physical contact and no staining or chemical preparation is required (34).
6.	**Near-infrared spectroscopy**	Fruits are cleaned and dried to minimise interference from surface moisture. The intact fruit is placed directly in front of the NIR sensor for spectral acquisition (35).
7.	**Surface plasmon resonance**	DNA, proteins or fungal biomarkers are extracted from infected tissue. The purified sample is introduced onto the sensor surface where binding interactions are measured in real time (36).
8.	**Electronic nose**	Fruits are placed in a sealed chamber for a short period to allow volatile organic compounds (VOCs) to accumulate. The sensor then measures the headspace gases released by the fruit (37).
9.	**Reflected UV imaging**	Fruit surfaces are gently cleaned and dried before imaging. The intact fruit is illuminated with UV light and reflected signals are recorded using a UV-sensitive camera (38).
10.	**Biomarker-based detection**	Tissue samples are collected from infected fruits and processed to extract specific metabolites, proteins or nucleic acids. The purified extract is analysed using the selected biomarker detection platform (39).

PCR: polymerase chain reaction; qPCR: quantitative polymerase chain reaction; LAMP: loop-mediated isothermal amplification; ELISA: enzyme linked immune sorbent assay.

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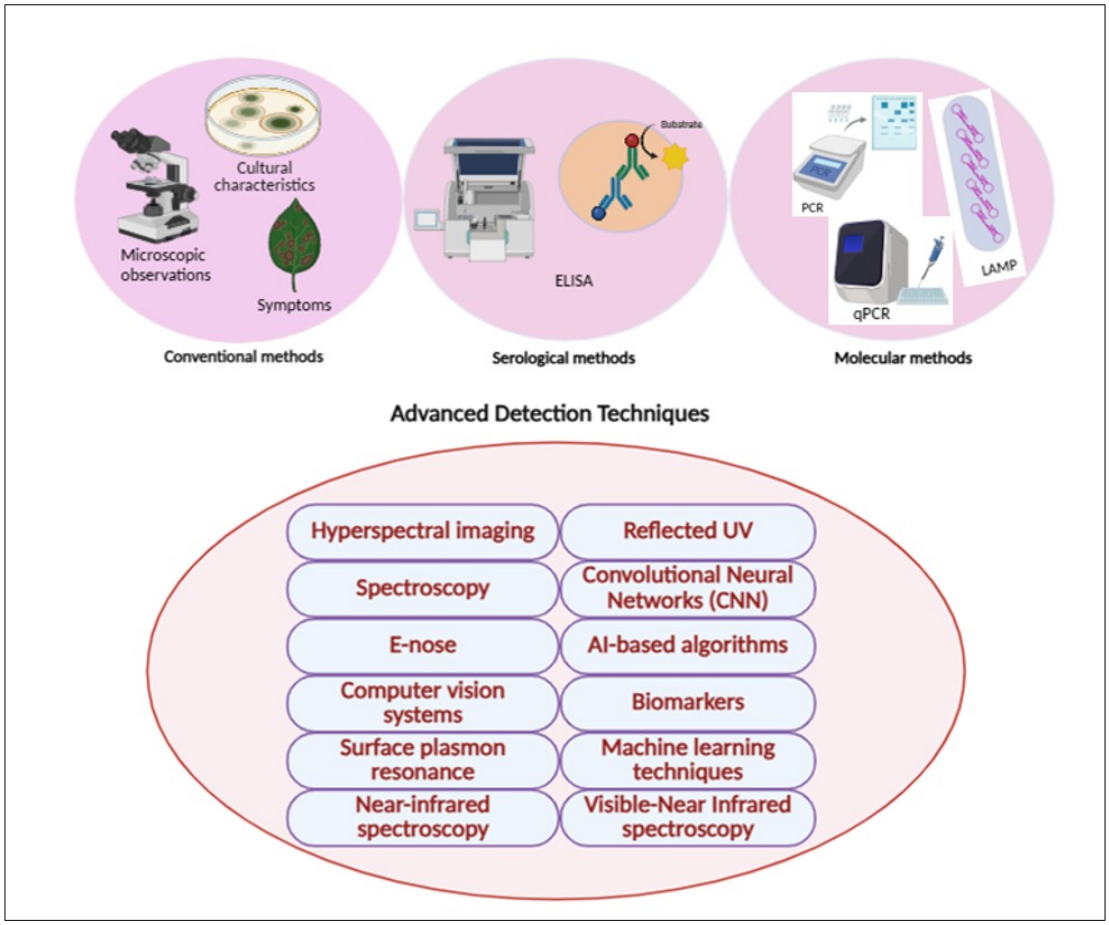

Fig. 3. Overview of conventional and advanced detection methods for anthracnose in mango and banana.

In mango, visual assessment is carried out on leaves, flowers, inflorescences and fruits, reflecting the broad host tissue range affected by anthracnose (44). In banana, visual diagnosis is mainly performed during the post-harvest stage, where infected fruits typically develop brown to reddish-brown spots that gradually enlarge into characteristic sunken lesions. Following symptom-based screening, pathogen identity is confirmed by isolating the fungus from lesion-margin tissues and examining colony characteristics and conidial morphology, with pathogenicity further verified through Koch's postulates (12).

Critical evaluation: Traditional methods such as visual inspection, microscopy and pathogen isolation via culture are cheap, simple, time-consuming and require pathogen sporulation or symptom development and are not suitable for detecting latent infections. By contrast, PCR-based molecular assays offer high sensitivity and specificity for the detection of the pathogen DNA, even where symptoms have yet to be seen. It is therefore concluded that molecular methods are better suited for the early and precise diagnosis and traditional methods are suitable for routine confirmation in laboratories with low resources.

Molecular detection methods

Principle: Molecular detection methods are based on the detection and analysis of pathogen-specific nucleic acids (DNA or RNA) to accurately identify the causal *Colletotrichum* species. These involve the use of conserved or species-specific regions of the genome, such as the internal transcribed spacer (ITS), β-tubulin (TUB2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT), calmodulin (CAL) and chitin synthase (CHS-1) genes, by means of molecular markers and nucleic acid amplification techniques including PCR, qPCR, LAMP and DNA sequencing. Two new species of *Colletotrichum* associated with mango anthracnose in Central Thailand have been identified. Amplification or detection of these genetic markers allows for sensitive, specific and rapid identification of the pathogen, even in the latent or quiescent phase of infection and prior to the appearance of visible symptoms (10). Molecular methods provide greater accuracy than traditional morphology-based approaches for differentiating closely related *Colletotrichum* species.

Molecular assays are often more sensitive than traditional diagnostic processes and are quicker. For instance, the LAMP assay was able to detect *C. asianum* and *C. musae* at 65 °C in 45 min, showing its potential for rapid diagnosis. In addition, it was stated that LAMP was able to detect *Colletotrichum* species up to 10 times more sensitive than conventional PCR (45). The potential of using LAMP for early and sensitive detection of *Colletotrichum* pathogens is also demonstrated in the case of tea anthracnose-causing fungus *C. siamense* (46).

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Polymerase chain reaction is used to identify anthracnose by amplifying specific DNA sequences of anthracnose pathogens (*C. gloeosporioides*) with species-specific primers. The pathogen DNA is first isolated from infected plant tissues and primers are specific sequences that bind to unique regions of the pathogen genome. The target DNA is amplified according to an exponential law during the thermal cycling, generating a typical amplicon which is indicative of the presence of the pathogen. Since PCR identifies the genetic material of the pathogen, it can identify the pathogen before clinical signs are seen. Previous research showed that *C. gloeosporioides* could be distinguished from other species of the genus *Colletotrichum* with high specificity and reliability using species-specific primers which can be used for early detection of mango anthracnose (47).

For the closely related *Colletotrichum* species, however, the ITS region is often not sufficient for accurate species identification and multiple genetic regions must be amplified. To enhance species discrimination additional markers like GAPDH, TUB2, ACT, CAL, CHS-1 and the ApMat region are widely used. These are useful for reliable species differentiation between members of the *C. gloeosporioides* and *C. acutatum* species complexes (48). Previous researchers successfully amplified the ITS, ACT, GAPDH, TUB2 and GS regions and conducted phylogenetic analysis to determine the species of *Colletotrichum* found in banana anthracnose. This method enabled the separation of species which were not reliably distinguishable by morphology or ITS alone and led to the characterisation of *C. musae*, *C. plurivorum* and *C. siamense* as the cause of banana anthracnose. The results indicate that multilocus PCR could give better taxonomic resolution and enhance the reliability of the identification of anthracnose and epidemiological studies (49). For mango, PCR of partial TUB2 detected the mutations responsible for carbendazim resistance and PCR- restriction fragment length polymorphism (RFLP) quickly screened the *C. asianum, C. gloeosporioides* and *C. siamense* that were highly resistant to carbendazim (50). To improve the accuracy of species identification within the *Colletotrichum* species complexes, multiple genetic markers are routinely used. Table 5 summarises the major multilocus markers employed for the detection and identification of *Colletotrichum* species associated with mango and banana.

Critical evaluation: Molecular techniques enable rapid and accurate species-level identification of *Colletotrichum*, overcoming the limitations of morphology-based methods, which are often influenced by environmental conditions and require considerable taxonomic expertise. Highly sensitive techniques such as PCR and qPCR can detect minute quantities of pathogen DNA, allowing the identification of infections at an early stage while reducing the likelihood of false-negative results. Unlike imaging and spectroscopic approaches, which detect disease-induced changes in host tissues, molecular methods directly identify the pathogen through its genetic material, providing high specificity for disease diagnosis. Despite these advantages, molecular techniques require specialised laboratory equipment, skilled personnel and high-quality DNA, making them more expensive and less suitable for routine field applications than conventional diagnostic methods. In addition, DNA-based assays cannot distinguish between viable and non-viable pathogens, which may lead to an overestimation of disease risk. Therefore, future research should focus on developing cost-effective, portable and field-deployable molecular diagnostic platforms capable of providing rapid, real-time detection for improved disease surveillance and management.

Table 5. Multilocus genetic markers used for the detection and identification of *Colletotrichum* species in mango and banana

Sl. No.	Species complex	Molecular marker/gene	Taxonomic resolution	References
1.	*Colletotrichum acutatum*	ACT CHS-1 GAPDH HIS3 nrITS TUB2	Species complex to species level	48
2.	*C. gloeosporioides*	APN2 APN2/MAT-IGS CAL GAP2-IGS GAPDH GS TUB2	Species complex and species delimitation	48
3.	*C. musae*	ACT GAPDH GS	Species-level identification	18

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		ITS TUB		
4.	*C. plurivorum*	ACT GAPDH GS ITS TUB2	Species-level identification	18
5.	*C. siamense*	ACT GAPDH GS ITS TUB2	Species-level identification	18
6.	*C. fructicola* *C. cliviicola* *C. siamense* *C. karstii* *C. musae*	ITS GAPDH ACT TUB2 CHS-1 CAL ApMAT	Species-level identification	49
7.	*C. gloeosporioides* spp.	RAPD ISSR	Intraspecific / strain-level differentiation	17
8.	*C. asianum* *C. fructicola* *C. siamense* *C. tropicale* *C. scovillei*	ACT CAL CHS GAPDH ITS TUB2	Species complex and species-level resolution	51
9.	*C. alienum* *C. asianum* *C. fructicola* *C. siamense* *C. tropicale*	Apn2/MAT	Species complex discrimination and species-level identification	52

Serological methods

Principle: This serological technique is based upon the principle of the highly specific antigen–antibody reaction. *Colletotrichum gloeosporioides* is a fungal pathogen with unique antigenic proteins that can be specifically detected by polyclonal antibodies against the pathogen. These antibodies will bind selectively to their antigen(s) to generate an antigen–antibody complex. This specific immunological recognition allows the discrimination of the target pathogen from other microorganisms and mango tissues, ensuring high specificity and sensitivity by being able to detect latent infections prior to the occurrence of evident disease symptoms (53).

Early detection of latent infection of anthracnose has been developed in both mango and banana using immunoassay-based technique. An immunoassay method was developed to detect latent postharvest anthracnose of mango caused by *C. gloeosporioides* (54). To achieve this, polyclonal antibodies were raised against a 40-kDa polypeptide, as well as against unfractionated mycelial protein (UMP) and after optimising antigen and antibody concentrations, the antibodies were used in enzyme-linked immunosorbent assay (ELISA), dot immunobinding assay (DIBA) and Western blot analysis. The assays were able to detect the antigens of *C. gloeosporioides* with relatively low cross reactivity, thus allowing the detection of latent *C. gloeosporioides* infection prior to symptom emergence (54). Likewise, anti-purified 65 kDa protein polyclonal antibodies were produced to develop an immunoassay for banana anthracnose (32). A sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on protein extracts to determine the presence of proteins specific to the isolates and the purified antigen was used to immunise New Zealand White rabbits. Immunological assays were used to detect *C. musae* in infected banana fruits and proved to be a rapid and specific method for the diagnosis of latent postharvest anthracnose of banana; these assays detected *C. musae* in infected banana fruit using ELISA and immunoblotting (32). A species-specific SCAR (Sequence-characterized amplified region) was developed for the molecular detection of *C. musae* in banana (55). A 588-bp RAPD fragment was cloned and sequenced with primer OPA-01 and new primers (SCAR-FP and SCAR-RP) were designed. The primers

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specifically amplified *C. musae* DNA and yielded a good detection of *C. musae* in infected banana tissues with a detection limit of about 30 ng infected banana tissues per reaction, which was a rapid and reliable molecular diagnostic tool for banana anthracnose (55).

Critical evaluation: Pathogen-specific detection is achieved by both molecular and serological methods. Molecular assays are more sensitive and can be used to differentiate closely related *Colletotrichum* species by analysing the nucleic acids. Serological methods (ELISA, immunostrips) are less complicated, faster, less expensive and rely on the presence of highly specific antibodies but can exhibit cross reactivity with closely related pathogens. Hence molecular methods are preferred for the precise diagnosis and serological methods are advantageous for large scale screening.

Spectroscopic analysis

Principle: The principle of spectral analysis is related to the interaction of the light with fruit tissues and changes in their optical properties due to the fungal infection. Due to biochemical differences, cell structure, pigment content and water status of healthy and anthracnose affected mango tissues, their ability to absorb, reflect and transmit light at different wavelengths is different. In the latent stage of anthracnose infection, these physiological and biochemical changes take place before symptoms are visible and are manifested as distinct spectral signatures. The reflectance spectrum of the fruit at different wavelengths can be measured and the spectrum patterns analysed to differentiate healthy fruit from infected fruit. Therefore, spectral analysis is a non-destructive, rapid and objective technique for the early diagnosis of anthracnose, which is based on the optical differences of healthy and diseased mango tissues (33).

Although conventional spectroscopy is rapid, inexpensive and non-destructive, it provides only averaged spectral information from the sample and lacks spatial resolution. Consequently, its ability to localise anthracnose lesions is limited compared with hyperspectral imaging. In addition, conventional spectroscopic approaches are highly dependent on calibration models and controlled measurement conditions, which may restrict their reliability under commercial storage environments. Therefore, spectroscopy is primarily suitable for rapid preliminary screening rather than definitive pathogen identification. Among the advanced spectroscopic approaches, hyperspectral imaging and NIR spectroscopy have been extensively investigated for fruit disease detection. Hyperspectral imaging integrates spectral and spatial information, enabling the visualisation and localisation of disease-associated changes, whereas NIR spectroscopy provides rapid and non-destructive assessment based on the spectral responses of infected tissues. The following sections discuss the principles, applications, advantages and limitations of hyperspectral imaging and NIR spectroscopy for anthracnose detection in mango and banana.

Hyperspectral imaging

The principle of hyperspectral imaging (HSI) to detect anthracnose in fruits is that the healthy and diseased tissues have different biochemical composition and cellular structure which interact differently with the electromagnetic radiation. Anthracnose infection alters fruit water status, pigments, cell integrity, other metabolites and chlorophyll, causing changes in the extent to which the fruit absorbs, reflects and scatters light at various wavelengths. These differences are reflected in the way the light reflects off the living cells as a characteristic spectrum of light in a continuous waveband, so subtle changes due to the disease can be detected even prior to the clinical signs. The difference between the spectral signatures of healthy and infected fruit tissues yields a basis for distinguishing anthracnose infection, since each tissue type has its own unique spectral response. So, the major tenet of HSI is that the spectral reflectance within the plant changes from normal as a result of the disease, which can be measured in a non-destructive manner to detect anthracnose in an early stage (56).

Spectroscopic-based techniques have gained considerable attention for monitoring plant quality and detecting diseases due to their rapid, non-destructive and information-rich characteristics (57). Among these approaches, HSI combines spectroscopy and imaging techniques to generate three-dimensional hyperspectral data cubes, where each pixel contains both spatial coordinates and spectral information across multiple wavelengths. Unlike conventional spectroscopy, HSI provides spatial distribution information of biochemical and physiological changes within the sample, enabling the localisation and characterisation of disease-associated alterations (58). However, the large volume of spectral-spatial data generated by HSI requires efficient data processing and analytical approaches. Machine learning (ML) and image-processing techniques have therefore been increasingly integrated with HSI to improve classification accuracy, automate analysis and enhance the reliability of disease detection systems (59).

Hyperspectral imaging has been extensively applied for evaluating mango quality attributes and internal fruit characteristics due to its ability to capture subtle spectral variations associated with physiological and pathological changes (60, 61). In a study using visible and near-infrared (Vis/NIR) hyperspectral images (450–980 nm) acquired through a liquid crystal tunable filter (LCTF), different classification algorithms, including random forest (RF), extreme gradient boosting (XGB), quadratic discriminant analysis (QDA) and artificial neural network-based models such as simple perceptron (SLP) and multi-layer perceptron (MLP), were evaluated for disease detection. Among these approaches, neural network models successfully identified early anthracnose infection in mango fruits within 48 hr using both full-spectrum data and selected characteristic wavelengths (56). Similarly, an automated HSI-based system was developed for the rapid

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detection of citrus fruit rot caused by *Penicillium digitatum* using spectral data collected from 325–1100 nm, demonstrating the broader potential of HSI for post-harvest disease diagnosis (62). Beyond disease detection, HSI has also been utilised to predict mango physicochemical properties, including firmness, acidity and total soluble solids, highlighting its potential as a comprehensive tool for simultaneous quality evaluation and disease monitoring (63).

The integration of HSI with machine learning algorithms has further improved the detection and classification of anthracnose infections. Hyperspectral imaging combined with PCA and support vector machine (SVM) could differentiate anthracnose-infected mango fruits and classify disease severity (34). The spectral signatures obtained from HSI reflected biochemical changes associated with fungal infection, including metabolite accumulation and tissue degradation. Notably, the HSI-SVM approach enabled detection of anthracnose infection before the development of visible symptoms and generated symptom distribution maps for improved visualisation and interpretation of disease progression (34).

Recent advances have focused on developing automated and intelligent HSI-based detection systems. A Vis/NIR hyperspectral imaging system integrated with discriminant analysis was developed for early detection of mango anthracnose. The model extracted relevant spectral features from hyperspectral images of healthy and infected fruits and successfully classified anthracnose infection before visible symptom development, demonstrating the potential of HSI as a rapid and non-destructive diagnostic tool for post-harvest disease management (64). Furthermore, a three-dimensional convolutional neural network (3D-CNN) was incorporated with HSI to enhance early anthracnose detection in mango. Unlike conventional machine learning approaches, the 3D-CNN simultaneously extracted spatial and spectral features from healthy, asymptomatic and symptomatic fruits. The model achieved an overall classification accuracy of 95.24 % and was further interpreted using gradient-weighted class activation mapping (Grad-CAM) to identify important spectral regions and fruit areas contributing to disease prediction. These findings demonstrate the potential of deep learning-assisted HSI for accurate, rapid and interpretable anthracnose detection (65).

Near infrared spectroscopy

Principle: The principle of Vis/NIR spectroscopy for anthracnose detection is that the interaction of visible and NIR light is different for healthy fruit tissue and infected tissue. Absorption and/or reflection of Vis/NIR radiation on the surface of the fruit is wavelength dependent because of the fruit's chemical composition and physical structure. The anthracnose infection causes physiological and biochemical changes such as degradation of chlorophyll, cell wall degradation, moisture changes and changes in carbohydrates, proteins and other metabolites. These changes alter the optical parameters of the diseased tissue and cause different spectral signs from those of healthy tissue. The visible region is mostly sensitive to changes in pigments (e.g., chlorophyll and carotenoids) while the NIRS region is sensitive to the molecular vibrations related to the O–H, C–H and N–H bonds, which are associated with water, carbohydrates, proteins, etc. and other organic compounds. The disease can therefore be detected as small changes in the spectral reflectance even though no symptoms are visible, during the latent stage of the infection. The basic principle of Vis/NIR spectroscopy is that the differences in these spectra, caused by disease are good indicators for the early detection and are not destructive (64).

Near-infrared spectroscopy has been proposed as an alternative to traditional inspection of anthracnose disease. It is a technique that uses macro- and micro-compounds present in the product that absorb electromagnetic energy, the technique allowing the quantitative and qualitative prediction of the physicochemical quality (66). Near infra-red spectroscopy with wavelengths between 800 and 2500 nm has been recently used for detection of anthracnose at an early stage in fruits such as olive (58) and Kent mango (64) compared to mid-infra-red spectroscopy (MIR) with wavelengths in the range of 2500–15000 nm (67).

The use of Vis/NIR spectroscopy as a rapid and non-destructive method was investigated for the early detection of anthracnose in sugar mango (*M. indica*) (33). Spectral reflectance data were collected from both healthy and anthracnose-infected fruits across the visible and NIR wavelength range. To improve data quality, the spectra were pre-processed to reduce noise before being analysed using chemometric methods. Principal component analysis was employed for feature extraction, while SVM and artificial neural network (ANN) models were used to classify healthy and infected fruits. The infection caused subtle changes in the spectral signatures, allowing the models to distinguish diseased fruits from healthy ones even before visible symptoms developed. The findings highlighted the potential of combining Vis/NIR spectroscopy with machine learning as a fast, accurate and non-destructive approach for early anthracnose detection, with promising applications in postharvest quality monitoring and automated fruit sorting (33).

Similarly, the application of Vis/NIR spectroscopy integrated with both conventional machine learning and deep learning techniques to monitor the progression of *C. musae* infection in bananas (68). Spectral data obtained from healthy and infected fruits were analysed using three feature extraction methods-successive projections algorithm (SPA), competitive adaptive reweighted sampling (CARS) and principal component (PC) loading. These features were then classified using partial least squares discriminant analysis (PLS-DA), SVM and a one-dimensional convolutional neural network (1D-CNN). The developed

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four-class (control, acceptable, mouldy and highly mouldy) and five-class (control and days 1–4 after infection) models achieved impressive classification accuracies of 93.98 % and 94.47 % respectively. The high performance of these models was attributed to disease-induced alterations in the Vis/NIR spectra, including chlorophyll degradation, tissue browning, changes in moisture content, disruption of cellular structure and variations in biochemical components such as sugars, phenolic compounds and proteins. These spectral changes enabled the models to accurately detect anthracnose at an early stage, before obvious visual symptoms appeared, demonstrating the strong potential of Vis/NIR spectroscopy as a rapid and non-destructive technique for early disease detection and quality assessment in bananas (68).

Critical evaluation: Hyperspectral imaging and NIRS are promising non-destructive techniques for the early detection of anthracnose by identifying infection-induced biochemical and structural changes before visible symptoms appear. The HSI provides both spatial and spectral information, enabling highly accurate disease localisation and classification, whereas NIRS offers rapid, simple and cost-effective analysis based on spectral characteristics alone. However, HSI is limited by its high cost, complex instrumentation, large data volume and the need for advanced machine learning and controlled imaging conditions. In contrast, NIRS lacks spatial imaging capability and its performance can be affected by variations in fruit cultivar, maturity, moisture content and environmental conditions, requiring robust calibration models. Overall, while HSI offers superior diagnostic accuracy, NIRS is more practical for routine screening and further advancements in portable sensors and artificial intelligence are expected to enhance the commercial application of both techniques for anthracnose detection. Although conventional spectroscopy is rapid, inexpensive and non-destructive, it measures only average spectral information from the sample and lacks spatial information. Consequently, its ability to accurately localise anthracnose lesions is lower than hyperspectral imaging. The technique is also highly dependent on calibration models and controlled measurement conditions, which limits its robustness under commercial storage environments. Therefore, spectroscopy is best suited for rapid preliminary screening rather than definitive pathogen identification.

Surface plasmon resonance

Principle: Surface plasmon resonance is a label-free optical biosensing technique that enables the real-time detection of biomolecular interactions by measuring changes in the refractive index at the surface of a thin metal film, usually gold. When a target molecule binds to a complementary probe immobilised on the sensor surface, the resulting increase in biomolecular mass changes the local refractive index. This change causes a shift in the resonance angle or wavelength of the surface plasmons, which is detected by the SPR instrument. The magnitude of this shift is directly proportional to the amount of target analyte bound to the sensor surface, allowing rapid and highly sensitive detection without the need for PCR amplification or fluorescent labelling (36).

A label-free SPR biosensor was developed for the rapid detection of a necrotrophic DNA biomarker of *C. gloeosporioides* in harvested produce (36). The biosensor employed a complementary DNA probe immobilised on an optimised bimetallic plasmonic sensor to specifically recognise the pectatelyase gene, a necrotrophic marker associated with host tissue maceration and disease progression. Hybridisation of the target DNA with the immobilised probe increased the biomolecular mass on the sensor surface, generating a measurable SPR response that enabled highly specific pathogen detection. The biosensor showed a working range from picomolar (pM) to micromolar (µM), with a detection limit of 7 pM and a response time of less than 30 min at room temperature. Its high sensitivity, specificity, rapid detection capability and portability highlight its potential as a valuable tool for real-time field diagnosis and large-scale postharvest screening of anthracnose caused by *C. gloeosporioides* (36).

Critical evaluation: Surface plasmon resonance is a highly sensitive and specific biosensing technique for anthracnose detection because it directly identifies pathogen-specific DNA or biomolecular interactions in real time without the need for fluorescent or radioactive labels. Compared with conventional PCR, SPR provides faster analysis and enables label-free detection, while maintaining high sensitivity for detecting *Colletotrichum* spp. Unlike imaging techniques such as hyperspectral imaging, NIRS or E-nose systems, which detect indirect changes in fruit physiology, chemistry or volatile compounds, SPR confirms the presence of the pathogen itself, reducing the likelihood of false-positive results caused by environmental or physiological factors. However, SPR requires expensive instrumentation, specialised sensor chips, skilled personnel and careful sample preparation, limiting its routine use in field conditions and large-scale commercial screening. In contrast, optical and volatile-based sensing methods are more suitable for rapid, non-destructive, high-throughput inspection but generally provide lower pathogen specificity. Therefore, SPR is best suited as a highly accurate confirmatory diagnostic tool, while imaging and sensor-based techniques are more appropriate for preliminary screening. Future integration of SPR with portable biosensor platforms, microfluidics and nanotechnology could improve its affordability and expand its application in rapid post-harvest anthracnose diagnosis.

Electronic nose

Principle: The E-nose is a non-destructive sensing technology that mimics the human sense of smell by detecting volatile organic compounds (VOCs) emitted by fruits during normal physiological processes and disease development. The underlying principle is that

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fungal infection alters the host's metabolism, leading to the production of a unique VOC profile that differs from that of healthy fruit. These VOCs are detected by an array of non-specific gas sensors, commonly metal oxide semiconductor (MOS) sensors, which generate characteristic response patterns or "odour fingerprints". The resulting sensor signals are then analysed using multivariate statistical techniques, particularly PCA, to distinguish healthy fruits from infected ones and to detect disease before visible symptoms appear (69–71).

Based on this principle, an E-nose equipped with MOS sensors was evaluated for the detection of banana anthracnose caused by *C. musae*. The system successfully identified changes in the VOC profiles released by infected banana fruits and PCA clearly separated infected samples from healthy controls, demonstrating the effectiveness of the E-nose for rapid and early anthracnose diagnosis (37). Other studies have also shown that VOC fingerprinting can differentiate various stages of anthracnose infection, including the pre-symptomatic stage, highlighting the potential of E-nose technology for continuous disease monitoring. Although its application to mango anthracnose is still relatively limited, previous research has reported that mango fruits produce consistent VOC fingerprints that change with physiological and pathological conditions, indicating that the technology could also be useful for early disease detection in mango (72, 73). Compared with conventional analytical methods such as gas chromatography–mass spectrometry (GC–MS), E-nose systems offer a faster, highly portable and cost-effective approach for postharvest disease surveillance and large-scale fruit quality assessment (74).

Critical evaluation: Compared with optical techniques such as hyperspectral imaging, NIR spectroscopy and UV imaging, the E-nose offers a rapid, non-destructive and real-time approach for detecting anthracnose by analysing VOCs released during fungal infection. It requires minimal sample preparation, is relatively simple to operate and has the potential for portable, on-site monitoring. Unlike imaging techniques that rely on visible or spectral changes in the fruit, the E-nose can detect metabolic changes through VOC profiles, enabling the identification of infection at an early stage. However, the technique has limited specificity because VOC patterns can also be influenced by fruit cultivar, ripening stage, storage conditions, temperature, humidity and other microorganisms. In contrast, molecular methods such as PCR and biosensors such as SPR provide higher pathogen specificity by directly detecting fungal DNA but are more expensive, require specialised laboratory facilities and are less suitable for rapid field screening. Therefore, while the E-nose is highly valuable for fast, non-invasive screening of anthracnose, its performance is improved when combined with chemometric analysis or artificial intelligence and used alongside complementary diagnostic techniques to achieve greater accuracy and reliability.

Reflected ultraviolet

Principle: Reflected ultraviolet (RUV) imaging is a non-destructive optical technique that detects surface defects by exploiting differences in the reflection of UV light from healthy and diseased fruit tissues. When the fruit surface is illuminated with UV light, changes in tissue structure, pigmentation, moisture content and biochemical composition caused by anthracnose infection alter the reflected UV intensity and pattern. These variations are captured by a UV-sensitive camera as digital images and subsequently analysed using image-processing algorithms to distinguish healthy and infected tissues. Unlike conventional RGB imaging, RUV imaging can reveal subtle surface defects that are not readily visible to the naked eye, enabling early disease detection (38, 72).

A computer vision system (CVS) was developed for the early detection of anthracnose in mango (38). The system consisted of an image acquisition setup equipped with UV-A illumination and a UV-sensitive camera to capture reflected UV images from fruit surfaces. Image segmentation algorithms were then applied to distinguish diseased areas from healthy tissues. The developed system successfully identified small anthracnose lesions that were not easily visible under normal lighting conditions, highlighting its potential for automated and non-destructive fruit inspection (38). Further studies improved this approach by combining UV-A imaging with linear discriminant analysis (LDA), which enabled anthracnose detection one day earlier than conventional visual evaluation by experts. Similarly, another UV-based computer vision system achieved a classification accuracy of 94.69 % and effectively categorised mango fruits based on disease severity, showing better performance than traditional RGB imaging for postharvest disease evaluation (58, 59). Compared with hyperspectral imaging, reflected UV imaging offers a simpler and more economical approach for detecting surface-level lesions, as it requires only a UV illumination source, a UV-sensitive camera and suitable image-processing techniques, without the need for complex hyperspectral data acquisition and analysis. However, direct applications of reflected UV imaging for banana anthracnose detection have not yet been documented and further research is required to evaluate its effectiveness for identifying infections caused by *C. musae* (57).

Critical evaluation: Compared with conventional visual inspection, reflected UV can reveal lesions earlier and improve disease detection accuracy. However, its detection capability is mainly limited to surface or near-surface infections because UV light has poor penetration into fruit tissues. In comparison, hyperspectral imaging and NIR spectroscopy can detect deeper biochemical changes and latent infections with higher sensitivity, while molecular methods such as PCR and biosensor techniques such as SPR provide much greater pathogen specificity by directly detecting fungal DNA. Reflected UV is also influenced by fruit cultivar, maturity stage, peel characteristics, moisture

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and ambient lighting conditions, which may result in false-positive or false-negative detections. Although it is less expensive and easier to implement than hyperspectral imaging, it generally provides lower specificity and diagnostic accuracy. Therefore, reflected UV is most useful as a rapid preliminary screening tool in packing houses or storage facilities, whereas more advanced spectral or molecular techniques are required for confirmatory diagnosis and reliable early detection of anthracnose.

Biomarkers

Principle: Biomarker-based detection involves the identification of pathogen-specific or host-induced molecular, biochemical, physiological and defence-related change associated with *Colletotrichum* infection. These biomarkers include alterations in gene expression, proteins, enzymes, metabolites and VOCs, which occur during the early stages of host–pathogen interaction. Since these molecular responses are initiated before visible symptom development, biomarker-based approaches provide a sensitive and reliable means for the early diagnosis of anthracnose while also offering insights into the underlying mechanisms of plant defence (39).

One of the promising host-derived biomarkers for banana anthracnose detection is phospholipase C (PLC)**,** an important signalling enzyme involved in plant defence responses. Unlike pathogen-based detection methods, this approach relies on identifying changes in the host's defence signalling mechanisms after infection. During infection by *C. musae*, PLC hydrolyses phosphatidylinositol 4,5-bisphosphate, generating the secondary messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). These signalling molecules promote intracellular calcium release and activate several downstream defense responses, including reactive oxygen species (ROS) accumulation, defence-related gene activation and synthesis of antimicrobial compounds. Since these biochemical responses are initiated before the appearance of visible symptoms, changes in PLC activity and its gene expression pattern can serve as early indicators of anthracnose infection (39).

The involvement of PLC in banana defence responses against anthracnose was investigated by inoculating banana fruits with *C. musae* and analysing physiological and molecular changes during disease development (39). The study examined fruit firmness, membrane permeability, PLC activity, levels of DAG and phosphatidic acid (PA), along with the expression patterns of *MaPLC-1* and *MaPLC-2* genes during infection. Compared with healthy fruits, infected bananas showed rapid fruit softening, increased membrane permeability, increased PLC activity, higher accumulation of DAG and PA and significant upregulation of *MaPLC-1* and *MaPLC-2* transcripts. These results confirmed that activation of the PLC-mediated signalling pathway is closely associated with the banana defence response and suggested its potential application as an early biomarker for anthracnose detection (39).

In addition to host defence-related biomarkers, metabolite-based biomarkers have also been investigated for anthracnose diagnosis. Gas chromatography–mass spectrometry-based metabolomic analysis of mango fruits revealed several disease-associated VOCs, including thujol, which was detected only in *Colletotrichum*-infected fruits and increased ethanol levels in diseased samples. These volatile compounds enabled differentiation between healthy and infected fruits; however, the reported cross-validation accuracy of 67–75 % suggested lower diagnostic reliability compared with molecular and spectroscopic approaches. Nevertheless, VOC profiling provides a promising complementary strategy by offering valuable information on metabolic changes associated with anthracnose development (75).

Critical evaluation: Biomarker-based detection methods offer high specificity and sensitivity for anthracnose diagnosis by identifying pathogen-specific DNA, RNA, proteins, metabolites or volatile biomarkers associated with *Colletotrichum* infection. Unlike conventional visual inspection and imaging techniques, which rely on disease-induced physiological or optical changes, biomarker-based approaches can detect the pathogen during the latent stage before visible symptoms develop, improving diagnostic accuracy. Molecular biomarker methods, including PCR, qPCR, LAMP and biosensors such as SPR, provide direct evidence of pathogen presence and reduce false-positive results. However, these techniques generally require specialised laboratory equipment, skilled personnel and sample preparation, making them more expensive and less suitable for routine, high-throughput screening than non-destructive methods such as hyperspectral imaging, NIR spectroscopy, E-nose or computer vision. In contrast, optical and sensor-based techniques enable rapid, non-invasive assessment of large numbers of fruits but may be influenced by cultivar, fruit maturity, storage conditions and environmental factors, resulting in lower pathogen specificity. Therefore, biomarker-based methods are best suited for confirmatory diagnosis and precise pathogen identification, while imaging and sensing technologies are more appropriate for rapid preliminary screening. Future integration of biomarker detection with portable biosensors, microfluidics and artificial intelligence is expected to improve the speed, affordability and field applicability of anthracnose diagnosis.

Advantages and limitations of detection methods

Each detection method offers unique advantages and has certain limitations that influence its suitability for anthracnose diagnosis. Table 6 summarises the advantages and limitations of the different detection techniques.

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Fig. 4 illustrates an overview of advanced detection technologies for anthracnose disease in mango and banana. Healthy, latent (asymptomatic) and symptomatic fruits are evaluated using various advanced approaches, including hyperspectral imaging, biosensors, NIR spectroscopy, SPR, LAMP and light spectrum-based sensing. The acquired data are further processed and analysed using machine learning approaches to enable rapid, accurate and early detection of anthracnose.

Comparative analysis of advanced detection methods over traditional methods

Traditional detection approaches, such as visual examination, morphological identification and culture-based pathogen isolation, continue to be commonly used for anthracnose diagnosis due to their simplicity, affordability and limited requirement for advanced equipment. However, these methods have several limitations, including lengthy detection periods, dependence on visible symptom development and the need for expert interpretation. As a result, they often fail to identify latent or asymptomatic infections, which can contribute to delayed management practices and increased post-harvest losses. In comparison, molecular techniques such as PCR, qPCR, LAMP, SCAR markers and biosensor-based methods provide rapid, sensitive and pathogen-specific detection by targeting pathogen nucleic acids or disease-associated biomarkers. These approaches enable early identification of infections before symptom appearance; however, their practical application is often restricted by the requirement for specialised laboratory facilities, trained personnel and higher operational costs.

Table 6. Advantages and limitations of different detection methods for anthracnose

Sl. No.	Detection techniques	Advantages	Limitations
1.	Traditional	Traditional detection is cheap and accessible because it relies on symptom observation, routine sterilisation, plating and microscopy rather than specialised imaging or multigene sequencing (12).	Misses latent infection Low species resolution Requires expertise Slow, labor-intensive and frequently lack sufficient sensitivity. Moreover, these techniques usually miss infections in the early stages, when quick action could reduce disease spread (54).
2.	Molecular detection methods	Detect anthracnose before visible symptoms, fast (45).	Requirement of skilled personnel, risk of contamination, sample quality dependence (76).
3.	Serological techniques	Species-specific, rapid (55).	Availability of highly specific antibodies is less (55).
4.	Spectroscopic analysis	Spectroscopy is non-destructive and rapid method of detection (34).	Performance often depends on controlled experiments with artificial inoculation, which do not fully represent commercial infection biology (77).
	Hyperspectral imaging	Combines spectral and spatial information for early detection and mapping (34).	Data are redundant and processing is complex (34).
	Near infrared	Fast, easy, lower cost than many alternatives (64).	Sensitivity to environmental conditions, high equipment cost (78).
5.	Surface Plasmon resonance	Fast turnaround High sensitivity Portable potential Scalable screening (36).	SPR has an intrinsic sensing-depth limit of about 300 nm, which reduces direct detection efficiency for large microbial targets (79).
6.	E-nose	Rapid, non-invasive, portable screening of VOC fingerprints, with real-time or near-real-time operation and low-cost potential (37).	Sensor selectivity, drift, atmospheric interference and open-field deployment remain unresolved (37).
7.	Reflected UV	Non-destructive, early disease detection, rapid and real-time analysis, no chemical reagents required (80).	Limited penetration depth, affected by fruit characteristics, requires controlled illumination, lower specificity, equipment cost (80).
8.	Biomarkers	It captures host response to infection, not just visible symptoms. It offers several candidate markers at once, including biochemical and gene-expression readouts plus conductivity and firmness changes. It appears especially relevant to peel, since (39).	Not a detection validation High cost Relies on environmental conditions, which may affect the reproducibility and reliability of detection.

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Biomarker analysis also requires laboratory-based molecular techniques, specialised equipment and skilled personnel, limiting its applicability for rapid, on-site and large-scale postharvest screening (39).

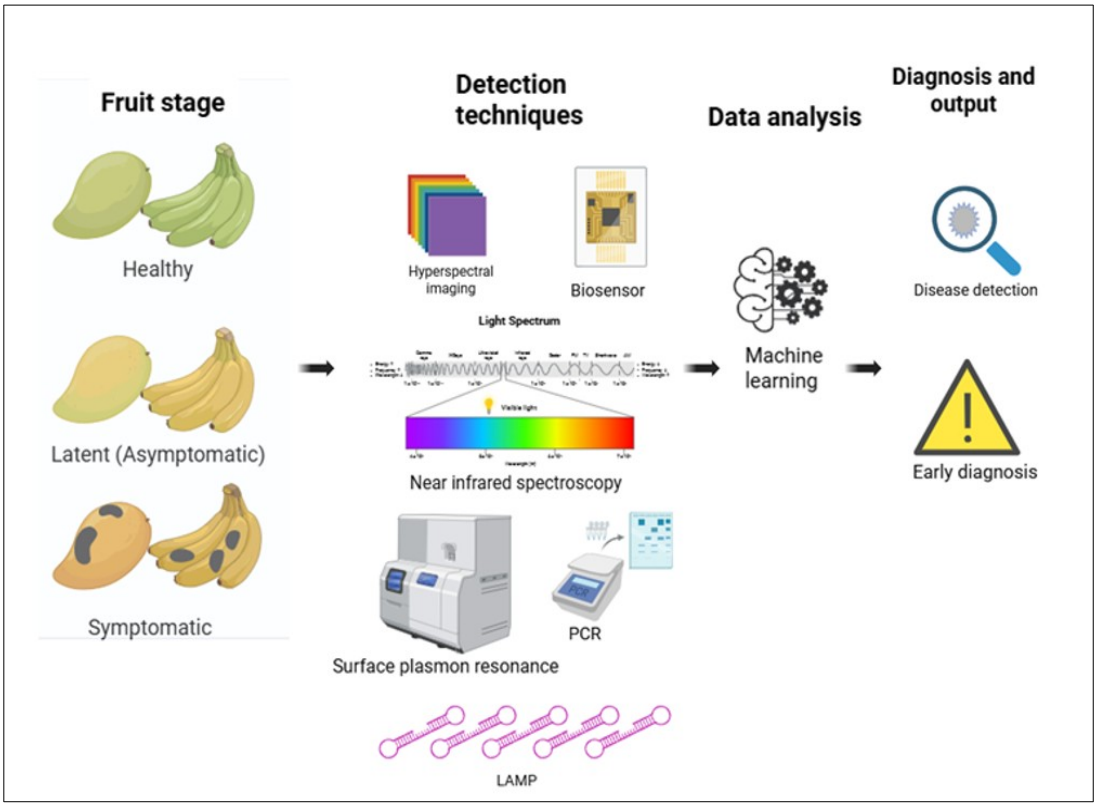

Fig. 4. Integrated workflow for anthracnose detection using molecular, spectroscopic, biosensor and artificial intelligence-based approaches.

Recent advancements in non-destructive detection technologies, including hyperspectral and multispectral imaging, Vis/NIR and NIR spectroscopy, reflected UV imaging, E-nose and biomarkers have provided promising alternatives for rapid anthracnose assessment. These techniques detect disease-associated changes in fruit physiology, biochemical composition and spectral properties without damaging the produce. When combined with chemometric tools, machine learning or deep learning algorithms, they allow rapid screening of large numbers of fruits and facilitate early disease detection, making them suitable for automated post-harvest quality monitoring. However, their accuracy can be influenced by factors such as fruit variety, maturity stage, storage environment and field conditions. Moreover, successful implementation requires reliable calibration models, standardised operating procedures and relatively costly imaging or sensing equipment.

Overall, molecular techniques remain highly valuable for accurate and species-specific confirmation of anthracnose infection, whereas imaging and spectroscopy-based approaches offer greater potential for rapid, non-destructive screening at commercial scales. Therefore, the integration of traditional methods with molecular diagnostics and sensor-based technologies may provide a more reliable and comprehensive strategy for early anthracnose detection and effective disease management in mango and banana production systems.

3. Conclusion

Anthracnose, caused by *Colletotrichum* spp., continues to be one of the major post-harvest diseases affecting mango and banana, leading to considerable economic losses through deterioration of fruit quality, reduced shelf life and decreased market value. This review summarises the recent advances in anthracnose detection, covering the transition from conventional culture-based and morphological identification methods to advanced approaches involving molecular diagnostics, biosensors, hyperspectral imaging, spectroscopy, E-nose technology, computer vision and artificial intelligence-based techniques. Compared with traditional methods, these advanced technologies provide improved sensitivity, specificity and the ability to detect infections at early or latent stages before the development of visible symptoms, thereby supporting timely disease management and reducing postharvest losses. However, no single detection technique can fulfill all requirements of accuracy, rapidity, affordability, portability and ease of application. Therefore, combining

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complementary approaches, including molecular, imaging, spectroscopic and biosensor-based technologies, offers a more effective and reliable strategy for anthracnose detection and management in mango and banana production systems.

Future prospects

Future research should focus on developing rapid, non-destructive, highly sensitive and cost-effective diagnostic tools capable of detecting anthracnose during the latent stage under commercial storage and field conditions. Greater emphasis should be placed on integrating artificial intelligence, machine learning and deep learning with imaging and spectroscopic technologies to enhance diagnostic accuracy, automation and real-time decision-making. The identification and validation of reliable host- and pathogen-derived biomarkers through genomics, transcriptomics, proteomics, metabolomics and other multi-omics approaches will facilitate the development of portable biosensors and precision diagnostic platforms. Emerging technologies such as lab-on-a-chip devices, microfluidics, nanotechnology-based biosensors and surface plasmon resonance systems should be further explored for rapid, on-site detection with minimal sample preparation. In addition, the establishment of standardised spectral libraries, annotated image databases and biomarker repositories across diverse cultivars, environmental conditions and *Colletotrichum* species will improve the robustness and reproducibility of diagnostic models. Future efforts should also focus on developing multimodal detection platforms that integrate molecular diagnostics, spectroscopy, hyperspectral imaging, E-nose technology, computer vision and biosensors to improve detection accuracy and reliability. Translating these technologies into portable, affordable and user-friendly devices suitable for commercial packing houses, storage facilities and field applications will be essential for minimising post-harvest losses and supporting sustainable fruit production.

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Authors' contributions

MTM was responsible for conceptualisation, writing the original draft, data curation, formal analysis, visualisation, figure preparation and methodology. IN was responsible for supervision, conceptualisation, writing, review and editing, methodology and validation. KA was responsible for investigation, data curation, writing the original draft, providing resources and formal analysis. AJ was responsible for investigation, data curation, writing, review and editing and visualisation. SN was responsible for methodology, writing, review and editing, formal analysis, software and data curation. RE was responsible for investigation, writing, review and editing, providing resources and data curation. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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During the preparation of this work, the authors used Grammarly to improve the language and readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publications' content.

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