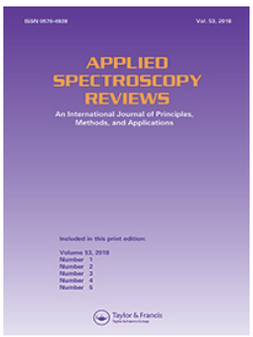

Applied Spectroscopy Reviews

ISSN: 0570-4928 (Print) 1520-569X (Online) Journal homepage: https://www.tandfonline.com/loi/laps20

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To cite this article: Alfadhl Yahya Khaled, Samsuzana Abd Aziz, Siti Khairunniza Bejo, Nazmi Mat Nawi, Idris Abu Seman & Daniel Iroemeha Onwude (2018) Early detection of diseases in plant tissue using spectroscopy – applications and limitations, Applied Spectroscopy Reviews, 53:1, 36-64, DOI: 10.1080/05704928.2017.1352510

To link to this article: https://doi.org/10.1080/05704928.2017.1352510

Published online: 08 Aug 2017.

APPLIED SPECTROSCOPY REVIEWS
2018, VOL. 53, NO. 1, 36–64
https://doi.org/10.1080/05704928.2017.1352510

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Early detection of diseases in plant tissue using spectroscopy – applications and limitations

Alfadhl Yahya Khaled[a], Samsuzana Abd Aziz [a,b], Siti Khairunniza Bejo [a,b], Nazmi Mat Nawi[a,b], Idris Abu Seman[c], and Daniel Iroemeha Onwude[a]

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ABSTRACT

Plant diseases can greatly affect the total production of food and agricultural materials, which may lead to high amount of losses in terms of quality, quantity and also in economic sense. To reduce the losses due to plant diseases, early diseases detection either based on a visual inspection or laboratory tests are widely employed. However, these techniques are labor-intensive and time consuming. In a view to overcome the shortcoming of these conventional approaches, several researchers have developed non-invasive techniques. Recently, spectroscopy technique has become one of the most available non-invasive methods utilized in detecting plant diseases. However, most of the studies on the application of this novel technology are still in the experimental stages, and are carried out in isolation with no comprehensive information on the most suitable approach. This problem could affect the advancement and commercialization of spectroscopy technology in early plant disease detection. Here, we review the applications and limitations of spectroscopy techniques (visible/infrared, electrical impedance and fluorescence spectroscopy) in early detection of plant disease. Particular emphasis was given to different spectral level, challenges and future outlook.

KEYWORDS

Plant diseases; spectroscopy techniques; VIS/IR spectroscopy; impedance spectroscopy; fluorescence spectroscopy

Introduction

Plants are the main sources of foods for human around the world. Plants are also useful in creating a balance between human and the environment (1, 2). However, during cultivation, plants can be affected by different kinds of diseases. These diseases could affect the production yield of plant fruits, and reduce the total bulk of available plants for human utilization, hence, reduction in the economic value in terms of quantity and quality (3, 4).

To date, a number of studies have reported more than 50,000 parasitic and non-parasitic plant diseases are available all over the world (5). Parasitic diseases are all the disorders that occur in plants as a result of severe attacks by an organism known as parasite (6). While

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non-parasitic diseases are physiological diseases that arise as a result of imbalance in the physiology of the vital plant parts due to factors such as inappropriate plant growth and environmental conditions (7, 8). These diseases cause a great deal of damage and are characterized by wilting, scabs, mold coatings, rusts, blotches and rotted tissue (9). The most common form of plant diseases include anthracnose (10), apple scab (11), bacterial canker (12), blossom (*Monilinia laxa*) (13), end rot (14), brown rot (*Monilinia fructicola*) (15), cedar apple rust (*Gymnosporangium juniperi-virginianae*) (16), club root (17), corn smut (*Ustilago maydis*) (18), crown gall (*Agrobacterium tumefaciens*) (19), damping off (20), downy mildew (21), early blight (*Alternaria solani*) (22), fire blight (23), fusarium wilt (*oxysporum f. sp. Cucumerinum J. H. Owen*) (24), gray mold (*Botrytis cinerea*) (25), late blight (*Phytophthora infestans*) (26), leaf curl (27), leaf spot (28), mosaic virus (29), potato scab (30), grapevine leaf-roll (*Grapevine leafroll-associated virus*) (31), citrus variegated chlorosis (CVC) (32), Fiji leaf gall (33), powdery mildew (*Erysiphe necator*) (34), wheat rust (*Puccinia striiformis f. sp. tritici*) (35), huanglongbing (HLB) (36), basal stem rot (BSR) (37) and verticillium wilt (38).

Major production and economic losses caused by these plant diseases in agricultural and food sectors have been reported to be over 40% of the total production losses in most developing countries (39). For instance, Sharma (39) also reported about USD 622,805 losses per year resulting from wheat rust (*P. striiformis* f. sp. *tritici*) in India. In fact, this figure reaches up to USD 7.79 million during the years of *epiphytotics* plant disease. Similarly, BSR disease caused by *Ganoderma boninese* was reported to cause immense damage to most oil palm plantations in Malaysia each year, with yield losses up to 80% in the infected areas (40). Generally, high percentage of losses is incurred during agricultural production due to the effect of plant diseases. These losses can be reduced or eradicated by early detection, monitoring and management of these plant diseases.

Several methods ranging from conventional to advance techniques have been used in early detection of plant diseases (41). These methods includes: visual inspection, laboratory tests and non-invasive techniques as illustrated in Figure 1. Visual inspection involves the identification of affected plants based on the appearance of pathological symptoms. For example, *Ganoderma* disease on oil palm trees can be identified based on fungus fruiting bodies on affected trees. This approach can detect the disease distribution within a wide range of the field (42). However, this method is labor-intensive, time consuming, inefficient

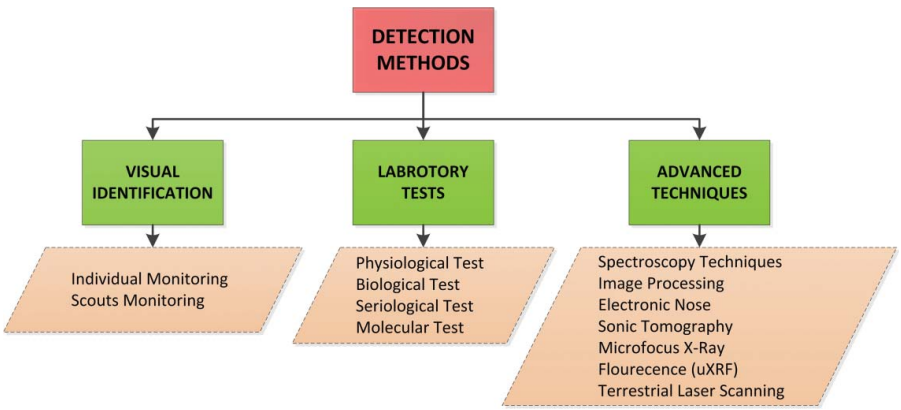

Figure 1. Different methods of plant disease detection.

and expensive in the early stages of infection (43–47). On the other hand, laboratory based methods used in the detection of plant diseases include: physiological, biological, serological and molecular tests (48–51). However, the most common of the laboratory tests are serological test, such as enzyme-linked immunosorbent assay (ELISA), based on the use of protein in the detection of causative diseases, and also molecular test, such as polymerase chain reaction (PCR) used in detecting plant diseases based on DNA sequence of the pathogen (52, 53). The DNA-based sequencing involves the identification of nucleotide within the plant DNA molecule in a precise order thereby enabling the detection of diseases before any visual symptoms appear. This biological method of plant disease detection is widely applied in identifying the severity level of different plant diseases, such as bacteria, fungi and genetically modified organisms. A typical DNA based sensor, which operates based on specific nucleic acid hybridization of the immobilized DNA probe can be used as a reliable and accurate testing device for plant genetics and disease detection (51, 54, 55). Nonetheless, there are limitations to the application of these techniques in early detection, control and management of plant diseases. This is due to the complexity of the methods and the time required (56, 57). In addition, these techniques are expensive and lack rapidity for the detection of plant diseases.

In view of the above drawback, non-invasive techniques have gained much attention in recent years as reported in the literature (41, 45, 58–61). These techniques, such as terrestrial laser scanning (46), image processing (62), electronic nose (63), sonic tomography (49), microfocus X-ray fluorescence (uXRF) (64), GanoSken technology (65), and spectroscopy have recently been applied in detecting plant diseases (58). Nevertheless, most of these techniques have numerous limitations. Some of the drawback to the application of these techniques in detecting plant diseases include (i) cumbersome process, (ii) long setup process, (iii) high-cost, (iv) sensitive to the change in environment condition and (v) low selectivity and high specific software requirement (51, 66). However, the advantages of spectroscopy over other novel techniques can be attributed to simplicity, rapidity and affordability. Therefore, the application of spectroscopy technique in the detection of plant diseases becomes indispensable.

Spectroscopy techniques are widely differentiated into molecular and atomic based on their mode of application (67, 68). Besides, they can be classified based on the nature of their interaction. Example of molecular spectroscopy include visible (VIS), infrared (IR), nuclear magnetic resonance (NMR), mass spectroscopy (MS) and electrical impedance (EI). On the other hand, the atomic spectroscopy includes fluorescence spectroscopy (FS). Most of these spectroscopy techniques have been widely applied for plant disease detection (59, 69–72). For example, VIS/IR spectroscopy was used to detect olive leaf spot (OLS) disease in olive trees (73). On the other hand, several studies reported that IR spectroscopy is used for identifying the disorders that affect the molecular structures and properties of plants (58, 74, 75). FS is stated as a tool to evaluate and determine the plant diseases at early stage (76–79). More so, EI technique has been used as a promising approach for plant disease detection (80, 81). Taken together, many researchers have justified the potential of molecular and atomic spectroscopy techniques for analyzing and monitoring the changes in the quality of agricultural and food products, and most importantly in detecting plant diseases at the early stage.

Despite the advantages associated with the application of spectroscopy technique in plant disease detection, most of the studies are carried out in isolation with no comprehensive

information on the most effective type of spectroscopy technique, and applied advanced statistical approach. These drawbacks limit the use of spectroscopy technique in detecting plant disease both in small and large scale applications. Thus, this paper attempts to make an assessment of the application and limitation of spectroscopy techniques (VIS/IR, EI and F) spectroscopy in plant disease detection.

Spectroscopy techniques for detection plant diseases

The rapid developments in advanced agricultural technologies have increased the demand for the application of non-invasive technique in detecting plant diseases. From the literature reviewed, most spectroscopy application in detecting plant diseases fit these criteria; rapid, non-invasive and specific to a particular type of disease whereby, the sensitivity for detection at the early stage of the infection are taken into consideration in the design and development of the said non-invasive technique (82, 83).

Spectroscopy is the study of the interaction of electromagnetic waves, including ultraviolet, visible and infrared spectra with matter, as illustrated in Figure 2 (84–86). Spectroscopy data is often described by an emission or absorption spectrum, and the result can be illustrated as a function of wavelength or frequency. Presently, several studies have reported the design, development and application of different spectroscopy techniques as an effective and practical tool for large-scale real-time plant disease detection under field conditions (45, 58, 66, 73, 76, 87–91).

Visible and infrared spectroscopy

Visible and infrared (VIS/IR) spectroscopy (400–100,000 nm) is one of the most promising non-invasive techniques which has obtained extensive acceptance in many areas due to its advantages over other analytical techniques. It is an effective approach to reveal quality of agro-products (93). Much of the current literature on VIS/IR pays particular attention to determine the quality of agricultural products and few publications have addressed the use

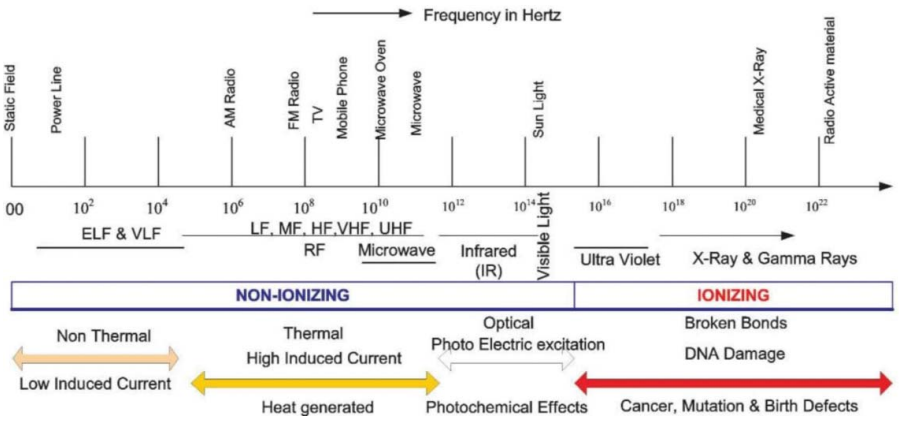

Figure 2. Infrared, visible and ultraviolet allocation in the electromagnetic spectrum. ELF: extremely low frequency, VLF: very low frequency, LF: low frequency, MF: medium frequency, HF: high frequency, VHF: very high frequency, UHF: ultra-high frequency and RF: radio frequency (92).

of this technique for detecting plant diseases (73). However, among many available disease detection techniques, a rapid and non-invasive method like VIS/IR spectroscopy is preferable.

Spectral reflectance analyses have proven to be very useful in detecting plant stress due to changes in the absorption of incident light in the VIS and IR range of the electromagnetic spectrum (94–99). In addition, the influence of the pathological status of a crop on its spectral characteristics can be visible or detectable in the VIS and/or the IR regions of the electromagnetic spectrum (100, 101). Several studies have used VIS/IR spectroscopy technique for detecting and monitoring plant diseases at early stage. These diseases included yellow rust in wheat, spot in wheat, parley and olive leaves, BSR disease in oil palm trees, HLB and CVC disease in citrus, verticillium wilt in cotton, leaf-roll in grape (*Grapevine leafroll-associated virus*), scab in apple, Fiji leaf gall in sugarcane, powdery mildew (*E. necator*) in wheat, pathogen in tomato, fungal infection in corn, crown rot in tomato and leaf folder infestation in rice (102–105).

Normally, VIS and IR spectroscopy system consists of four components, namely light source, light-isolating mechanisms, detector and sampling devices (106). The data acquisition using VIS and IR techniques depends on the type of mode used. There are 3 different modes of data acquisition, namely reflectance, interactance and transmittance mode. The main difference between these modes is the location of the light source and detector as illustrated in Figure 3. In reflectance mode, the detector is situated at the same side of the light source, in interactance mode the detector and the light source are positioned parallel to each other, while in the transmittance mode, the detector is located at the opposite side of the illumination (107). In reflectance mode, the wavelength is wider compared to the other modes, while in transmission mode the wavelength is narrowest. Figure 4 shows the different wavelength in the 3 modes whereby the vertical transmission scale is only approximate and the vertical interactance axis shows raw intensity on an arbitrary scale (106).

Based on the wavelength of VIS/IR spectroscopy technique in plant disease detection 4 different VIS/IR regions have been identified. They are visible, near-infrared (NIR), mid-infrared (MIR) and far-infrared (FIR) regions. In order to effectively apply VIS/IR spectroscopy in detecting plant diseases, the ideal wavelength must be known. This is because the wavelength in VIS/IR can be changed in response to many factors such as nutrients,

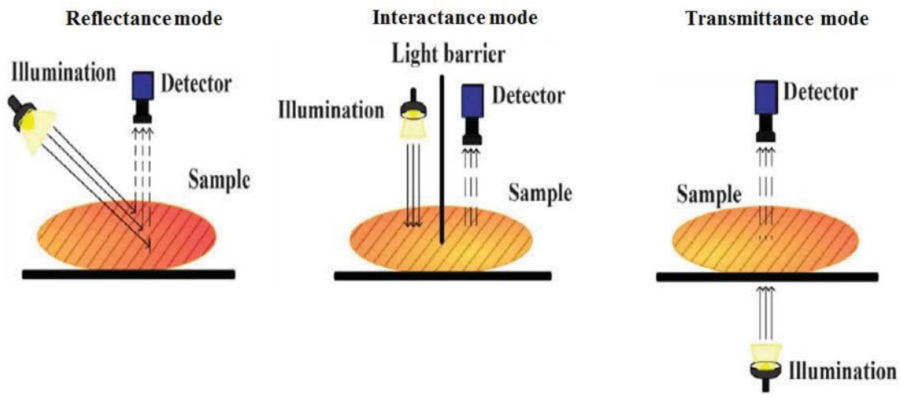

Figure 3. Mode of data acquisition in the VIS/IR system (106).

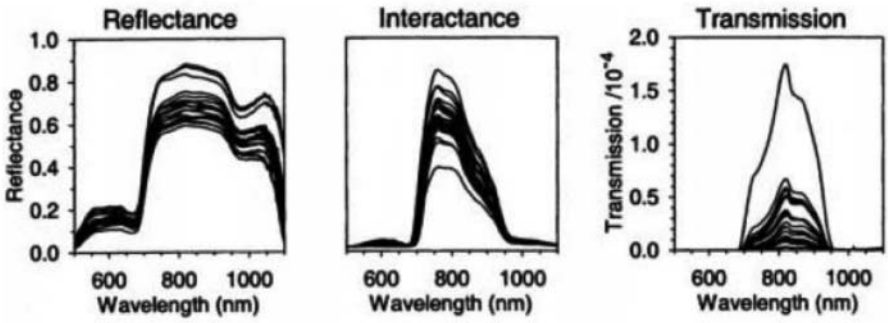

Figure 4. Typical reflectance, interactance and transmission for VIS/NIR spectra. The reflectance spectra range from 500 to 1100 nm (107).

temperature, color and water activity in plants (45, 66). According to the American Society of Testing and Materials (ASTM), the spectrum for VIS covers the wavelength range of 400–750 nm, NIR of 780–2500 nm, MIR of 2500–25,000 nm and FIR of 25,000–100,000 nm. VIS spectrum has blue-green band region (400–650 nm), which has been used to report differences between healthy and affected tissues in plants (51, 77). NIR consist of 2 important regions, short-wavelength (SW-NIR) (750–1300 nm) and long-wavelength (LW-NIR) (1300–2500 nm) where every region is associated with special content in the plants (90). MIR consists of 2 regions including water absorbance region and carbohydrate region with wavelength of 2500–6500 nm and 8500–11,500 nm, respectively (102, 108, 109).

Overall, the reflectance properties of individual leaf depend on the interaction of pigment content, leaf structures and water content with electromagnetic radiation (EMR) (110, 111). In VIS spectrum, the reflectance of leaf is low because of the absorption by photosynthetic pigments mainly chlorophylls and carotenoids. Similarly, SW-NIR region has no strong absorption features whereby the magnitude of reflectance is governed by structural discontinuities encountered in the leaf. Schaare and Fraser (107) studied the response of wavelength of VIS and SW-NIR (450–1150 nm) at the 3 different modes (reflectance, interactance and transmittance). It was found that the reflectance at VIS was weak and the reflectance increased at the SW-NIR as illustrated in Figure 4. However, LW-NIR region presents variable-reflectance values mainly linked to the absorption characteristics of water and other compounds (100, 112). MIR region of the spectrum has also been applied to detect changes in infected plants, when comparing to healthy or non-infected plants (108).

The changes in VIS/IR reflectance spectra due to the plant diseases and pathogens can be clarified by impairments and the variation of chemical composition inside the affected tissue that can be seen, such as the appearance of typical fungal structures like powdery mildew hyphae. Also, changes in MIR spectra can be attributed to water and carbohydrate vibrational bands present in the leaves include various sugar, starches and cellulose. In addition, plant diseases produce fungal and bacterial structures in the leaves surface, which can influence spectra characteristics of the plant-pathogen interaction. Thus, the measured patterns enable the identification of diseases based on the spectral properties of plants.

Previous works have described the application of IR spectroscopy in detecting amino groups (113–115). The amines compounds can be characterized by absorption bands between 3300 and 3500 cm^{-1}. The results of studies in the scientific literature have shown

that measurements of bandwidth, frequency and intensity permit much greater refinement in the structural identification of these compounds, because the OH stretching vibration in monomeric alcohols. In 2007, Schulz and Barunska (115) investigated the amino acids in the plant material using IR spectroscopy. It was found that amino acid structure provides helpful information for a reliable interpretation of the registered IR spectra. Consequently, the vibrational bands corresponding to amine groups can be useful information and reference in detecting the plant disease in plant.

Application of VIS/IR on plant disease detection

Typically, the quantitative and qualitative analyses of VIS/IR spectroscopy data require the application of multivariate calibration algorithms and statistical methods in order to model VIS/IR spectral response with chemical or physical reference sample parameters. VIS/IR spectral data needs careful attention because the absorption bands are typically wide, extensively interfered and weak. The available instruments of spectroscopy normally have high resolution, with spectral data, which can be thousands of variables having noises generated from the instrument and/or environment. Therefore, very specific methods such as partial least squares (PLS), principle component regression (PCR) and multiple linear regressions (MLR) can be used to treat the VIS/IR large data and also extract relevant information (116, 117).

Several studies have reported significant findings on the use of VIS/IR method in plant disease detection as shown in Table 1. Abu-Khalaf and Salman (118) investigated the feasibility of using of VIS/NIR spectroscopy on sensing tomato's pathogen. It was demonstrated that the lowest classification rates using VIS/NIR spectroscopy were 90%, 80% and 74% for pathogens, antagonistic and their combinations, respectively. Liaghat et al. (58) investigated mid-infrared spectroscopy for early detection of BSR disease in oil palm. In their study, the overall accuracy was found to be 92% using linear discriminant analysis (LDA) classification model. In 2011, Sankaran et al. (103) applied VIS/NIR spectroscopy for detection of HLB in citrus orchards. The results shown that quadratic discriminant analysis (QDA) classified between healthy and symptomatic HLB-infected leaves with accuracy higher than 90%, also 88% accuracy was found when asymptomatic leaves were included in the classification. Similarly, Hawkins et al. (102) studied the effects of IR spectroscopy to detect the HLB disease in its earlier pre-symptomatic stages. It was reported that this method can be a substitute method of a PCR test, as it took minutes rather than hours to measure a sample with accuracy of 95%. In addition, Chen et al. (119) applied VIS/IR spectroscopy for revealing and evaluating verticillium wilt in cotton. It was found that the wavelength range of 731–1317 nm shown the maximum determination coefficient of 74%. Naidu et al. (78) examined the feasibility of applying VIS/NIR spectroscopy for the detection of grapevine leaf-roll (*Grapevine leafroll-associated virus*) disease. It was observed that the accuracy of the classification ranged from 73% to 81% relying on characteristics (vegetative indices) used for healthy leaves and detecting infected either symptomatic or asymptomatic. In 2012, Cardinali et al. (104) applied IR spectroscopy (2500–1428 nm) as a potential tool to detect the CVC disease. It was highlighted that the accuracy found greater than 90%. Kos et al. (87) studied the effect of MIR spectroscopy on detection of fungal infection on corn. It was depicted that two clusters made up of contaminated and blank kernels were obviously distinguishable by principle component analysis (PCA) on ATR averaged spectra with accuracy of 79%. Xu et al. (90) tested the efficacy of NIR spectroscopy to diagnose miner disease in

Table 1. Previous studies on plant disease detection using visible/infrared spectroscopy technique.

Plant	Disease/ pathogen	Infrared measurement	Wavelength (nm)	Sample grouping	Equipment	Statistical approach	Significant results	References
Spinach leaves	Bacterial contamination	VIS/NIR spectroscopy	456–950	Healthy Contaminated	A line-scan push-broom hyperspectral imaging system	Partial Least Squared-Discrimination Analysis (PLS-DA)	The accuracy was found more than 84%	(75)
Wheat and parley fields	Spot disease	VIS/NIR spectroscopy	360–900	Severity and Density samples	Spectroradiometer	Nearest neighbor classifier (kNN)	The accuracy is higher than 91.9%	(101)
Wheat	Yellow rust	VIS/ NIR spectroscopy	350–2500	Healthy and 1, 10, 20, 30, 45, 60, 80 and 100% covered by rust	Pushbroom Hyperspectral Imager (PHI)	Regression analysis	The R^2 was found to be higher than 90%	(120)
Olive leaves	OLS	VIS/NIR spectroscopy	350–2500	Healthy leaves OLS-infected leaves (1 (1 lesion), 2 (2 lesions), 3 (3–5 lesions), 4 (6–10 lesions) or 5 ($>$ 11)	Fiber optic spectrometer	PLS-DA, SVM	The overall classification accuracy is 86%	(73, 118)
Tomato	Fusarium wilt Root and crown rot Gray mold	VIS/SW-NIR spectroscopy	550–1100	Control samples Samples inoculated with fungus Samples inoculated with bacterium	Fiber optic spectrometer	PCA, SVM	The classification accuracy were found more than 85%	(59)
Wheat	Stripe rust pathogen Leaf rust pathogen	NIR spectroscopy	1000–2500	—	FT-NIR MPA spectrometer	Discriminant-PLS, Quadratic-PLS	The accuracy were found more than 90%	(121)
Oil palm leaves	BSR	MIR spectroscopy	2550–25,000	Healthy Mild Moderate Severe	FT-IR spectrometer	PCA, LDA, QDA, kNN, Naive-Bayes (NB)	The highest overall classification accuracy is 92% based on LDA-based model resulted	(58)

(*Continued on next page*)

Table 1. (*Continued*)

Plant	Disease/ pathogen	Infrared measurement	Wavelength (nm)	Sample grouping	Equipment	Statistical approach	Significant results	References
Citrus	HLB (greening)	VIS/NIR spectroscopy	350–2500	Healthy leaves Diseased leaves	SVC HR-1024 spectroradiometer	Stepwise discriminant analysis (SDA), Stepwise regression analysis (SRA), QDA, Soft independent modeling by class analogy (SIMCA)	The highest overall accuracy is 80%. Up to 85% based on QDA, and 83% based on SIMCA.	(103, 108)
Citrus	HLB	MIR spectroscopy	5,882–14,285	HLB-infected Non-infected	Thermoelectron Nicolet Magna 850 FT-IR spectrometer	PCR, PLSR	The model could predict positive or negative with accuracy more than 90%	(102)
Wheat	Yellow rust	VIS/NIR spectroscopy	460–900	Healthy Infected	spectrograph spectrometer	Analysis of covariance (ANCOVA) F-test QDA	The overall accuracy was 94%	(122)
Cotton	Verticillium wilt	VIS/NIR spectroscopy	350–2400	Normal Mild degree Moderate degree Severe degree Most severity degree	ASD Field spec Pro FR 2500 spectrometer	Quantitative correlation, RMSE	The highest estimative accuracy was found of 82.4%	(119)
Grape	Grapevine leaf-roll	VIS/NIR spectroscopy	400–2500	Healthy samples GLR-infected samples	portable spectrometer	Step-wise selection of variables, Discriminant classifier	The highest accuracy of classification was 81%	(78)
Sweet orange trees	HLB CVC	MID spectroscopy	2500–14,285	Healthy CVC symptomatic HLB-symptomatic HLB-asymptomatic	Perkin Elmer spectrometer	PLSR	The overall accuracy of the classification is 94%	(104)
Sugarcane	Fiji leaf gall	NIR spectroscopy	909–2500	Resistance Susceptible to FLG (Fresh and dried leaf samples)	spectrometer	PLS, scanning electron microscope (SEM)	SEV = 0.98 (R^2 = 0.97) SEP = 1.20 (R^2 = 0.88)	(123)
Corn	Fungal infection	MIR spectroscopy	5263–16,666	RWA2 Blanks Contaminated with *Fusarium graminearum*	Bio-Rad FTS-60A mid-infrared spectrometer	PCA, Cluster analysis	Accuracy of 79% found from ATR averaged spectra.	(87)
Tomato	Leaf miner damage	NIR spectroscopy	800–2500	Scale 0 Scale 1 Scale 2 Scale 3 Scale 4	Nexus FT-NIR spectrometer	Regression analysis	The overall accuracy was 78.33%	(90)

Oil palm	BSR	VIS/NIR spectroscopy	273–1099	Healthy Infected	Spectroradiometer	Descriptive statistics	The overall accuracy was 80.8%	(124)
Rice	Brown planthopper and leaffolder infestation	VIS/NIR spectroscopy	350–2400	Healthy Infected	Fiber-optic probe attached to Fourier transform infrared spectrometer	Linear regression models	$R^2 = 0.922$	(125)
Wheat	Fusarium fungi	MIR spectroscopy	2500–15,384	Monika Blanks Contamination	Thermo Nicolet Nexus 670 spectrometer	PCA Cluster analysis PLS MLR	The overall correlation coefficient was found 93%	(88)
Citrus	HLB	MIR spectroscopy	5150–10,720	Healthy Nutrient-deficient HLB-infected	InfraSpec VFA-IR spectrometer	QDA kNN	The overall accuracy was found higher than 94%	(108)
Wheat	Powdery mildew	VIS/NIR spectroscopy	250–1300	S0 = control S1 = 2500 S2 = 5000 S3 = 10,000 spores per leaf	—	ANOVA Correlation and regression analyses	$r^2 = 0.74$	(126)
Apple	Apple scab	VIS/NIR spectroscopy	350–2500	Healthy and infected leaves	FieldSpec Pro JR spectroradiometer	PLS-LDA, T Tree-based modeling	The overall accuracy was 88%	(127)

tomato leaf. Moreover, reflectance at 1450 and 1900 nm was highly correlated with miner severity level ($R^2 = 0.98$ and 0.96, respectively).

It can be seen that VIS/NIR spectroscopy can detect up to 66–90% of different types of plant diseases, while NIR spectroscopy can detect the diseases with accuracy ranging from 90% to 96%. However, MIR spectroscopy can sense up to 79–92% of plant diseases. In addition, from Table 1, VIS/NIR spectra and the NIR region were applied in detecting plant diseases with a percentage of 75% and the MIR region was used with a percentage of 25%. These results show that VIS/NIR, with wavelength in the range of 400–2500 nm, was used by exactly three-quarters of the studies reviewed and the applied spectroscopy technique. However, fewer studies have been reported on the application of MIR, which covers the wavelength between 2500 nm and 25,000 nm, for plant disease detection. This limitation in the use of MIR in detecting plant diseases may be associated with the high cost and complexity of the equipment. These can be due to the type of plant disease infection, which also affects the plant tissue contents, such as chlorophyll, water content and plant structure. These can lead to changes in the appearance of plant leaves based on certain symptoms, such as color or spot.

Impedance spectroscopy

Impedance spectroscopy (IS) or electrical impedance spectroscopy (EIS) has been used as a powerful measurement approach in many applications, including biology and medicine, material science, electrochemistry, fuel cell and battery, semiconductor industry and sensors (129). Besides that, IS has been established as a promising technique for plant diseases detection and monitoring of food quality (81, 130). IS measures the electrical properties of samples as a function of frequency. Since IS can be measured over a wide range of frequency, this raises the informational basis that can be obtained during test. The working principle of IS involves the application of an external field upon a material for example plant tissue or leaf to measure impedance or energy stored, then the results were interpreted and showed as the change of electrical properties as a function of frequency (131).

In plants, the electrical properties of tissues significantly rely on the composition and distribution of cell and extracellular fluid. Both extracellular and intracellular fluids comprise electrolytes, water, salts, free ions and other components; thus, their electrical behavior is generally resistive. Nevertheless, the double lipid layer located in the cell membrane works as an interface between extracellular and intercellular media. Because of the existence of the double layer, the cell membrane has a capacitance behavior. These two behaviors affect the electrical impedance of biological tissues (132).

There were several studies describing the ability of using electrical impedance as a diagnosis criterion to reveal the physiological responses (81, 133–136). Although most recent attention regarding the application of IS on food and agricultural materials focused on analysis and determination of quality attributes (137–141), the applications of IS in plant disease detection and determination of physiological dysfunction and membrane damage are being introduced at an increasing rate (130, 138, 142–144).

The application of IS to determine the physiological dysfunction, tissue and membrane damage commonly implemented at two frequencies, namely low frequency and high frequency (145). The low frequency ranges from 50 Hz to 1 kHz, while the high frequency ranges from 100 Hz to 1 MHz Coleman (146) even though Ando et al. (144)

argued that high frequencies generally ranged from 100 MHz to 10 GHz. At low frequencies the cell membrane acts as an insulator and blocks the current flow, causing a very high impedance modulus. While at high frequencies, the current flow indiscriminately through the cell structures (147–149). However, it can be observed that the frequency area in which the properties of cell structures appear is approximately from 100 Hz to 10 MHz.

Application of IS on plant disease detection

Table 2 presents some findings of the application of IS on plant disease detection. Previous studies have reported that impedance measurements are appropriate for plant disease detection (143, 150, 151). In 2009, Mendes et al. (152) published a paper on detection of Asian rust disease on soybean leaves extract at the early stages using EIS. In their study, biosensor was developed based on surface plasmon resonance, which the antibody was covalently immobilized on a gold substrate via a self-assembled monolayer. The measurement of the impedance was conducted at frequency range from 100 kHz to 100 MHz. The results found that, the correlation coefficient was 0.995. It was stated that the EIS can be used as a tool for the early diagnosis of soybean rust. Similarly, Huirong et al. (153) examined the application of EIS to detect *cucumber mosica virus red bean*disease in tomato leaves. In their study, the impedance measurements were taken in the frequency range from 1 Hz to 10 MHz. It was found that as frequency increased the impedance decreased.

Borges et al. (143) applied bioelectric IS for early detection and monitoring of disease on pine. The authors developed electrical impedance equipment that would be able to perform AC scans in frequency range between 1 kHz to 1 MHz. In their study, the bioimpedance was identified of healthy pine individuals and tested the feasibility of using IS in early detection of nematodes disease. They found that the initial bioimpedance tests in young pine samples revealed some classification between healthy samples and those infected with nematode disease. It was being arisen that EIS system can be used as a suitable technique to diagnose plant diseases.

In 2014, Borges et al. (154) investigated the differential impact of IS technique to evaluate the physiological state of plants. It was designed a portable electrical impedance system to stand for biological application purpose. In their study, the samples were chosen from three different plant species: chestnut (*Castanea sativa*), pine (*Pinus pinaster*) and physic nut (*Jatropha curcase*) affected by ink, pinewood and esca disease, respectively. The healthy samples were selected to be eight while some individuals were inoculated with disease for assessing the affected plants. The hydric stress which is the internal hydration condition of the plant was measured. In addition, it was measured the impedance of the samples using the EIS in a frequency range between 1 kHz and 1 MHz. It was reported that the results found from EIS can determine three various physiological states namely as plants with disease, plant with high level of hydric stress, and healthy and watered plant. Similarly, in 2014, Repo et al. (155) investigated the applicability if IS to detect the mycorrhizal colonization in scots pine roots. It was measured the impedance values from healthy root samples and from affected root samples by hebeloma sp (H) and suillus luteus (SL) fungus at a frequency range between 5 Hz and 100 kHz. It was stated that there was obvious change between affected and non-affected root samples. In their results, the impedance values were classified correctly for more than 95% of the samples. As we can see from the previous studies and their results, the IS can detect the plant disease with high significant level.

Table 2. Previous studies on plant disease detection using impedance spectroscopy technique.

Samples	Disease/ pathogen	Frequency range	Electrical variable measured	Modeling	Statistical approach	Significant results	Reference
Vineyard Pinewood	Esca disease Nematode disease	1 kHz to 1 MHz	Imaginary and real impedance	Cole–Cole model	Cole–Cole diagram	—	(142)
Pine	Pinewood nematode	1 kHz and 1 MHz	Impedance, Reactance, Resistance	Cole model	Cole–Cole plot	The accuracy higher than 80%	(153, 158)
Tomato	Cucumber mosica virus red bean (CMV-RB)	1 Hz to 10 MHz	Impedance	Double shell model	Correlation plot between impedance and frequency	The higher of frequency, the lower impedance value	(152)
Nicotiana glutinosa leaves	Local lesions	1 kHz 1 MHz	Reactance, Resistance	—	Mean differences	*P-value* of the samples was significantly different (<0.05)	(159)
Potato, Carrot, Banana, Apple	Dysfunction of physiological tissue (ruptured cells)	3 kHz and 50 MHz	Electrical conductivity	Equivalent circuit model	Disintegration index	—	(150)
Scots pine	Symbiotic mycorrhizal fungi (*Hebeloma sp. And Suillus luteus*)	5 Hz to 100 kHz	Real and imaginary impedance	Equivalent circuit model	ANOVA and PCA	Impedance data classified correctly for more than 95% of the samples	(154)
Maize	Arbuscular mycorrhizal fungal (AMF)	100 Hz to 10 kHz	Impedance Capacitance	—	Correlation algorithm	The highest r^2 is 0.9405	(160)
Gum-Arabic-chitosan	—	1 Hz to 1 kHz	Impedance	Equivalent-circuit model, Buttler–Volmer model	Smoluchowsky mathematical model	—	(161)
Soybean	Soybean rust	100 kHz to 100 MHz	Impedance	Randle's equivalent circuit	The correlation coefficient	$r^2 = 0.995$	(151)

Many factors, including frequency, temperature and moisture content, influence the dielectric properties of plant tissues (156, 157). Knowledge of the relationship between frequency and dielectric properties is helpful in determining the optimum frequency range for early detection of disease applications (158). From Table 2, almost 66% of the previous studies have used frequency ranges between 1 Hz and 1 MHz. On the other side, 34% from the previous studies used high frequencies of IS in disease detection. Therefore, frequency range between 1 Hz and 1 MHz is widely applied from previous studies for disease detection in plant. This is largely due to the electrical conductance of plant tissue which consists of extracellular fluid and cells containing the intracellular fluid inside the cell membrane, and it rely on the moisture content and the water or liquid available in the tissues. In biological tissue the conductivity of the double-layer plasma membrane can be neglected, which leads to a very high value of membrane resistance. The application of this technique is affected by the type of disease infection, since impedance measurement is highly influenced by water content, the challenge in disease detection is therefore to verify that the changes was actually due to plant disease infection or merely due to effect of water stress/water uptake of the plant.

Fluorescence spectroscopy

FS is a spectroscopy method employed to measure the fluorescence from certain substances after excitation with a beam of light (usually ultraviolet spectra, wavelength from 10 to 400 nm). The absorbed light is invisible to the human eye, while the emitted light being in the visible region, gives the fluorescent substance a distinct color that can only be seen when exposed to UV light. The operating mechanism involves using a beam of light to excite the electrons in the molecules of the substances, causing them to emit light (162). FS has many applications in life (163–167). It has been employed in monitoring the physiological states and stress levels in plants (41). The leaves of green plants possess chlorophylls, and as such are able to emit two different kinds of F; blue-green F having a wavelength range of 400–600 nm and chlorophyll F with a range of 650–800 nm (76). Generally, the application of FS in plant sciences, particularly plant diseases and nutrient deficiencies has received considerable attention (76, 77, 79, 168–170).

The FS method is used as a tool to sense and determine plant-pathogens at an early stage. For example, it is used to sense plant-pathogen interactions in spring barley (79), powdery mildew infection and leaf rust in wheat (77), to detect cucumber diseases (170), detection of HLB, citrus canker and mechanical injury in citrus orchards (76, 78, 171), to visualize and analyze the infection of banana with *Fusarium oxysporum* f.sp. cubense (168) and to detect and quantify infection symptoms on detached grapevine leaves (172). The mechanism of this is that at spectra data, the intensity of samples inoculated with diseases are different from the normal and healthy samples due to the accumulation of pathogen or resistant specific compounds, such as lignin, and/or the production of waxes, which affects the F emission and changes the intensity of measurement (79).

Different types of devices and equipment of FS have been used to measure the data from plant samples. These devices include fiber-optic fluorescence spectrometer, imaging multispectral fluorescence and portable multiparametric F sensor (79). However, the most effective kind of F used in detecting plant diseases is the fiber-optic fluorescence spectrometer as compared to other techniques (77, 169, 170, 173). In F, there are four excitation wavelengths

used: UV, blue (B), green (G) and red (R), and the emitted F is detected in yellow YF, red RF and far-red FRF (79). Although various F ratios are determined from the spectra ranges, the ratios which yields the most promising results to sense and diagnose the fungal diseases in plants are simple fluorescence ratio (SFR), which is the inverse ratio of the chlorophyll fluorescence ratio F680/F730, and blue-to-far-red fluorescence ratio (BFRR), which is dependent on the blue and far red F (174). This can be explained due to the fact that diseases cause minor changes in the SFR and obvious change in BFRR, this can be attributed to the biotrophic relationship of the pathogens with their host. Whereas the changes in SFR can be explained due to the distribution of photosynthetic quantum conversion and consequently the chlorophyll content will be decreased.

In the investigated literature, different types of statistical approaches have been used to measure and analyze the spectral data measured using FS. These approaches include: Gaussian cure fitting, back propagation artificial neural networks (BP-ANN) algorithm, PCA, NB, bagged decision trees (BDT), SVM, ANOVA and *t*-tests (77, 79, 168–171). In addition, these statistical methods facilitated the analysis by removing possible data distortion, and reducing the dimensionality of measured spectral data, classified tools and tools to find the significant difference of health and infected plants at different spectral range.

Application of fluorescence on plant disease detection

Burling et al. (77) analyzed the application of three F ratios including, (F451/F522) blue-to green, (F451/F687) blue-to-red and (FF451-F736) blue-to-far-red for early detection of powdery mildew infection in susceptible and resistant leaves in wheat varieties. In their study, it was found that the accumulation of defense-related secondary compounds of pathogen infection lead to longer F decays. In addition, it was reported that the mean lifetime in spectral range from 500 to 620 nm was significantly increased in inoculated leaves as compared to control leaves. Also, Leufen et al. (79) highlighted that the rust diseased spring barley leaves show a lesser green and blue F intensity as compared to powderly mildewed leaves. Moreover, it was noted significant differences between healthy and diseased leaves. Additionally, Romer et al. (169) studied the potential application of F spectra for presymptomatic wheat leaf rust infection. In their study, a wavelength ranging from 370 to 800 nm was recorded. The accuracy of 93% was found in their study. It was highlighted that a spectral range from 550 to 630 nm could separate between healthy and inoculated leaves; however, the range from 650 to 800 nm has limited effects on the results. Similarily, Burling et al. (173) conducted an experiment to assess the change of F induced by the pathogens of leaf rust (*Puccinia triticina*) in wheat leaves. Their results indicated that more pronounced increase of green F as compared to the rise in blue F were observed early after inoculation. Furthermore, spectral range from 560 to 620 nm measured longer mean lifetime due to pathogen infection.

Yao et al. (175) demonstrated the relationship between F emissions of corn kernels inoculated with *Aspergillus flavus* spores and aflatoxin contamination levels. In their research, the results emphasized that contaminated corn kernels illustrates specific F emission peaks around 470 nm related to the presence of aflatoxin in the samples. In addition, low contaminated corn kernels got high F response around 470 nm while high contaminated samples showed lower F. It was stated that F hyperspectral imaging was applicable to estimate the total aflatoxin concentration in individual corn kernels. In 2012, Yang and Yu (170) investigated the potential of F technique to detect cucumber diseases and insect pests. Cucumber

samples were divided into four classes, namely healthy, downy mildew, aphid and downy mildew and aphid. It was found that BP-ANN showed significant identification with an accuracy of 100%. Also, it was reported that chlorophyll F spectrum could be a promising tool to diagnose cucumber diseases and insect pests. The detection of powdery mildew (*E. necator*) infection symptoms on detached grapevine leaves was investigated using UV-induced F technique (172). In their experiments, the grapevine plants were grown in a greenhouse under controlled environment and then the selected leaves were divided into controlled and inoculated leaves. The authors applied different excitation/emission wavelength combinations. It was found that the ratio between blue and green (F440/F520 nm) F intensity of healthy and diseased areas of leaves displayed significant difference after three days post-inoculation process. Also, it was reported that the detection was increased in the spatial average of F440/F520.

In 2011, Li et al. (168) discussed the usage of green fluorescent protein (GFP)-tagged transformed to visualize and analyze the infection of banana with *Fusarium oxysporum* f.sp. cubense. The authors mentioned that epidemal cells of banana roots was invaded by Foc race 4, and fungal hyphae could pentrate cell walls immediately to grow inside and outside cells. In their study, samples of nine cultivars were inoculated with the GFP-transfored pathogen. The authors have pointed to GFP-tagged foc race 4 as an effective tool to monitor and evaluate resistance in banana to foc race 4.

In 2008, Belasque et al. (76) employed FS to detect stress caused by citrus canker and mechanical injury. In their experiment, the measurement probe was located 2 mm above the leaf in order to collect data from different samples during the study period (60 days). The excitation wavelength applied was 532 nm and different wavelengths ratios were used to detect the stress caused by bacterial infection. In their research, three different ratios of F intensity were used including: 452/685 nm, 452/735 nm and 685/735 nm. The leaves samples were grouped into four classifications: leaves with disease, leaves with no stress, leaves with mechanical stress and leaves with disease and mechanical stress. Similarly, Lins et al. (89) used FS to monitor citrus canker in citrus plants. In their study, the same approach as in the previous study was used. It was stated that FS could detect disease and would be able to discriminate between mechanical and diseased stress. The above study could classify healthy leaves from citrus canker-affected ones. But were unable to identify water stress and distinguish between variegated chlorosis and citrus canker-infected leaves. It was not presented yet any statistical analysis to evaluate the ability of the technique to discriminate or classify different plant conditions. On the other hand, Sankaran and Ehsani (171) used handheld F spectrometer sensor to collect data from healthy, nutrient-deficient and HLB-infected leaves of two different sweet orange, namely Hamlin and Valencia. In their study, it was applied four excitation wavelength called UV, blue, green and red, and from these wavelength yellow, red and far-red F was measured. NB and BDT were used as classifiers with 85% and 94% accuracy, respectively. In addition, it was reported that the best overall accuracy was higher than 94% for field HLB detection. The BDT classifier resulted in better performance as compared to NB; however, it required more time for the computation process, at least 10 times greater than the NB. Also, some asymptomatic leaves were incorrectly detected as healthy leaves.

Leufen et al. (79) highlighted that the most significant changes between healthy and affected (mild powderly and leaf rust diseases) leaves were detected in the blue-green spectra with 75% accuracy as compared to minor alterations in the spectra with 25% for powdery mildew (*E. necator*) disease. Leaf rust disease showed 68% in blue-green spectra compared

to 32% for chlorophyll F spectra. Similar results were reported by Ludeker et al. (176), who stated that chlorophyll concentration has less change with fungal infection that was 30% as compared to blue-green spectra that was 70%. Similarly, Romer et al. (169) revealed that blue-green spectra (550–630 nm) found feasibility of detecting the separation between healthy and inoculated leaves with highest accuracy of 93%, whereas chlorophyll spectra (650–800 nm) has only very limited impact. In addition, Burling et al. (173) found accuracy of almost 83.33% as the significant difference between healthy leaves and those inoculated with pathogen infected wheat leaves at blue-green region (F451/F522) and 33.33% at chlorophyll region (F687/F736).

In contrast, Burling et al. (77) reported that for detecting the powdery mildew the result was 68.75% at chlorophyll F spectra and 87.5% at blue-green spectra. This increasing result at chlorophyll F spectra can be due to the reduction of chlorophyll content causes by the distribution of photosynthetic quantum conversion. Similarly, Kuckenberg et al. (177) used chlorophyll F spectra (F686/F740) to study the damage in apple leaves, with R^2 of 0.73. In the same vein, Yang and Yu (170) diagnosed cucumber disease in the area of green spectra and found accuracy between 76% and 89%, while the accuracy of 95–98% was found in the chlorophyll spectra. Moreover, Belanger et al. (172) mentioned that at chlorophyll region particularly ratio of F690/F740 did not present significant visual differences between infected and healthy grapevine leaves and the mean was found 25%. Also, Marcassa et al. (178) and Belasque et al. (76) applied the ratio of two chlorophyll F bands F685/F735 to detect and discriminate mechanical and water stress in citrus limonia osbeck and orange leaves and they found R^2 was almost more than 80%. More information is given in Table 3. These studies revealed the prospective of the FS technique in conjunction with advanced statistical models for detecting different diseases and health conditions in plants. This indicates that the F emitted spectral range released by plants could be used as a disease monitoring tool for rapid and early detection of plant diseases.

Challenges and future direction

All studies reviewed in the literature supports the notion that spectroscopy techniques, which largely depends on frequency and spectral reflectance, can be used for non-invasive field detection of plant diseases. However, there are several limitations on the wide acceptance and commercialization of spectroscopy techniques in plant disease detection. One of the challenges includes the effect of environmental conditions. Griffin and Burke (185) reported that environmental conditions could affect the spectral reflectance from the object under test. Moreover, noises and high light intensity can interrupt the frequency and wavelength and increase the noises to signal ratio. Therefore, there is a need to identify suitable approaches to overcome this problem. One of the ways that can be applied to overcome this drawback is to identify a specific wavelength range or index that is not only sensitive to a specific plant disease but also is least affected by the changes in the environmental condition.

Probes or test fixtures play a very important role in using the IS technique in measurements. This importance comes because the probe is the medium between devices and objects to be measured. Collecting data depends on the properties of the probe. In other words, the probe is the sensor or the transducer, the maximum reading that can be obtained from the probe is considered the highest limitation to accurate measurement. For example, Lizhi et al. (186) observed that the precision of inductance (L), capacitance (C) and resistance (R) of the

Table 3. Previous studies on plant disease detection using fluorescence spectroscopy technique.

Plant	Disease/ severity levels	Excitation wavelength (nm)	Emitted spectral range (nm)	Optimum spectral range (nm)	Repetition rate (Hz)	Energy per pulse	Significant results	Reference
Rice	Gram-negative bacterial strains	280	300–400	330 and 348	—	—	—	(179)
Barley	Healthy leaves Powdery mildew Leaf rust	337	410–560	410, 440, 470, 500, 530 and 560	30	2–3.5 μJ	Accuracies more than 65%	(79)
Rosemary	Protein aggregation	295	300–400	—	—	—	Significantly different in wavelength trends	(180, 181)
Wheat	Powdery mildew; Susceptible leaves Resistant leaves	337	370–800	530 and 560	20	1–2 μJ	All the three F ratios were found significantly different ($p < 0.05$)	(77)
Cucumber	Healthy Downy mildew Aphid, Downy mildew and aphid	473	504–900	501, 685 and 740	—	—	BP-ANN showed significant identification with an accuracy of 98%	(170)
Wheat	Health Leaf rust	337	370–800	550–630	20	1–2 μJ	The accuracy was 93%	(169)
Wheat	Health Leaf rust	337	370–800	451, 522, 687, 687, and 736	20	1–2 μJ	The accuracy was 83.33% as the	(173)
Corn kernel	Aspergillosis	365	400–600	485	—	—	The coefficients of determination, r^2 was 0.72	(175)
Walnut	Fusarium head blight	365	425–775	425, 470, 515, 560, 615, 660, 745, and 775	—	—	The overall accuracy was 90.6%	(182)
Cantaloupe	Fecal Contamination	320–400	425–774	520, 550, 595, and 675	—	—	The accuracy range from 79% to 96%	(183)
Maize	Toxigenic and atoxigenic fungal strains	365	400–700	450–500	—	—	The overall higher classification accuracy was 94.4%	(184)
Nutmeg	Fungal infection	200–900	200–900	420 and 450	50/60		The coefficients of determination, r^2 was higher than 0.75	(185)
Grapevine	Powdery mildew	360 436	300–1000	440, 520, 690, and 740	50	—	ANOVA found ($P < 0.05$) significantly different between F ratios	(172)
Citrus	Citrus canker	532	500–850	735	—	—	—	(76, 89)
Wheat	Rust infection	355	400–800	470 and 685	12	30 mJ	Blue-green spectra found accuracy of 70% with fungal infection	(176)

Table 4. Comparison of spectroscopy techniques in plant disease detection.

Techniques	Method accuracy	Applicability for rapid detection	Applicability for field work	Speed of detection	Advantages	Limitations	Other aspects
Visible/infrared spectroscopy	Higher visible symptoms, gave higher accuracy of the technique	It has the ability to rapid detection	Fairly ruggedness, the ruggedness of the technique depends on the sensitivity of sensor applied	Few minutes is required to detect the disease after the setup, speed depends on the computational process of the computer and on the scanner speed	Rapid method, less expensive than conventional methods, environmental friendly, and can be used in processing lines	Photobleaching, complex processing, and difficulty to distinguish diseases on crops	This method can be automated, this technique can be used as preliminary investigating of diseases, the methods in combination with other methods as molecular detection can be effective in rapid detection of plant diseases.
Impedance spectroscopy	Accuracy depends on the properties of the electrodes or probes and also on the physical structure of the object	Applicable for rapid detection if it combine with software	Challenging to be used in the field, unless it provided in portable device	Few minutes is required to detect the disease after the setup, speed depends on the properties of the probe used in the analyzer	Precise, reliable method, and less expensive	Limit of the frequency range that can be used in measurement	This method can be automated, this technique can be used as preliminary investigating of diseases
Fluorescence spectroscopy	Accuracy is currently unknown, as this method is in the developmental stages and has been utilized in recent years	The technique shows the potential for rapid plant disease detection	Moderately rugged, depending on the detector used.	Speed depends on the detector speed and computational speed	High sensitivity	Autoflourescence, photobleaching and preparation of plants has to follow a strict protocol, thus FS is difficult to implement in field environment	This method can be automated, and Fluorescence assay is dependent on the instrument in use

LCR meter (4284A) manufactured by the Agilent Technology Company can produce frequency more than the maximum limitation of the probe of solid test fixtures, which can measure up to a maximum of 30 MHz. This limitation can be overcome by creating a probe that can measure at a larger frequency in order to collect data at wider range of frequency or combine two techniques of spectroscopy to improve efficiency. The available probes and devices used in IS methods require tedious and complex processes for the calibration. The calibration is required for every single test. Thus, completing all steps of a calibration procedure is time consuming. Therefore, reducing and simplifying the calibration process can help in getting enough time for measurement. In order to tackle this matter, developing automated system software that can automatically calibrate the entire process becomes essential. In addition, regarding IR, the application of Fourier transformation can also serve as a potential tool for rapid and accurate calibration.

Another major challenge for FS techniques is photobleaching. Photobleaching is a general term for any photochemical process that causes the molecule to eventually change to another form and stop absorbing and/or emitting photons. This chemical phenomenon affects the integrated signal on application of spectroscopy, thereby resulting in decreased sensitivity, inaccurate recording and data collection. This phenomenon was observed in the application of FS on plant disease detection (51). However, this challenge may be overcome by reducing the intensity and duration of light exposure, using more encompassing-less photobleaching-sensitive fluorophores, and increasing the concentration of fluorophores. In addition, the ratio of power/time/signal to that of digital noise must be carefully considered. Pulsing the probing laser at low duty cycle with long intervening dark periods may be a very important technique of overcoming the non-linear photobleaching effect.

The deployment of a spectroscopy technique in on-site detection of plant disease is still carried out manually. This can affects the efficiency of using this technique in term of time and work required in data collection. Thus, it is feasible to incorporate the VIS/IR, I and F spectroscopy techniques into an autonomous machine. These methods can further be integrated with an automatic agricultural vehicle to be used as real-time monitoring for plant diseases, if these techniques are well established for a specific disease detection application. Therefore, more study in this regard is required. The overall comparisons of three major techniques are summarized in Table 4.

Conclusion

The main goal of the current paper was to review and summarize the spectroscopy techniques that have been used for plant disease detection. The three major categories for non-invasive monitoring of plant diseases are the (i) VIS/IR technique, (ii) IS technique and (iii) FS technique. This study has shown that spectroscopy techniques have the ability to be used as tools for monitoring and identifying different levels of infection in plants and trees.

The scientific literature reviewed have shown that the application of IS depends on the properties of the electrodes or probes and also on the physical structure of the products. The most significant advantage of using this method is the potential for rapid detection if combine with artificial learning software. However, its use in industrialization and commercialization is limited by its low frequency range limits. This can be overcome by combining IS with either VIS/IR or FS technique. Similarly, the application of VIS/IR in plant disease detection has been shown to be a promising novel technology due to higher accuracy when

compared to IS and FS. Nevertheless, its long image processing time still limits its overall efficiency. More so, the investigation on applied FS technique has further revealed that it has great potential in detecting plant diseases.

In terms of cost and practical difficulties in adopting these technologies, the VIS/IR is the least expensive to set up and easy to adapt when compared to other spectroscopy techniques. More so, in terms of field configuration and equipment optimization, the VIS/IR and IS are the most adequate, while the FS is less suitable for field deployment.

In conclusion, this review suggests that spectroscopy techniques have the potential to be applied on plants, as a non-invasive disease detecting tool. Although they are generally limited by (i) the effect of environmental light to the measured spectra data on the field, (ii) photobleaching, (iii) optimization of the technique for a specific plant/tree and disease and (iv) limit of frequency and wavelength range, they can however be automated and easily commercialized, especially the VIS/IR technique. Thus, more studies should focus on enhancing their acceptability, commercialization and automation.

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