Journal of Biotechnology and Crop Science
Vol. 10, No. 15, January-December, 2022, 102-110

doi: 10.5958/2582-5089.2022.00012.3

Research Article

Fourier transform infrared spectroscopic characterization of powdery mildew infection in mulberry leaves

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ABSTRACT

Mulberry leaves are affected by a variety of fungal diseases, which alter the biochemical constituents and make the leaves for silkworm feeding. Powdery mildew (PM) infection is one of the most important fungal diseases in the sericulture industry. FTIR spectra of healthy and powdery mildew infected leaves of different mulberry varieties (AR_{12}, AR_{14}, BR_2, S_1, S_{13}, $S_{146,}$ $S_{1635,}$ TR_{10} and V_1) were recorded in this study to obtain possible spectral traits in PM infection detection and characterization. FTIR spectra were used to determine the types of vibrations (stretching and bending), visible intensities (v, sh, m, w) and infra red (IR) wave numbers (4000 cm^{-1} – 400 cm^{-1}) of prominent peaks. The effect of powdery mildew infection was attributed to the probable differences in the spectra of healthy and infected leaves. In different varieties of mulberry leaves FTIR data reveal the presence of bonded O–H, N–H, C–H, C–C, C–O, C=O, C–O–C, SO_3, and O–N=O functional groups and biochemical constituents such as phenol, amine, alkene, alkane, alkyne, carboxyl, ester, ether, polysaccharide, nitrite and sulfur compounds. The bonded O–H stretch observed in AR_{12} and V_1 infected mulberry leaves at 3734 cm^{-1} and 3600.5 cm^{-1}, respectively attributes the production of phenol due to powdery mildew infection. Similarly, the bands observed at 2967.8 cm^{-1} – 2958.3 cm^{-1} due to C–H asymmetric stretching only in S_{146}, S_{1635}, AR_{12} and V_1 corresponds to the powdery mildew infection. Also, the weak absorption at 2378.9 cm^{-1} to 2325.1 cm^{-1} observed in AR_{12}, AR_{14}, V_1, S_1, S_{13}, S_{146} and S_{1635} varieties represents the effect of powdery mildew infection and production of carboxyl group (C-O) in different varieties of mulberry leaves. The wave number of the prominent peaks of powdery mildew infected and healthy leaves differed significantly (t = 9.63, df = 80, p 0.001).

Keywords: FTIR spectroscopy, functional groups, fungal infection, mulberry leaves, powdery mildew

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INTRODUCTION

Mulberry (*Morus* spp.) is the exclusive food plant of silkworm [*Bombyx mori* (L.)]. The leaf quality of mulberry plays an important role in silkworm rearing (Muthulakshmi et al., 2010). Physical and chemical properties of leaves directly affect the amount of leaf consumed and digested by the silkworm (Das & Vijayaraghavan, 1990). Mulberry crops are affected by a variety of fungal diseases such as powdery mildew, leaf blight, leaf spot, leaf rust and dwarfing (Sharma et al., 1993; Reddy et al., 2009). Powdery mildew is caused by *Phyllactinia corylea* (Itoi et al., 1982; Braun, 2002; Takamatsu et al., 2008; Bizhannia et al., 2010;

Chattopadhyay et al., 2011). The powdery mildew disease is characterized by white dust-like mycelia that develop over abaxial leaf surfaces. The heavily infected tissues develop chlorosis and senesce prematurely (Gupta, 2001). Hyphae of *P. corylea* produce two kinds of special branches on the host surface: adhesion bodies which serve as fungal attachment and stomatopodia which enter the leaf through stomata (Babu et al., 1999). Feeding of diseased leaves prolongs larval period and reduces cocoon yield (Noamani et al., 1970; Umesh Kumar et al., 1993).

The morphological characters, mainly the reproductive structures were traditionally used for identification of filamentous fungi. Though, molecular analysis is an effective tool for chemical characterization but expensive and time consuming. FTIR spectroscopy is one of the oldest, convenient, non-destructive and well established experimental technique needs less sample preparation (Kong & Yu, 2007). The FTIR spectroscopy was used to observe the changes in chemical structure of fungal infected wood (Pandey & Nagveni, 2007). Santos et al. (2010) reported that the Fourier Transform Infrared Spectroscopy (FTIR) is a powerful technique for characterizing chemical compositions of microorganisms. Hawkins et al. (2010) used FTIR spectroscopy to study the spectrum of infected citrus plants. FTIR spectroscopy has been recognized as a valuable tool for determination of protein in both solution and dried states (Elliott & Ambrose, 1950; Krimm & Bandekar, 1986; Surewicz & Mantsch, 1988). The current study aimed to use FTIR to investigate the biochemical constituents in healthy mulberry leaves as well as the biohemical changes in powdery mildew infected mulberry leaves

MATERIALS AND METHODS

The study was carried out at Dr. Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India during August 2012 to February 2013. Healthy and powdery mildew infected mulberry leaves of different varieties of *Morus alba* (AR_{12}, AR_{14}, BR_2, S_1, S_{13}, S_{146}, S_{1635}, TR_{10} and V_1) taken from BBAU germplasm centre Lucknow were carefully collected and placed separately in polythene bags. The samples were air dried in a clean environment at 25°C for 12-13 days. The dried sample was crushed into fine powder by using a clean mortar and pestle, and 1 mg of sample was mixed with 100 mg of dry potassium bromide (KBr). The KBr based pellets were compressed into thin disks using a hydraulic press (PCI Analytic, India) by establishing about 15 ton pressure. The disks were fixed into the sample holder of FTIR spectrometer (Nicolet 6700, Thermo) and analyzed the spectra with a total of 32 scans against KBr background. The FTIR spectra were collected for the wave number 4000-400 cm^{-1}. Three replications were used for each variety of healthy and powdery mildew infected mulberry leaves. Peak heights of spectra were measured using OMNIC software. The IR spectra of healthy and powdery mildew infected leaves were assigned by following (Dyer 2006). The wave number of prominent peaks of healthy and infected leaves were compared using paired '*t*' test.

RESULTS

The symptom of powdery mildew infection was characterized by white powdery patches on the lower surface of mulberry leaves. The white powdery patches spread to the entire leaf surface and the leaf turned into blackish brown. The powdery mildew infection was higher during rainy and moist period of the year. The powdery mildew infection was observed in AR_{12}, AR_{14}, BR_2, TR_{10}, S_1, S_{13}, S_{146}, S_{1635} and V_1 mulberry varieties. The FTIR spectra of healthy and powdery mildew infected leaves were assigned to wave number 4000 cm^{-1}–400 cm^{-1} and the details are given in (Tables 1 and 2). The stacked FTIR spectra of healthy and powdery mildew infected leaves of different mulberry varieties (AR_{12}, AR_{14}, BR_2, S_1, S_{13}, S_{146}, S_{1635}, TR_{10} and V_1) are shown in (Figure 1, 2, 3, 4 and 5) respectively. The wave number of prominent peaks of powdery mildew infected

Table 1: Wave numbers and functional groups of infected and healthy leaves of AR_{12}, AR_{14}, BR_2 and V_1 mulberry varieties

Functional groups and vibrations	AR_{12}		AR_{14}		BR_2		V_1	
	I	H	I	H	I	H	I	H
O–H stretching, v & sh (Alcohols & Phenols)	3734.0						3800.5	
N–H stretching, v, s & sh (Primary Amine)	3397.9	3388.1	3380.1	3358.5	3429	3388.0	3377.0	3405.1
C-H asym. stretching, v & s (Alkene & Alkane)	2967.8						2960.7	
C-H asym. stretching, v & s (Alkane)	2920.1	2921.8	2922.1	2922.4	2922.4	2922.0	2928.6	2920.1
C-H symm. stretching, w (Alkene)	2852.9	2853.4	2853.4	2855.0	2852.7	2853.3	2853.8	2852.2
C-O bands, m (Carboxyl group)	2350.4	2366.3, 2331.4			2363.2		2360.7	2375.7
C-C stretching, m (Alkyne)	2251.5				2123.7		2287.1	
C=O stretching, s (Aliphatic esters)	1733.0	1729.8	1729.8	1733.9		1733.0	1739.3	1734.5
N-H bending, s (Amine)	1643.4	1649.6	1647.4	1549.3	1637.9	1650.3	1649.9	1651.9
C-O/O-H bending, w (Alcohols & Phenols)	1419.7	1414.1	1421.1	1415.2	1452.1	1417.8	1437.4	1415.4
C-H bending, s (Alkane)					1372.6		1372.6	
S=O stretching, v (Sulphone)	1316.9	1316.9	1316.2	1316.2		1315.0		1318.6
C-O, bonded, s (Ether)	1241.6	1254.9	1243.5		1249.2	1245.0	1242.0	1248.6
C-O-C symm. stretching, s (Polysaccharides)		1131.4, 1056.8		1054.2			1150.4	1056.8
SO_3 stretching, s (Sulfur compounds)	1075.5	1096.6	1073.2		1069.5	1073.1	1074.1	
C-H bending, m (Aromatic compound)	779.5	779.9	778.2	767.3	770.4	776.6	795.8	779.9
O-N=O bending, w (Nitrite)	615.3	681.8	628.0			662.8	662.8	
S-S stretching, w (Sulphide)	535.4	598.5, 533.0	534.8	533.3	557.5	534.0	578.7	532.5

Note: I – powdery mildew infected mulberry leaves, H – healthy mulberry leaves, m - medium, sh - sharp, s - strong, v - variable, w - weak, asym - asymmetric, symm - symmetric. Dyer (2006) and Mohan (2005)

leaves and healthy leaves showed a significant difference (t = 9.63, df = 80, $p < 0.001$).

The absorption bands observed only in AR_{12} and V_1 infected mulberry leaves at 3734 cm^{-1} and 3800.5 cm^{-1}, respectively due to bonded O–H stretching which show the presence of phenols and alcohols. The strong absorption bands observed between 3400.1 cm^{-1} and 3358.5 cm^{-1} due to the bonded N–H stretching show the presence of primary amine in all the mulberry variety.

The variable strong bands observed at 2928.6 cm^{-1}–2920.1 cm^{-1} due to asymmetric stretching of bonded C–H show the presence of alkene (CH_2) and alkane (CH_3) groups in the leaves of all mulberry varieties. However, the multiple bands observed at 2967.8 cm^{-1}–2958.3 cm^{-1} due to bonded C–H asymmetric stretching attribute the presence of alkene and alkane mainly in infected leaves of $AR_{12,}$ S_{146}, S_{1635} and V_1 varieties (Table 1). In addition, the bands observed at 2855.0 cm^{-1}–2847.5 cm^{-1} in all mulberry varieties due to C–H symmetric stretch show the presence of alkene as a common biochemical constituent.

The C–O bands at 2378.9 cm^{-1}–2325.1 cm^{-1} due to carboxyl group were predominantly observed in powdery mildew infected mulberry leaves of AR_{12}, AR_{14}, BR_2, S_1, S_{13}, $S_{146,}$ S_{1635} and V_1 varieties. The multiple bands observed at 2140 cm^{-1}–2100 cm^{-1} and at 2287.1 cm^{-1}–2190.2 cm^{-1} due bonding of C–C stretching show the

Table 2: Wave numbers and functional groups of infected and healthy mulberry leaves of S_1, S_{13}, S_{146}, S_{1635} and TR_{10} mulberry varieties

Functional groups and vibrations	S_1		S_{13}		S_{146}		S_{1635}		TR_{10}	
	I	H	I	H	I	H	I	H	I	H
N–H stretching, v, s & sh (Primary Amine)	3366.9	3396.4	3380.5	3404	3400.1	3384.0	3365.7	3379.6	3386.9	3404
C-H asym. stretching, v & s (Alkene & Alkane)					2958.3	2961.5	2958.3			
C-H asym. stretching, v & s (Alkane)	2922.0	2921.2	2921.5	2922.9	2920.4	2922.9	2921.9	2924.5	2922.2	2921.9
C-H symm. stretching, w (Alkene)	2853.4	2853.2	2852.9	2854.2	2852.5	2854.3	2853.5	2847.5	2853.7	2853.6
C-O bands, m (Carboxyl group)	2359.9		2325.1		2363.1		2378.9			
C-C stretching, m (Alkyne)	2131.9					2269.2			2290.2, 2103.4	
C=O stretching, s (Aliphatic esters)	1723.5	1734.2	1736.1	1735.1	1726.6	1733.0	1736.1	1742.5	1733.5	1735.4
N-H bending, s (Amine)	1650.4	1652.0	1649.6	1652.1	1643.5	1650.2	1648.0	1650.4	1649.5	1652.8
N-H bending, w (Amine)	1549.3			1549.3						
C-O/O-H bending, w (Alcohols & Phenols)	1418.0	1416.1	1417.0	1414.1	1427.2	1437.7	1420.4	1412.9	1415.6	1415.8
S=O stretching, v (Sulphone)	1316.3	1315.5	1316.8	1314.3	1308.7	1319.5	1319.0	1315.0	1313.9	1312.2
C-O, bonded, s (Ether)	1243.7	1248.8	1244.4	1252.2	1238.6	1242.6	1238.7	1250.9	1252.0	1255.7
C-O-C symm. stretching, s (Polysaccharides)		1055.8		1054.1				1054.5		1054.3
SO_3 stretching, s (Sulfur compounds)	1077.6		1072.3		1077.2	1073.4	1075.3		1068.5	
C-H bending, m (Aromatic compound)	776.3		774.8		783.9	778.5	780.4	773.6	770.9	
O-N=O bending, w (Nitrite)	675.5		685.0	669.1	656.5		685.0		650.1	
S-S stretching, w (Sulphide)	535.8	534.4	593.1, 534.5	593.8	529.8	597.4	537.8	598.7	533.5	533.9

Note: I - powdery mildew infected mulberry leaves, H – healthy mulberry leaves, m - medium, sh - sharp, s - strong, v - variable, w - weak, asym - asymmetric, symm - symmetric. Dyer (2006) and Mohan (2005)

presence of monosubstituted and disubstituted alkyne, respectively in AR_{12}, BR_2, S_1, TR_{10}, and V_1. The spectral band observed at 1742.5 cm^{-1}–1723.5 cm^{-1} due to C=O stretch vibration corresponds to presence of saturated aliphatic esters.

The secondary structure of amine due to N–H bending observed between 1652.8 cm^{-1}–1549.3 cm^{-1}. The bands observed at 1452.1 cm^{-1}–1412.9 cm^{-1} may be due to bonded C–O / O–H bending in alcohols and phenols. The band observed at 1372.6 due to C–H bending shows the presence of alkane in infected V_1 and BR_2 mulberry varieties. The IR spectral range observed at 1319.5 cm^{-1}–1308.7 cm^{-1} due to S=O stretching shows the presence of sulphone in the leaves of different mulberry varieties.

The bonded C–O absorbed IR at 1255.7 cm^{-1}–1238.6 cm^{-1} show the presence ether functional groups in mulberry leaves. The strong bands observed at 1150.4 cm^{-1}–1054.2 cm^{-1} due to C–O–C symmetric stretching shows the presence of polysaccharides. The spectral band observed at 1077.6 cm^{-1}–1068.5 cm^{-1} due to SO_3 symmetric stretching shows the presence of sulfur compounds. The medium bands observed at 795.5 cm^{-1}–770.9 cm^{-1} due to C–H out-of-plane bending show the presence of aromatic compound with substitution of hydrogen. The band observed at 685 cm^{-1}–615.3 cm^{-1} due to O–N=O bending shows the presence of nitrite. The weak absorption bands observed at 578.7 cm^{-1}–529.8 cm^{-1} may be due to S–S stretching show the presence of sulphide and disulphide compounds.

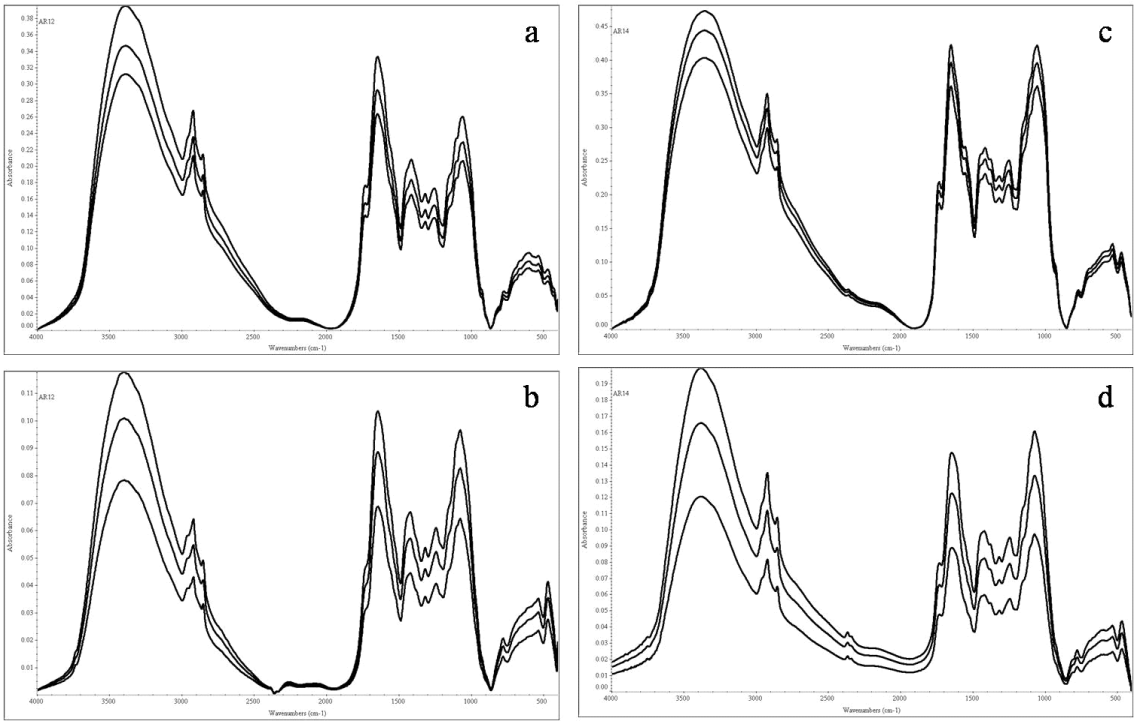

Figure 1: The stacking FTIR spectra of AR_{12} (a) and AR_{14} (c) healthy and AR_{12} (b) and AR_{14} (d) infected mulberry leaves. Each variety of healthy and infected mulberry leaves was replicated three times and stacked.

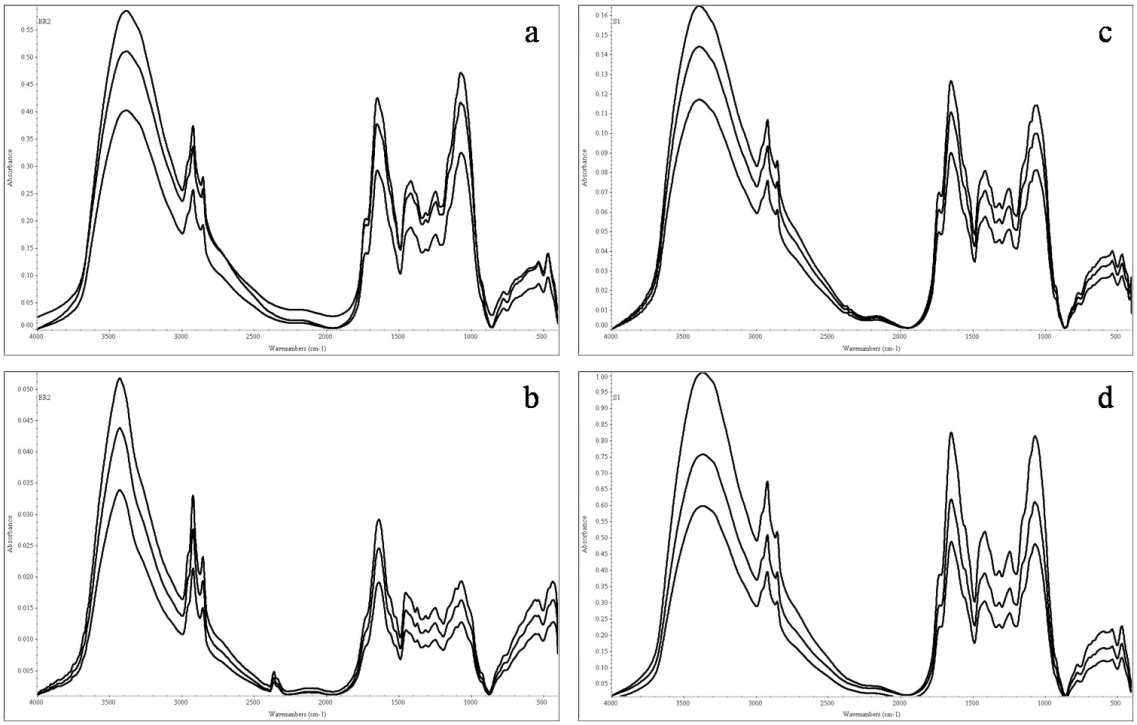

Figure 2: The stacking FTIR spectra of BR_2 (a) and S_1 (c) healthy and BR_2 (b) and S_1 (d) infected mulberry leaves. Each variety of healthy and infected mulberry leaves was replicated three times and stacked.

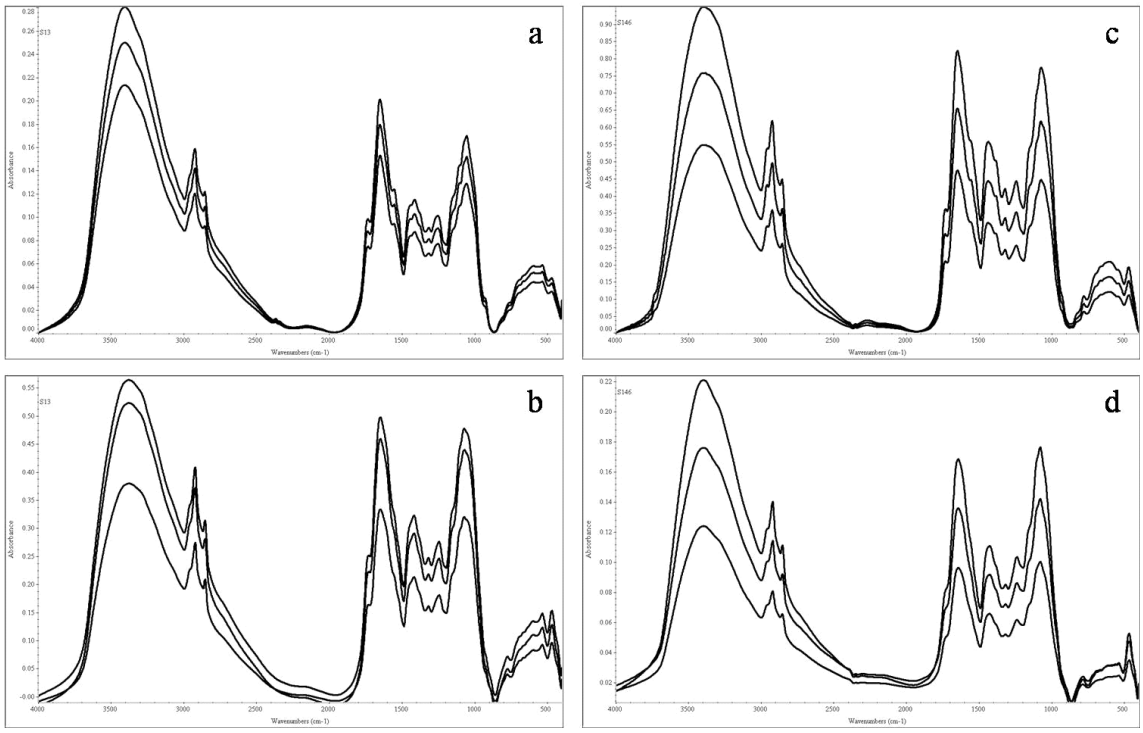

Figure 3: The stacking FTIR spectra of S_{13} (a) and S_{146} (c) healthy and S_{13} (b) and S_{146} (d) infected mulberry leaves. Each variety of healthy and infected mulberry leaves was replicated three times and stacked.

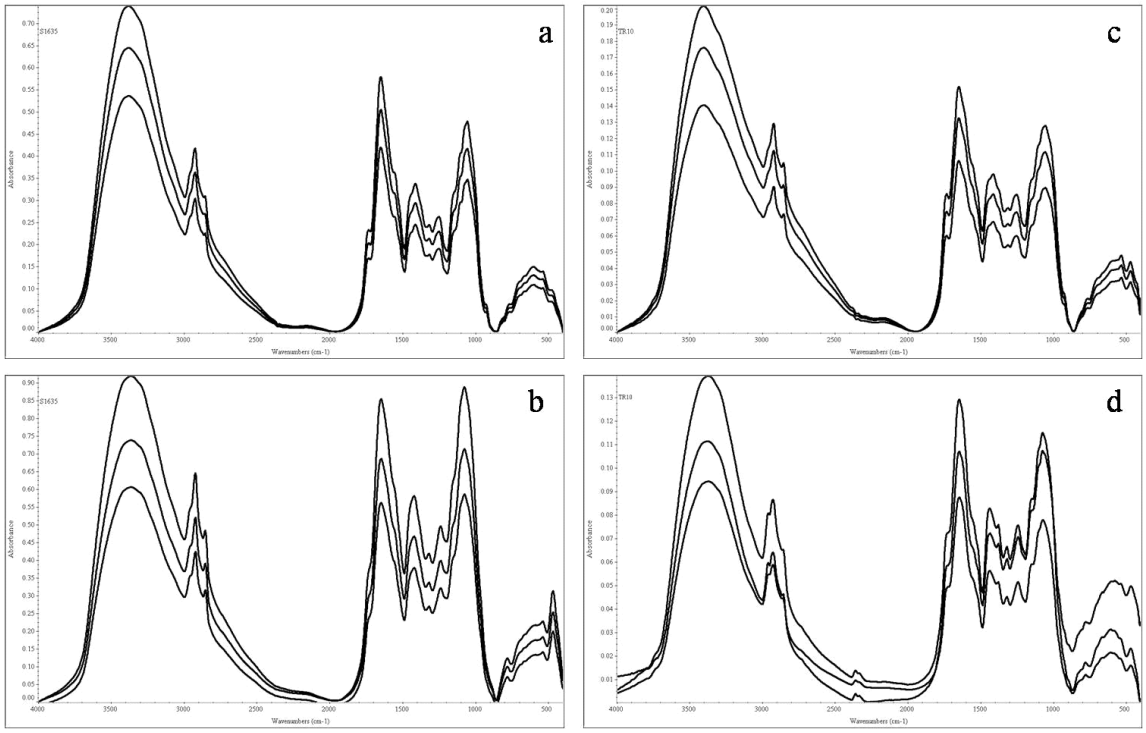

Figure 4: The stacking FTIR spectra of S_{1635} (a) and TR_{10} (c) healthy and S_{1635} (b) and TR_{10} (d) infected mulberry leaves. Each variety of healthy and infected mulberry leaves was replicated three times and stacked.

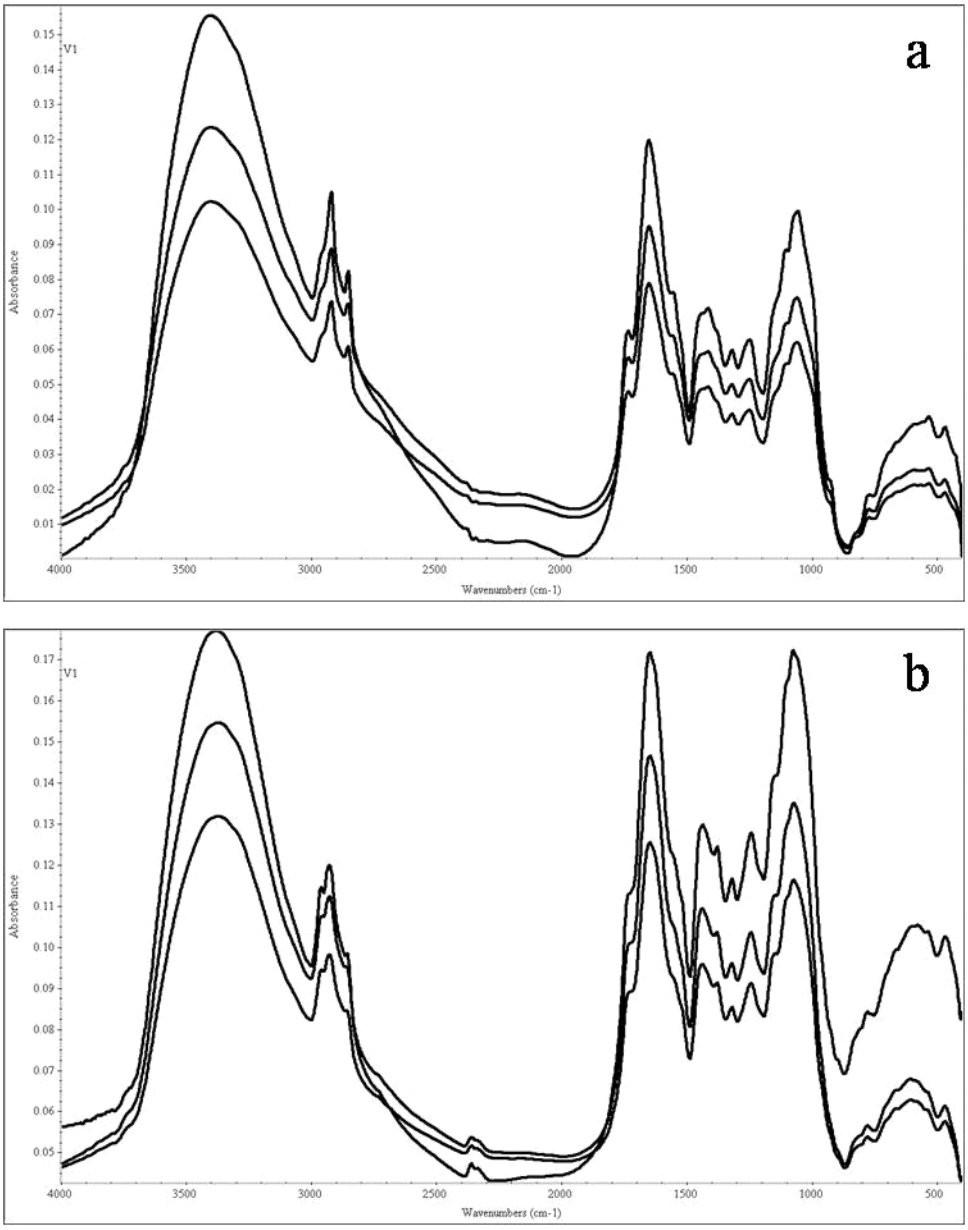

Figure 5: The stacking FTIR spectra of V_1 (a) healthy and V_2 (b) infected mulberry leaves. Each variety of healthy and infected mulberry leaves was replicated three times and stacked.

DISCUSSION

Severely affected leaves become yellowish and fall prematurely on leaf spot of Mulberry plants could be influence of *Alternaria alternata* The results reveal that the powdery mildew infection deteriorates the mulberry leaf quality. The fungal degradation resulted a significant change in the biochemical properties and thus in the intensities of IR spectra of infected mulberry leaves (Tables 1 and 2). Further, it gives detailed information on functional groups and biochemical compositions of the leaves of different mulberry varieties. The spectral bands observed between 3400 cm^{-1}–3300 cm^{-1} assigned in the present study as N–H stretching shows the

presence of primary amine in all mulberry varieties and the assignment of wave number consistent with earlier reports (Muruganantham et al., 2009; Silverstain & Webster, 1998). The bands observed between 2928.6 cm^{-1}–2920.1 cm^{-1} as well as 2855.0 cm^{-1}–2847.5 cm^{-1} in the present investigation correspond to the earlier studies which assigned the wave number to C–H stretch vibrations (asymmetric and symmetric) and confirm the presence of alkene (CH_2) groups (Rao, 1963; Dyer, 2006; Muruganantham et al., 2009). Silverstain & Webster (1998) observed multiple bands between the wave number 2140 cm^{-1} and 2100 cm^{-1} and reported the presence of monosubstituted alkyne.

The O–H bonds observed only in AR_{12} and V_1 infected mulberry leaves at 3734 cm^{-1} and 3800.5 cm^{-1}, respectively showed the production of phenol / alcohol due to powdery mildew infection. The multiple bands observed at 2928.6 cm^{-1}–2920.1 cm^{-1} due to C–H asymmetric stretching only in $AR_{12,}$ S_{146}, S_{1635} and V_1 corresponds to the powdery mildew infection. The weak absorption at 2378.9 cm^{-1}–2325.1 cm^{-1} observed in AR_{12}, AR_{14}, S_1, S_{13}, $S_{146,}$ S_{1635} and V_1 varieties attributes the effect of powdery mildew infection on biochemical property of the leaves. Harendra et al. (2021) reported that highest infection of powdery mildew was observed in the S_{146} Mulberry variety and least was in V_1 variety. The results of current study show a profound effect of powdery mildew infection on biochemical constituents of infected mulberry leaves. According to the findings of this study, powdery mildew infection alters the biochemical constituents of mulberry leaves such as phenol, amine, alkene, alkane, alkyne, carboxyl, ester, ether, polysaccharide, nitrite and sulfur compounds, which may affect the silkworm growth and development.

ACKNOWLEDGEMENTS

The authors are thankful to the University Science Instrumentation Centre for extending FTIR facility. The authors are grateful to Dr. Venkatesh Kumar, R., Department of Applied Animal Science for extending laboratory facilities during the course of research. HK and MK are recipients of UGC Rajeev Gandhi National Fellowship.

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