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# Application of Spectroscopic Techniques in Early Detection of Fungal Plant Pathogens

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## Abstract

Among the plant pathogens, around 85% of diseases in plants are caused by fungi. Rapid and accurate detection of fungal phytopathogens up to the species level is crucial for the implementation of proper disease control strategies, which were previously relied on conventional approaches. The conventional identification methods have been replaced by many rapid and accurate methods like high throughput sequencing, real-time polymerase chain reaction (PCR), serological and spectroscopic technique. Among these rapid pathogen detection techniques, spectroscopy is a rapid, cost-effective, non-destructive method and does not require sample preparation. Nowadays, visible, infrared and near-infrared rays are commonly employed for pathogen detection. Fluorescence Spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Attenuated Total Reflection (ATR)-FTIR spectroscopy, Raman Spectroscopy, Matrix-assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). Biocontrol fungus-like *Trichoderma* spp. can be detected with the help of MALDI-TOF MS. Fluorescence spectroscopy used fluorescence emanating from the sample and successfully used in the detection of powdery mildew (*Blumeria graminis*). Hyperspectral imaging is an advanced approach which uses artificial intelligence in plant disease detection. This literature discusses briefly about the features of above-mentioned spectroscopy techniques which may impel the general understanding and propel the research activities.

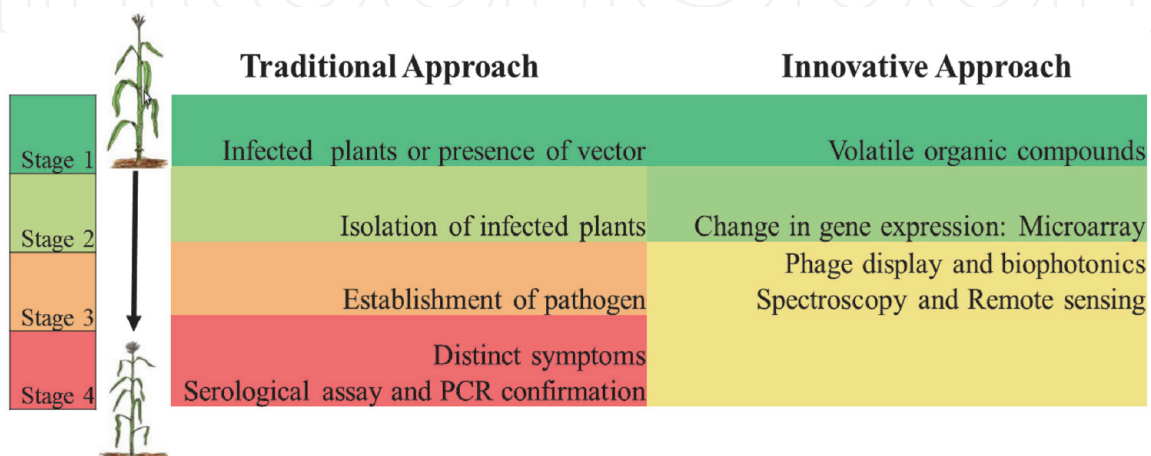
**Keywords:** diagnosis, fluorescence spectroscopy, fungal plant pathogens, infrared, near-infrared, spectroscopic techniques

## 1. Introduction

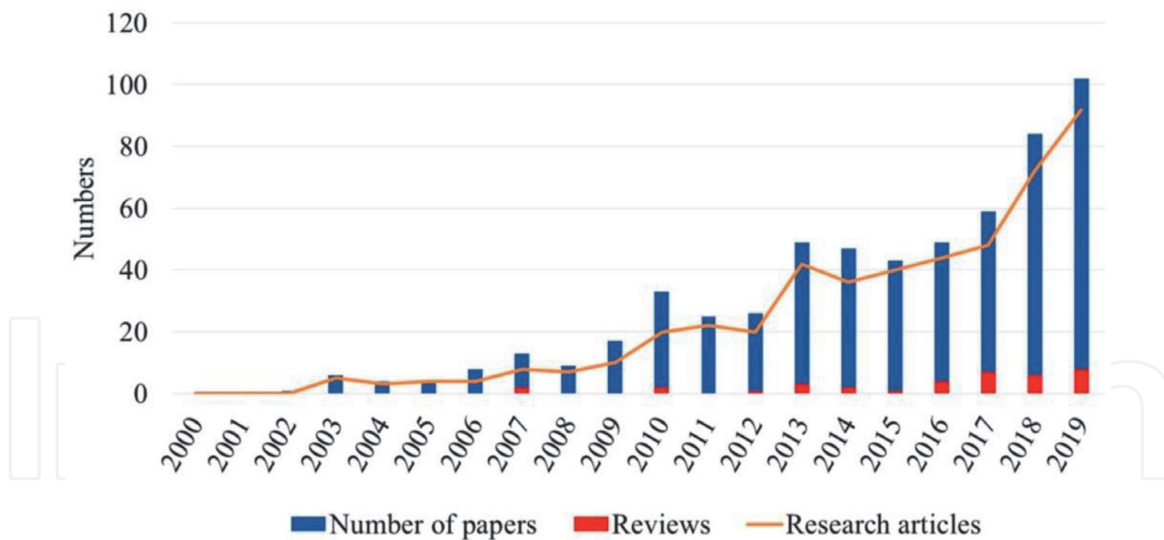
Fungi, bacteria, viruses, nematodes, and parasitic plants cause plant diseases, which result in a complex relationship between the host plant, the pathogen, and the environment. But most plant diseases (around 85%) are caused by fungi. More than 10,000 species out of 100,000 recognized fungal species may cause diseases in plants. The different strain types and the fungal pathogen's formae speciales make detection and identification more difficult, necessitating the use of specialized techniques. For the implementation of proper disease control strategies, rapid and accurate identification of phytopathogenic fungal pathogens up to the species level

is critical. For a long time, experts have used their skills and experience to identify crop diseases with their naked eyes. Finding a specialist and approaching them is not only a time-consuming and repetitive task, but it is also a lengthy and expensive procedure that can take a long time, making the disease very difficult to eradicate and time-consuming in the case of large areas [1]. These traditional methods available for phytopathogenic fungi detection and identification are not always very precise along and even time consuming, which can be shown by **Figure 1**. In order to prevent economic yield losses and safe crop production, advanced plant disease diagnosis can provide rapid, accurate, and effective early-stage identification of plant diseases. For their timely control, early detection and recognition of these phytopathogens are essential. The traditional methods of phytopathogenic fungi detection and identification were mainly based on symptoms, isolation and culture, accompanied by morphological observations along with their biochemical analysis [2]. The study of fungal biology and its relationship with the host plant has made considerable progress in recent years, thanks to the advent of modern holistic and high throughput techniques. The new technologies that are essential to detecting fungal diseases and sensor production are focused on spectroscopy and imaging, mass metabolites and volatile profiling. When “plant disease” and “hyperspectral” are used as key terms to scan for in all databases, according to Web of Science statistics, there are 651 related papers from 1990 to 2019 (**Figure 2**).

Spectroscopy, along with other methods, offers a platform for the creation of non-destructive approaches. The study of the relationship between matter and electromagnetic radiation is known as spectroscopy [3]. Spectroscopy was limited to the absorption, emission, and scattering of visible, ultraviolet, and infrared electromagnetic radiation at the end of the nineteenth century. Throughout the twentieth century, the definition of spectroscopy was extended to include other forms of electromagnetic radiation, such as X-rays, microwaves, and radio waves, as well as energetic particles like electrons and ions [4]. It’s ideal for plant disease detection tools to be fast, specific to a particular disease, and sensitive enough to detect symptoms as soon as they appear [5]. With rapid analysis, non-destructive methods meet these requirements, as minimal to no sample preparation is needed. Current research activities in agricultural engineering are working on developing certain technologies to establish a realistic method for large-scale real-time observation of diseases under field as well as semi-field conditions. There are several different kinds of spectroscopy techniques that include specific fungal pathogen detection methods (**Table 1**).



**Figure 1.** Comparison between the traditional and innovative approach of plant disease detection by considering four different stages and their timings.



**Figure 2.** Number of published articles by year on plant disease with hyperspectral data (adopted from Zhang et al., 2020).

Technique	Crop	Plant Disease	Spectral range	Reference
Visible and Infrared spectroscopy	Wheat	Powdery Mildew and Take all disease	490–540 nm	[6, 7]
	Kiwi	Root rot		[8]
	Orange	Sooty Mold	450–850 nm	[9]
	Grape fruit	Greasy Spot		
	Muskmelon	Powdery mildew		
Fluorescence Spectroscopy	Wheat	Powdery Mildew	blue-to-green (F451/F522); blue-to-red (F451/F687); blue-to-far-red ratio (F451/F736)	[10]
	Barley	Powdery Mildew	410–560 nm	[11]
Nuclear Magnetic Resonance (NMR) Spectroscopy		Mycorrhiza		[12]

**Table 1.** Spectroscopy techniques for the detection of fungal pathogens.

## 2. Plant-pathogen interactions make spectroscopy indispensable

From sowing and growing to harvest, multiple disease-causing pathogens can simultaneously affect plants, reducing the yield and quality of the cultivated plants. It is obvious that many diseases produce similar symptoms and signs on the basis of studies on plant disease detection study, but are caused by very different microorganisms or agents [13]. It can therefore be said that, particularly for non-invasive assay methods, pathogens themselves and plant-pathogen interaction processes are complex. This makes it impossible to use the naked eye or basic machine vision to discriminate against particular pathogens.

## 2.1 Visible and infrared spectroscopy

As a tool for pathogen detection, non-destructive methods based on visible, infrared and near-infrared spectroscopy are becoming more common as they are fast and cost-effective. In most cases, visible spectroscopy is paired with infrared/near-infrared spectroscopy to detect disease in plants. Dowell et al. [14] used NIR spectroscopy to predict scab, vomitoxin, and ergosterol in single wheat kernels. The application of NIR spectroscopy for mycotoxin measurements in cereals was identified by Pettersson and Aberg [15]. Erukhimovitch et al. [16] explored the ability of FTIR microscopy to differentiate easily and rapidly and to identify different fungi that are responsible for severe agricultural damage. For each of the fungi studied, the findings produced a specific and clear spectral marker. They showed that the spectral region can be regarded as a significant area for simple and accurate differentiation between the different fungi examined, ranging from 1,000 to 1,800  $\text{cm}^{-1}$ . Huang and Apan [17] used a portable spectrometer to collect hyperspectral data in the field to detect *Sclerotinia* rot disease in celery and found that adequate reflectance in the visible and infrared ranges from 400 to 1,300 nm produced similar results as the entire spectrum (400–2,500 nm).

## 2.2 Fluorescence spectroscopy

Fluorescence spectroscopy is a method of electromagnetic spectroscopy that analyses fluorescence from the sample of interest. The sample is excited by using a light beam that results in a lower energy light emission, resulting in an emission spectrum that is used to interpret results [18]. Green leaves generate two forms of fluorescence: blue-green fluorescence (about 400–600 nm range) and chlorophyll fluorescence (about 650–800 nm range). Fluorescence spectroscopy, with a high sensitivity and specificity rate, seems to be a promising diagnostic technique that makes it an ideal diagnostic method. Fluorescence spectroscopy can be used to track food shortages, environmental stress levels, and plant diseases [19]. In four genotypes of spring barley in healthy leaves, as well as leaves, inoculated with powdery mildew pathogen (*Blumeria graminis*), Leufen et al. [11] studied the ability of three optical devices, namely fluorescence lifespan, image-resolved multispectral fluorescence and selected indices of a portable multiparametric fluorescence system for the proximal sensing of plant-pathogen interactions (*Puccinia hordei*). Important variations were found between healthy and diseased leaves.

## 2.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

The introduction of a higher magnetic field has brought greater sensitivity and spectral resolution. Technology developments have made it possible to combine various NMR techniques that allow for metabolic, anatomical, and physiological knowledge. Another advantage of NMR measurements in researching the biochemistry of mycorrhizas is the ability to spectroscopically distinguish host from fungal metabolites without the need for separation or chemical derivatization. Through the implementation of high-resolution solid-state magic angle spinning nuclear magnetic resonance, intact tissue analysis was possible (HR-MAS NMR). Pfeffer et al. [12] studied the application of Nuclear Magnetic Resonance (NMR) to the two major types of mycorrhiza (ectomycorrhiza and arbuscular mycorrhiza) in order to address the physiological question of the sufficient discrepancy between these two mutualistic symbioses. They found that NMR isotopic labelling can be used to investigate the transfer of substrates between *in-vivo* and *in-vitro* symbionts, as well as the formation of secondary metabolites in response to colonisation. It can also be

used to evaluate the locations of biosynthesis and storage compound translocations in mycorrhizal fungi.

## 2.4 Fourier transform infrared (FTIR) spectroscopy

The Fourier transform infrared spectroscopy (FTIR) is one of the methods that has been successfully used to detect and recognise fungal plant pathogens [20]. This technique was shown in some studies to be capable of discrimination not only at the genus level but also at the species level [21]. The vast majority of these experiments involved both bacteria and fungi. Because of its sensitivity, rapidity, low cost, and simplicity, FTIR spectroscopy has the potential to be a very useful method for detecting and recognising fungal pathogens in agriculture [22].

Erukhimovitch et al. [23] used standard FTIR spectroscopy methods to identify control uninfected potato tubers and tubers naturally contaminated with fungal pathogens. To confirm the absence of fungal infection, samples from uninfected control potatoes were grown in the required growth medium. Thin potato samples were prepared directly from the surface of uninfected and infected potatoes for FTIR analysis. The FTIR spectra of both uninfected and contaminated samples collected from potatoes. Tubers indicate a disparity in spectra between infected and uninfected tissues, with unique clear spectral bands appearing in the spectra of infected tissues.

## 2.5 ATR-FTIR spectroscopy

Attenuated total reflection Fourier transform infrared abbreviated as ATR-FTIR spectroscopy imaging is a non-destructive imaging method which can be exploited for wide range of samples and system studies. It is highly versatile in nature and can be applied in biomedical sciences and horticulture industries for identification and interaction of pathogen with host.

ATR-FTIR has been used in characterization of fungal isolates of *Rhizoctonia*, *Verticillium*, *Colletotrichum*, *Fusarium* species [24, 25] and *Geotrichum candida* [26]. Diagnostic analysis and exploratory features of ATR-FTIR spectra offers potential detection of intact host–pathogen systems and other biological insights.

## 2.6 Raman spectroscopy

Raman spectroscopy (RS) is based on the Raman effect, which states that when incident light (750–850 nm) excites molecules in a tissue, the molecules will reflect light at a different wavelength. The wavelength of the reflectant light is unique to various chemical components, allowing for chemical synthesis to be identified by atheromatous plaque. It may distinguish between various plaque components including elastin, collagen, cholesterol, cholesterol esters, lipids, carotenoids, and calcium apatite deposits. To distinguish normal tissue from abnormal tissue, fluorescence spectra are obtained from a coronary artery by supplying excitation light and collecting emitted light through flexible optical fibers. RS is a nondestructive, label-free spectroscopic technique that offers knowledge about the chemical composition of examined specimens. Food chemistry [27], electrochemistry, forensics and materials science, and agricultural sciences are among its practical applications. Farber et al. [28] demonstrated that using a hand-held Raman spectrometer in conjunction with chemometric analyses, it is possible to differentiate between healthy and diseased maize (*Zea mays*) kernels, as well as between different diseases, with 100% precision. The study was compact and sample-agnostic, implying that it could be retooled and performed autonomously for other crops.

Van Duyne discovered in 1977 [29] that coherent oscillations of an electron cloud at the surface of nanoparticles would amplify Raman scattering by a factor of 108. This phenomenon, known as surface-enhanced Raman spectroscopy (SERS), allows for single-molecule detection and has thus been widely used to detect fungi-related toxins [30].

## 2.7 MALDI-TOF MS

With technological advancement new and reliable tools are emerging for detection and identification of plant pathogens, one of such technique is Matrix Assisted Laser Desorption Ionization-Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). Identification of several phytopathogenic fungal genera such as *Alternaria*, *Fusarium*, *Monilinia*, *Puccinia*, mildews and potential biocontrol fungus like *Trichoderma* and *Metarhizium* has been successfully done with MALDI system. Although identification of several genera of fungus is done by MALDI-TOF MS, still this technology is not yet considered a standard tool for the fungal identification and their functions (Table 2).

## 3. Some other uses of spectroscopy in plant pathogen detection

Sankaran et al. [42] used spectroscopy imaging technologies and made a distinction between normal healthy and diseased leaves of many plants. These systems have the advantage of being effective in detecting plant diseases. The challenges that these techniques face, include determining the best approach for a specific plant disease and automating techniques for continuous plant disease monitoring.

Ewis Omran [43] demonstrated a method for early detection of plant disease by focusing on the impact of fungal diseases such as leaf spots on peanut. *In-situ*

Fungus	Contribution	Reference
<i>Alternaria</i>	Detection of alternariol, alternariol monomethyl ether, and tentoxin from <i>Alternaria</i>	[31]
	Separation of <i>A. porri</i> , <i>A. dauci</i> , <i>A. tomatophila</i> and <i>A. solani</i> , from a complex	[32]
	Identification of 60 isolates, among them 12 were <i>Alternaria</i> species.	[33]
<i>Fusarium</i>	Identification and characterisation of <i>Fusarium verticillioides</i> and fumonisins.	[34]
	Differentiation of various <i>Fusarium</i> spp. based on spores	[35]
Downy and powdery mildew fungus	Identification of the <i>Bremia lactucae</i> and <i>Oidiumneo lycopersici</i> , from infected leave	[36]
<i>Bremia</i> , <i>Oidium</i>	Identification of ribosomal proteins and histones as markers for the biotyping of plant pathogens	[37]
<i>Gibberella</i>	Characterisation of <i>Gibberella zeae</i> conidia by on-target trypsin digestion	[38]
<i>Monilinia</i>	Identification of <i>Monilinia</i> brown rot fungi from infected fruits	[39]
<i>Trichoderma</i>	Direct identification of hydrophobins in <i>Trichoderma</i> isolates.	[40]

Adopted from Drissner and Freimoser [41].

**Table 2.**  
MALDI-TOF MS studies of agriculturally important fungi.

spectroscopy was used to identify early and late leaf indices. Thermal and spectral measurements were also used to differentiate between healthy and contaminated plant leaves. Later, a drop in plant chlorophyll was also observed.

Martinelli et al. [44–46] identified modern nucleic acid and protein analysis-based methods for identifying disease in plants. The authors also identified various mobility spectrometer and lateral flow devices that detect early infections directly on fluid, which summarised remote sensing technologies combined with spectroscopy-based methods and resulted in high spatialization whirlpools.

Ray et al. [47] used hyperspectral reflectance data from a spectro radiometer with spectral ranges of 770–860 nm and 920–1050 nm to detect late blight disease in potatoes and found a significant difference between healthy and diseased plants. Zhang et al. [48, 49] used a spectro radiometer with 32 spectral features and different models to compare normal and contaminated leaves' hyperspectral reflectance. Every model was thoroughly examined using t-tests, correlation analyses, and fisher linear discriminant analysis, and it was discovered that PLSR outperformed the MLR model. FLDA also included accurate information.

At an early stage, Romer et al. [50] proposed a method for distinguishing leaf rust wheat leaf from safe leaf. The authors provided pre-symptomatic identification, which was followed by classification using the support vector machine approach. It also shows how to collect different parameters using fluorescence detection using a fluorescence spectrometer. Further support vector machine was being used for classification for healthy and inoculated leaves.

#### 4. Spectroscopy based methods for remote sensing of plant disease

In remote sensing of plant diseases spectroscopy is among the most used methods which involves imaging or no-imaging sensors, visible wavelength, near infrared wavelength, and shortwave infrared wavelength. These techniques are considered reliable in crop disease monitoring as they are promising in operational instruments, efficacy, cost-efficiency and flexibility.

Under non-imaging spectroscopy approach of remote sensing of plant disease data is recorded based on inherent optical properties of leaf and leaf pigments, structural characteristics and chemical components [51]. Leaf spectra were collected either in field or laboratory to determine spectral regions (visible wavelength, near infrared wavelength, and shortwave infrared wavelength) to detect diseases. Some of the most studied fungal diseases using this method are wheat powdery mildew caused by *Erysiphe graminis* sp. *tritici* and take-all disease caused by *Gaeumannomyces graminis* sp. *tritici* [6, 7]. Apart from fungal diseases this method is also been used in detection of different viral diseases and numerous insect pest incidence in crop fields [44–46].

Nowadays, hyperspectral imaging instruments are being incorporated into monitoring and assessment of plant diseases. Some of the laboratory-based studies for imaging spectroscopy includes head blight and disease Fusarium fungal infection in wheat [52], early stage detection of diseases of sugar beet [53], and detection of sugar beet rust, *Cercospora* leaf spot and powdery mildew on sugar beet leaves [54]. A large range of statistical methods were applied under these studies for image analysis which includes principal component analysis (PCA), linear regression, support vector machine (SVM) classification and spectral angle mapper (SAM) classification producing high accuracy for detection of disease. Data obtained from both field and airborne hyperspectral were used to assess the severity of *Rhizoctonia* crown and root rot disease in sugar beet [55] and yellow rust in wheat [56].



## 5. Hyperspectral imaging

Image analysis is a new breakthrough in the field of plant disease identification and detection. Image analysis has huge potential in near as it is non-invasive and autonomus approach of detecting stress (biotic and abiotic) in plant [57]. It involves extraction of image from the images captured digitally. The image can be captured from a varied source viz., smart phones, digital camera, highly specialized cameras which are designed to extract variety of information from the image. Hyperspectral thermal, Multispectral, 3D sensor, Red Green Blue (RGB) method, Chlorophyll fluorescence are methods used in plant disease detection. Among these, Hyperspectral imaging and RGB is mostly preferred for plant disease identification [58]. In Hyperspectral imaging, camera is capable to capture light wavelength beyond visible range (400–700 nm). Human eye can perceive electromagnetic spectrum ranging from 400 to 700 nm but Hyperspectral imaging ranges from 250 nm (Ultraviolet, UV) to 2500 nm (Short-wave infrared range, SWIR). Camera is combined with some specific sensors to widen the coverage of the capturing spectrum. Usually, certain sub-range of electromagnetic range of radiation is captured by the camera viz., UV (250–400 nm) or visible and near infrared range (NIR) (400–1300 nm) or SWIR (1300–2500 nm). 400–700 nm wavelength are capable to detect changes in pigmentation of leaf while 700–1300 nm are to detect mesophyll cell structure, however extended range of wavelength 1300–2500 nm are needed to analyze content of water in plant.

## 6. Hyperspectral imaging technology

The image is captured in various way with the help of different hardware approach. The various hardware approaches include push broom, liquid crystal tunable filters, filter wheel others [59]. Among these, push broom technology involves incidence of light on a prism or convex grating leading to formation of narrow wavelength spectrum which further recorded on light sensitive chip (analogous to digital camera). A push broom device comprises of the camera, a lens and a spectrometer. This device involves simultaneous capture of single spatial line and whole range of colour spectrum. After scanning first line, camera moved to capture the next line and final image is formed. Camera act as a line scanner and after completion of scanning final image is formed. Snapshot is an alternative to push broom approach. Instead of providing point-and-click measurements, in Hyperspectral devices onus lies on the developer to develop capture process. Capture of image results in generation of large dataset sets which is further analyzed to obtain useful information. A simple and convenient way is to analyze this large dataset is to consider positions of small number in the captured wavelength. This approach facilitates countering the effects of relative changes in light by taking into account the ratios of data values. This is achieved by combining two or more wavelengths of light which is referred as “indices”. In order to interpret the captured data, numerous such indices have been formulated through pre-considered biological reasoning (eg. Knowledge that particular wavelength refers to the specific properties in cell structure) or because of limitations of particular wavelengths obtained from capture equipment (e.g. indices which are developed from data obtained from multispectral remote sensing, may have limited number of wavelength). When these indices are applied to plant material then referred to as vegetation indices. Several such indices (**Table 3**) exist and each indices uses distinct set of wavelength measurements to describe different physiological attributes of plant.

S.N.	Index	Formula	Information	Reference
1	Normalised difference vegetation index (NDVI)	$(RNIR - RRED) / (RNIR + RRED)$ RRED ~680, RNIR ~800	Range: - 1 to 1 Common range: 0.2-0.8 Broadband	[60]
2	Red edge NDVI	$(R750 - R705) / (R750 + R705)$	Range: - 1 to 1 Typical healthy range: 0.2 to 0.9 Narrowband (hyperspectral data)	[60]
3	Simple ratio index (SRI)	$RNIR / RRED$ RRED ~680, RNIR ~800	Range: 0 to >30 Typical healthy range: ~ 2-8 Broadband	[61]
4	Photochemical reflectance index (PRI)	$(R531 - R570) / (R531 + R570)$	Range: - 1 to 1 Typical healthy range: - 0.2 to 0.2 Vegetation health prior to senescence	[60, 62]
5	Plant senescence reflectance index (PSRI)	$(Red - Green) / NIR$	Range: - 1 to 1 Typical healthy range: - 0.1 to 0.2 >PSRI ~ canopy stress, onset of senescence, fruit ripening	[60]
6	Normalise dphaeophytinizationindex (NPQI)	$(R415 - R435) / (R415 + R435)$	Chlorophyll degradation 0.56-1.41 Unacidified and acidified solutions	[63]
7	Structure Independent Pigment Index (SIPI)	$(R800 - R445) / (R800 + R680)$	Range: 0-2 Typical healthy range: 0.8-1.8 Good with canopy variety	[60, 62, 64]
8	Leaf rust disease severity index (LRDSI)	$6.9 \times (R605 / R455) - 1.2$	Accuracy of 89% in study may vary with other data.	[65]

**Table 3.**  
 Vegetation indices, their formulae and information.

Normalised difference vegetation index (NDVI) is widespread and highly popular metrics used to measure the general crop health status [66, 67]. NDVI is used to detect biotic stress due to Sunn pest/cereal pest, *Eurygaster integriceps Put. (Hemiptera: Scutelleridae)* in wheat. There are many specific or disease centric indices which are helpful in detection and quantification of specific disease [61]. Leaf rust disease severity index (LRDSI) is an example of disease centric index having 87-91% accuracy in detection of wheat leaf rust (*Puccinia triticina*) [65].

Red edge approach is another commonly used method where abrupt rise in reflectance at the red/near infrared border is detected. The red edge position comprise of narrow section of electromagnetic spectrum (690-740 nm) where visible light spectrum ends and the NIR starts. This section of wavelength range showed good spectral response for green plant material. Chlorophyll has high absorption capacity and low reflectance for 700 nm light wavelength but it has strong reflection for infrared i.e., light wavelength starting from 720 nm. A red edge based disease index is largely used for detection of powdery mildew of wheat (*Blumeria graminis f.sp. tritici*). However, red edge approach has less accuracy than Partial least squares regression (PLSR) method. PLSR has a statistical approach.

## 7. Classification approach using subset of selected spectrum data

This approach involves subsampling of particular wavelength from the full spectrum. Unlike multispectral data, specific wavelength can be chosen

autonomously or manually from any position in the captured wavelength range. Wheat field experiment study involves NDVI response to remove all datasets except from the leaves followed by ANCOVA (Analysis of Co-variance) to detect specific wavelength of band which again followed by quadratic discriminant analysis (QDA) which distinguish the spectra of healthy plant from diseased leaves (yellow rust) [68]. This is the typical operation flow in hyperspectral image analysis. Use of QDA enhances accuracy up to 92% along with four bands [68]. Likewise, several techniques are used in plant disease detection in Hyperspectral imaging technology (**Table 4**).

Multi-layer Perceptron (MLP) approach detect yellow rust in wheat field uses a spectrograph of range 460–900 nm and 20 nm of spectral resolution [71]. The image is captured by the spectrograph by handheld system. Four significant light wavelengths were selected. The ‘variable selection’ method was employed for the selection of first two wavelengths using discriminant analysis and F-test. Another pair of wavelengths were selected by using NDVI wavelength. Moshou employed a neural network comprising four inputs, two outputs and one hidden layer consist of ten neurons. It has classification accuracy of 98.9% for healthy plants while 99.4%

S.N.	Technique	Plant disease	Accuracy	References
1	89 Quadratic discriminant analysis (QDA)	Wheat (yellow rust)	92%	[68]
		Avacado (laurel wilt)	94%	[69]
2	Decision tree (DT)	Avacado (laurel wilt)	95%	[69]
		Sugarbeet (cerospora leaf spot)	95%	[70]
		Sugarbeet (powdery mildew)	86%	
		Sugarbeet (leaf rust)	92%	
3	Multilayer perceptron (MLP)	Wheat (yellow rust)	98.9/99.4%	[71]
4	Partial least square regression (PLSR) Raw Savitsky-Golay 1st derivative Savitsky-Golay 2nd derviative	Celery (sclerotinia rot)	88.92%	[17]
			88.18%	
			86.38%	
5	Partial least square regression (PLSR)	Wheat (yellow rust)	92%	[72]
6	Fishers linear determinant analysis	Wheat (aphid)	60%	[48, 49]
		Wheat (powdery mildew)	90%	
		Wheat (powdery mildew)		
7	Erosion and dilation	Cucumber (downeymildew)	90%	[73]
8	Spectral angle mapper (SAM)	Sugarbeet (cerospora leaf spot)	89.01– 98.90%	[53]
		Sugarbeet (powdery mildew)	90.18–	[52]
		Sugarbeet (leaf rust)	97.23%	
		Wheat (head blight)	61.7%	
			87%	
9	Artificial neural network (ANN)	Sugarbeet (cerospora leaf spot)	96%	[70]
		Sugarbeet (powdery mildew)	91%	
		Sugarbeet (leaf rust)	95%	
10	Support vector machine (SVM)	Sugarbeet (cerospora leaf spot)	97%	[70]
		Sugarbeet (powdery mildew)	93%	[74]
		Sugarbeet (leaf rust)	93%	

**Table 4.**

*Techniques used in Hyperspectral imaging for detection plant diseases.*

for diseased plants. In machine learning, a highly sophisticated approach known as deep learning is gaining popularity. Deep learning consists of artificial neural network having a structure containing numerous layers. Each layer of neuron implicitly represent features obtained from the data which in turn complex information can be furnished from later layers and whole image features can be obtained from network. Convolutional neural networks (CNN) and Artificial neural networks (ANN) popularly used in deep learning. Using CNN, deep learning is reported to identify 26 diseases in 14 crop species [75]. AlexNet and GoogLeNet are two popular versions of CNNs having accuracy in disease detection up to 97.82% and 98.36% respectively. Both versions use datasets of 54306 images where 80% involve in training and 20% testing.

## **8. Disease identification**

Apart from detection of presence and absence of disease, research is focusing on distinguishing between different disease and identification of specific disease. Spectral information divergence classification is one of the approaches fulfilling this purpose. Comparative analysis is performed between observed spectra and available reference spectra (a library of diverse spectra). Spectral information divergence employed in detection of canker lesions on citrus. Greasy spot, melanose, insect damage, wind scar and scab were detected in grape with 95.2% classification accuracy [76].

## **9. Quantification of disease severity**

SAM (Spectral angle mapper) is an approach is used to quantify severity of plant disease. SAM approach matches pixel spectra to available reference spectra leading to classification of pixels. This classification involves calculation of angle between the spectra. These spectra further considered as n-dimensional vectors in the space [77]. This approach has moderate level of success and widely used by researchers. Yuhas et al. [77] recorded the Fusarium head blight severity in wheat using hyperspectral data of range 400–1000 nm and spectral resolution 2.5 nm. In quantification of disease severity, SAM accounts for 88% classification accuracy. Malhein et al. [78] also quantified disease severity of Cercospora leaf spot, powdery mildew, and rust in sugar beet using SAM approach.

## **10. Conclusion**

Early detection of plant disease plays key role in planning of plant disease management programme. Nowadays many non-destructive methods of plant disease detection are gaining popularity. Different spectroscopic methods offer a non-destructive method of plant disease detection. These methods use visible, ultraviolet, infrared and near-infrared lights to capture image of plant sample. Fluorescence microscopy, NMR, FTIR spectroscopy, ATR-FTIR spectroscopy, Raman spectroscopy, MALDI-TOF microscopy, and Hyperspectral imaging are different nondestructive and spectroscopy-based method of plant disease detection. Among all these methods visible, ultraviolet, infrared and near-infrared wavelength of lights are used for image analysis of diseased plant sample. In hyper spectroscopy imaging, image captured using visible, ultraviolet, infrared and near-infrared wavelength of lights were further analyzed by using artificial intelligence. As these methods are very much promising but still their accuracy needs to be improved.

Quantification of disease is also great concern. These methods are not very much promising in quantification of disease severity in plants. Future research must be focused on developing a system which give promising result regarding quantification of plant disease severity.

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