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FOURIER TRANSFORM - INFRARED (FTIR) SPECTRAL SIGNATURES OF *SESAMUM ORIENTALE* L. LEAVES FOR THE EARLY DETECTION AND DIFFERENTIATION OF *ALTERNARIA* LEAF SPOT DISEASE

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This study examines the potential of infrared spectral sensor systems for the non-destructive detection and differentiation of *Alternaria* leaf spot disease in *Sesamum orientale* L. IR reflectance spectra 4000-450/cm of leaves infected with the fungal pathogens *Alternaria* leaf spot were recorded repeatedly during pathogenesis with a Fourier Transform - Infrared (FTIR) and analyzed for disease-specific spectral signatures. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products. Absorption bands 1159, 1255, 1440, 1647 and 3456/cm were observed for both wild and cultivar sesame suggesting the FT-IR finger print for early identification of the disease. The pronounced peaks belonging to vibration of C=O, C-OH, C-N, and N-H were present in the spectra of diseased leaves indicating the presence of proteins, amino acids, carbohydrates, amides or phenols. Whereas, the marked reduction in intensities of the absorption peaks in the spectra of cultivar Thilarani treated indicates the reduction in the amount of compounds. Sesame may produce secondary metabolites and potentially other components in response to pathogen stress as indicated by changes in spectral features. Similarly, the wild and cultivar exposed to the pathogen, showed differential tolerance response towards *Alternaria* leaf spot disease.

Keywords : *Alternaria* leaf spot; *Alternaria sesami;* IR spectroscopy; *Sesamum orientale* L.

Introduction

Pests and pathogens cause serious damage to large number of crops with a significant negative feedback on economy. Early identification enables precise targeting a pathogen and enables the most effective treatment. Most commercially available identification systems are based on the physiological characteristics or on serological methods. Such identification systems are usually time consuming and not always very specific. Between the techniques offering possibilities for rapid analysis, molecular biology methods are considered the most rapid and sensitive methods for identification of pathogens, but they are not yet in large-scale use[1]. Recent developments in agricultural technology have lead to a demand for a new era of automated non-destructive methods of plant disease detection. It is desirable that the plant disease detection tool should be rapid, specific to a particular disease, and sensitive for detection at the early onset of the symptoms[2]. The spectroscopic and imaging techniques are unique disease monitoring methods that have been used to detect diseases and stress due to various factors, in plants. Recent reports in the literature support the notion that both volatile profiling and changes in spectral reflectance can be used for non-invasive field monitoring of plant diseases. Plants release organic compounds as a result of the metabolic activities taking place within its shoots, leaves, flowers, or fruits. The chemical profile of each plant due to pathogen infection differs significantly based on its physiological condition and the species. Various factors influence the profile from a particular plant, which may include changes in plant metabolism as a result of environmental changes, the age of plant, developmental stage of a plant, effect of stress on plants, and the presence of disease/herbivore in a plant[3]. Fourier Transform - Infrared spectra (FT-IR) promises to be of a great value because of its sensitivity, rapidity low expense and simplicity. This together with the large information already known about spectral peaks obtained from FTIR spectra of living cells make FTIR spectroscopy as an attractive technique for detection and identification of pathogens[4].

Leaf spot disease of sesame caused by *Alternaria sesami* appear mainly on leaf blades as small, brown, round

to irregular spots and is responsible for losses in grain yield of the *S. indicum*[5]. It is well established that the disease constitutes the single major threat in the yield level of sesame crop. Many researchers have reported the occurrence of this fungal disease in crops to be associated with the changes in the organic constituents. One of the important applications of the infrared spectroscopic study is the diagnostic value in establishing the presence of certain organic constituents in plants[6]. FT-IR spectroscopy provides more detailed chemical information on the samples composition because it measures the fundamental vibration. More recently FT-IR has been introduced as a metabolic fingerprinting tool for the plant sciences[7,8]. In such an attempt, either a frequency shift or the variation of the intensity of some characteristic absorption bands can be of some use. From the diseased leaves, compounds responsible for the disease are identified by the FT-IR analysis.

Material and Methods

Plant materials: Sesame cultivar Thilarani (susceptible to *A.sesami*) and wild species seeds were surface sterilized with two changes of sterile distilled water before sowing in 800 ml (16 cm height) pots containing a medium of soil : sand : FYM (Farm Yard Manure [decomposed cow dung] (2:1:1 v/v/v). Plants were maintained under controlled conditions. Irrigation was applied in the pots every third day with a beaker without touching the plant foliage. Wild and Thilarani the cultivar *Sesamum orientale* were raised from seeds in healthy conditions in a glasshouse.

For *in vitro* fungal inoculation studies, mature plants were inoculated with 20 µl of *Alternaria sesami* conidial suspension (1×10^3 conidia/ml) from pure culture or 20 µl of water (mock inoculation). The inoculated plants, along with their respective healthy controls, were then maintained at 30°C in a temperature controlled glasshouse under a photoperiod of 12/12 h (light/dark) and 60 % RH. After the development of symptoms in infected plants (after 10 and 14-d of inoculation in the wild and Thilarani plants respectively) the experiment was terminated and the plants harvested for all analysis.

IR spectroscopy: The leaves of each accession (approximately 3-4 cm) taken from plants were pooled as one sample. The samples were immediately dried in an oven for 2 days at 60 °C. Tablets for FTIR spectroscopy were prepared in an agate mortars, by mixing leaves powder (2 mg) with KBr (1:100 p/p). The absorbance spectra were measured between 300 and 4500/cm. At least three spectra were obtained for each sample[9].

A FTIR spectrometer (FTIR Shimadzu Prestige 21) was used to collect spectra. Spectra were obtained in 32 scans co-added, 4000 resolution, and 2.0 gains. The parameters for the Fourier self-deconvolution were a smoothing factor of 15.0 and a width factor of 30.0/cm. De-convolved and second-derivative spectra were calculated for Fourier self-deconvolution and the bands were selected and normalized to unity with Omnic 7 software. Curve-fitting of the original spectra was performed with Origin 7 software. The band position of functional groups was monitored with Knowitall 7.8 software. The spectral region between 3000 and 2800/cm was selected to analyze lipids. The spectral region between 1800 and 1500/cm was selected to analyze proteins. The spectral region between 1200 and 1000/cm was selected to analyze carbohydrates. Triplicate experiments (N = 3) were conducted and spectra from the first two times of experiments were used for establishment of chemometric models and the spectra from the third time of experiment were used for model validation.

Statistical analysis: Three independent replicate trials were conducted and significant differences ($P < 0.05$) between control plant and infected samples, band area of spectra, and regression coefficient of loading plot were determined by one-way analysis of variance (ANOVA) followed by t-test using Matlab.

Results and Discussion

FT-IR analysis: The IR spectrum of plant samples collected from wild and cultivar Thilarani (control and *Alternaria* infected) are shown in Fig. 1 A,B,C&D. A summary of the most characteristic absorption bands and their tentative assignments are given in Table 1 for the both treated and for the control sample. The FT-IR spectrum exhibits the characteristic finger print band features. IR spectra show absorption bands at 408.91, 621.08, 815.89, 1159.22, 1255.66, 1377.17, 1440.83, 1647.21, 2924.09 and 3456.44/cm for infected wild sesame plant. Similarly, for the infected cultivar Thilarani the IR bands are at 599.86, 734.88, 1070.49, 1155.36, 1257.59, 1440.83, 1458.48, 1658.78, 3348.72, 3369.64, 3390.86, 3408.22, 3427.51 and 3468.01/cm. Common FT-IR spectrum corresponding to all the *Alternaria* leaf spot diseased sesame caused by fungi *A.sesami* were visualized at the absorption bands 1159, 1255, 1440, 1647 and 3456 /cm.for both the infected sesame. The control plants showed 22 bands in the case of wild sesame while 25 in Thilarani sesame. Interestingly, no bands are common among the infected and control in both the cases.

The complexity of FT-IR spectra in the 1450 to 600 /cm region makes it difficult to assign all the absorption bands, and because of the unique patterns found there, it is often called the fingerprint region. Absorption

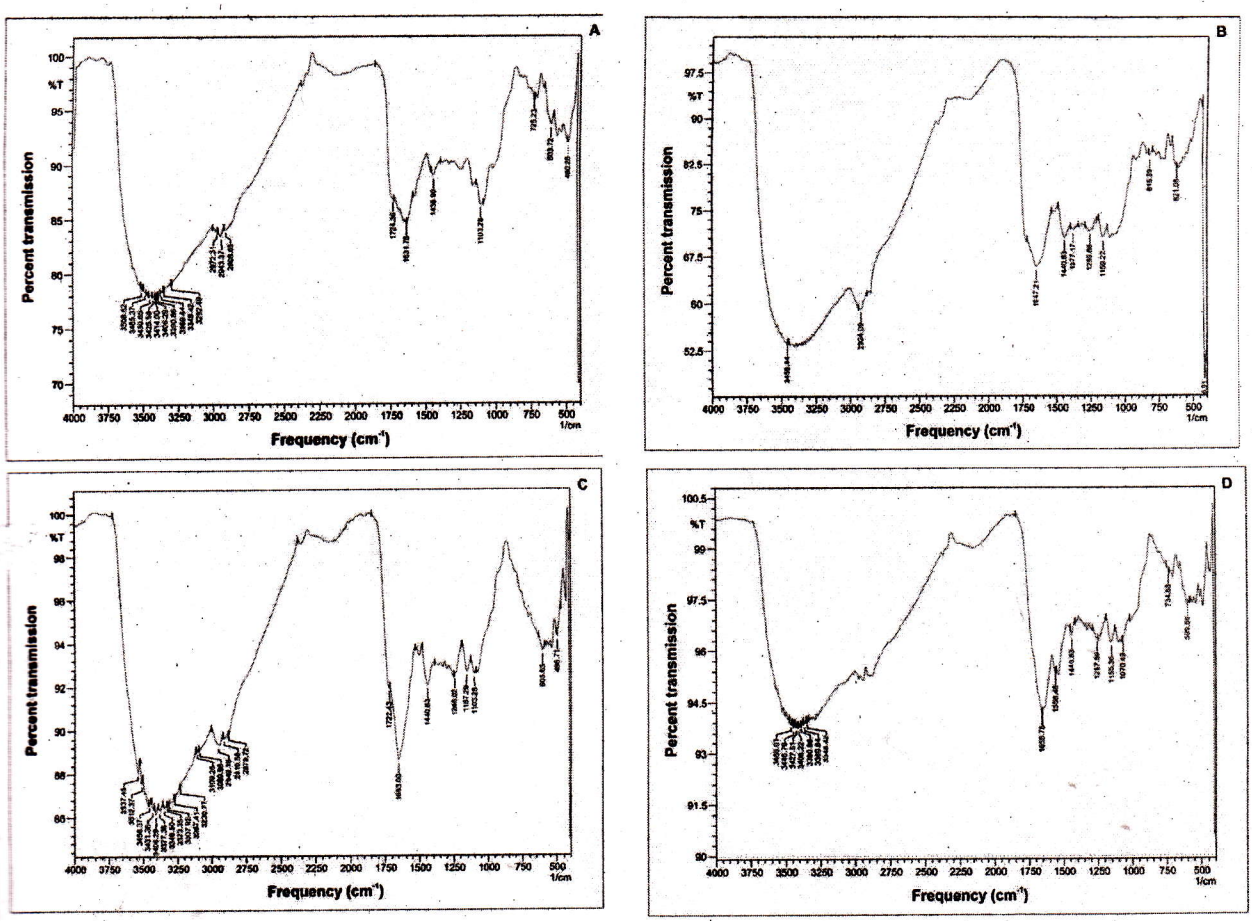

Fig.1. A, B, C & D. FT-IR Spectra of *Sesamum orientale* L. in wild (A - control & B - infected) and Thilarani cultivar samples (C - control & D - infected).

bands in the 4000 to 1450 /cm region are usually due to stretching vibrations of diatomic units, and this is sometimes called the group frequency region[10].

Alkanes are represented between 2850-3000 CH_3, CH_2 & CH; alkenes 1630-1680 C=C; alcohols and phenols 3200-3550, 970-1250 O-H (H-bonded), usually broad C-O; amines 3400-3500 N-H (1°-amines), 1000-1250 two bands, C-N carboxylic acids and its derivatives 2500-3300 (acids) overlap C-H O-H (very broad) 1210-1320 (acids) O-C (sometimes 2-peaks) 1000-1300 O-C (2-bands) 1630-1695(amides) C=O (amide I band) 1350-1470 CH_2 and CH_3 deformation 1370-1390 CH_3 deformation 780-850 =C-H and $=CH_2$ 600-700 C-H deformation 690-900 C-H bending and ring puckering 1330-1430 O-H bending (in-plane) 1550-1650 NH_2 scissoring (1°-amines) 660-900 NH_2 and N-H wagging (shifts on H-bonding) 1400-1450 α-CH_2 bending 1395-1440, C-O-H bending S-OR esters 700-900[11,12].

The absorption bands at 3420 and 3434/cm are representative for C-H, O-H and N-H stretching vibrations, characteristic of the presence of amino acids. In all samples, it is noticed that the bands at 2918, 2921 and 2928/cm are due to the stretching vibration of -CH_3 and -CH_2 groups[13,14] indicative of the chlorophyll groups. The 1632, 1640 and 1629/cm bands are due to stretching vibration of carbonyl group characteristic of the secondary amides and other compounds containing C=0 group[15,16]. The bands at 1434/cm and 1411/cm represent the bending vibrations of CH indicative of the lignins. The 1232 and 1216 /cm bands in all samples predict the presence of ester carbonyl[17-19]. The C-O-C groups exhibit strong bands at 1095/cm and very strong bands at 1101/cm respectively. The absorption bands at 1100 - 1000 /cm in the fingerprint region indicate several modes such as C-H deformation or C-O or C-C stretching, pertaining to carbohydrates. Carbohydrates in the leaves were the major constituents

Table 1. IR spectral analysis of control(C) and *Alternaria sesami* infected (I) wild and Thilarani sesame.

Peak	Wild		Thilarani	
	C	I	C	I
406.98	-	-	+	-
408.91	-	+	-	-
480.28	+	-	-	-
495.71	-	-	+	-
557.43	+	-	-	-
599.86	-	-	-	+
603.72	+	-	-	-
605.65	-	-	+	-
621.08	-	+	-	-
731.02	+	-	-	-
734.88	-	-	-	+
815.89	-	+	-	-
831.32	+	-	-	-
1070.49	-	-	-	+
1103.28	+	-	+	-
1155.36	-	-	-	+
1157.29	-	-	+	-
1159.22	-	+	-	-
1244.09	+	-	-	-
1246.02	-	-	+	-
1255.66	-	+	-	-
1257.59	-	-	-	+
1377.17	-	+	-	-
1438.9	+	-	-	-
1440.83	-	+	+	+
1558.48	-	-	-	+
1631.78	+	-	-	-
1647.21	-	+	-	-
1653.0	-	-	+	-
1658.78	-	-	-	+
1722.43	-	-	+	-
2879.72	-	-	+	-
2908.65	+	-	-	-
2910.58	-	-	+	-
2924.09	-	+	-	-

2943.37	+	-	-	-
2949.16	-	-	+	-
2973.31	+	-	-	-
3089.96	-	-	+	-
3109.25	-	-	+	-
3230.77	-	-	+	-
3267,41	-	-	+	-
3292.49	+	-	-	-
3307.92	-	-	+	-
3323.35	-	-	+	-
3346.5	-	-	+	-
3348.42	+	-	-	+
3369.64	+	-	-	+
3377.36	-	-	+	-
3390.86	+	-	-	+
3406.29	+	-	+	-
3408.22	-	-	-	+
3414.0	+	-	-	-
3425.58	+	-	-	-
3427.51	-	-	-	+
3431.36	-	-	+	-
3446.79	-	-	-	+
3450.65	+	-	-	-
3456.44	-	+	-	-
3458.37	-	-	+	-
3468.01	-	-	-	+
3485.37	+	-	-	-
3508.52	+	-	-	-
3512.37	-	-	+	-
3537.45	-	-	+	-

of these absorption bands[20,21]. The peak at 1032 /cm to 1070/cm in the spectrum also indicates the starch content in the sample. The stronger the relative intensity of the band, the higher the chemical constituents. The secondary peaks at 770-922/cm are assigned as characteristic absorption of the carbohydrate[22]. The absorbance bands at 837-721/cm represent C-H in plane and out of plane bending for the benzene ring and bands at 553-633/cm represent C-O-O and P-O-C bending of aromatic compounds (phosphates). The infrared spectrum is able to identify not only the major components in organic materials, but also to find some differences among them. These differences may be due to the industrial environment.

From the spectrum, one can notice that the bands 3485, 3568, 3512 and 3537/cm are present in the samples only in control and they are absent in treated samples. The bands 2924/cm indicating the chlorophyll groups are strongly present in wild treated sesame and was absent in the cultivar Thilarani which was also supported

morphologically as paleness suggesting the susceptibility nature of the cultivar. The secondary amides 1631 to 1653/cm are present in the samples in all infected samples and the absorption is also strong. It is also strong in the control sample. Likewise the lignins are present in the sample from strong absorption to weak absorption whereas it has medium absorption in the control sample. The presence of carbohydrate and starch (1100-1000/cm) in the samples is varying from strong to medium absorption, but they have medium absorption in the control sample. The intensive broad absorption band appears in the characteristic carbohydrate region with a maximum at 1058 and 1033/cm[20] . The phosphate groups are present with a medium to weak absorption band whereas in the control sample it possesses medium absorption through 544-633/cm.

FT-IR (4000 - 400/cm) spectra of the sesame leaves exhibit the absorption bands of chromophoric group characteristics of phenols, amino acids and proteins. The wild and cultivar showed diversity in peak intensities which in turn suggests the differential tolerance mechanism in the plant. The results suggest that this method of disease detection show a good potential with an ability to detect plant diseases accurately and early. The FT-IR technology could be integrated with an autonomous agricultural vehicle for reliable and real-time plant disease detection to achieve superior plant disease control and management. Further studies will focus on explorative multifactor approaches for investigating pathogen injury under various stresses, including DNA microarray, scanning electron microscopy, and vibrational spectroscopy.

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