FOURIER TRANSFORM – INFRARED SPECTRA (FT-IR) ANALYSIS OF ROOT ROT DISEASE IN SESAME (*SESAMUM INDICUM*)

B. GOKULAKUMAR , R. NARAYANASWAMY**#*

*Department of Physics, **Department of Engineering Physics,
Annamalai University, Annamalainagar – 608002, Tamilnadu, India
#profrns@rediffmail.com

Abstract. A study has been made for five different sesame varieties, namely CO-1, VRI-1, VRI-2, TMV-3 and TMV-4 which were grown in trial fields with three different manure treatments viz. control (T_1), chemical fertilizer (T_2) and organic manure (T_3). From the root rot diseased roots, the compound identified and quantified from the five sesame varieties by FT-IR analysis and the spectra were recorded for all the samples in the range of 4000 – 400 cm^{-1}. The pronounced peaks belonging to vibration of C=O, C–OH, C–N, and N–H were present in the spectra of diseased roots indicating the presence of diketopiperazine. Whereas, the marked reduction in intensities of the absorption peaks in the spectra of organic manure treated varieties indicates the reduction in the amount of diketopiperazine. Also an attempt has been made to correlate the extinction coefficient (*K*) values with the changes in diketopiperazine of the root rot diseased roots in sesame varieties.

Key words: FT-IR, sesame, root rot disease.

INTRODUCTION

Sesame (*Sesamum indicum*) is one of the major ancient oil seed crops in India. Although it has been cultivated for a long time, the significant increase in productivity is yet to be achieved. It is well recognized that the disease constitutes a major constraint in increasing the yield level of sesame crop. Root rot caused by *Macrophomina phaseolina* is the most devastating disease limiting the production in almost all the sesame growing areas of Tamilnadu. Root rots disease caused by *M. phaseolina* is an important disease of sesame (*Sesamum indicum*) causing 5 to 60 percent loss of yield under the field conditions [3]. Many researchers have reported the occurrence of this fungal disease in crops to be associated with the

Received: March 2008;
in final form June 2008.

ROMANIAN J. BIOPHYS., Vol. 18, No. 3, P. 217–223, BUCHAREST, 2008

changes in the organic constituents [6, 8, 13]. The root rot disease of sesame is too amenable for chemical management and most of the cultivators of sesame in Tamilnadu are susceptible to this pathogen. Therefore, with this background in mind, the present study was planned and conducted. One of the important applications of the infrared spectroscopic study is the diagnostic value in establishing the presence of certain organic constituents in plants [16]. 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 [9]. 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 roots, compounds responsible for the disease are identified and quantified by the FT-IR analysis [10].

MATERIALS AND METHODS

In the present study, five varieties of sesame, namely CO-1, VRI-1, VRI-2, TMV-3 and TMV-4 were obtained from Regional Research Station, Tamilnadu, India. All the varieties were grown in Kharif season and the soil location is clay. In the fields three manure treatments such as control (T_1) (without any manure treatment), chemical fertilizer (T_2) (NPK) and organic manure treatment (T_3) (farm yard manure and neem cake). All the diseased roots are powdered well and oven dried to remove the moisture content. The oven dried roots are ground well into a fine powder by using agate mortar. The Infrared Spectra of roots were recorded using KBr pellet technique in BRUKER IFS 66V model FT-IR spectrometer in the region 4000–400 cm^{-1}.

RESULTS AND DISCUSSION

Sample characterization using FT-IR spectroscopy concerned the correct assignment of the observed spectral characteristics to the most likely origin of the absorption bands. A summary of the most characteristic bands observed in diseased roots and their assignments are presented in Table 1. The corresponding FT-IR spectra of the descriptive sesame root rot diseased roots are presented in Fig. 1a, 1b, 1c, 1d and 1e. In FT-IR spectrum corresponding to all the fifteen sesame root rot diseased roots caused fungi *Macrophomina phaseolina* of absorption bands occur at 3368 cm^{-1}, 3359 cm^{-1} 2925 cm^{-1}, 1735 cm^{-1}, 1643 cm^{-1}, 1549 cm^{-1}, 1439 cm^{-1}, 1425 cm^{-1}, 1246 cm^{-1}, 1035 cm^{-1}, and 606 cm^{-1}. In all the fifteen FT-IR

spectrum, the following bands corresponding to the compounds responsibility for the disease are present [15]. Recently, the use of FT-IR spectroscopy in metabolomics has become increasingly important within the plant sciences field [7].

A strong broad absorption band around 3360–3390 cm^{-1} found in all samples may be due to the presence of hydrogen bond N–H stretching, characteristic of aminoacids [6, 12]. The absorption band 2924 cm^{-1}, corresponding to C–H stretching of the CH_2 groups, indicates the presence of various amino acids, this band may also be characteristic for the presence of aliphatic CH groups in these compounds [1, 11, 14]. The absorption band at 1735 cm^{-1}, characteristic of C=O stretching, indicates the carbonyl groups [5]. Bands 1643 cm^{-1} and 1560 cm^{-1} coupled with the presence of the band around 3368 cm^{-1} may be taken as indication of the presence of amino acids [5]. The absorption band appears at 1439 cm^{-1} due to the aromatic ring C–C stretching. The absorption bands 1240 cm^{-1} and 1052 cm^{-1} are due to the stretching vibration of C–O group of esters and phenols (15). The band 606 cm^{-1} belongs to C–C ring bending coumarine structure [2].

This quantitative infrared analysis of sesame root rot disease roots reveals the different presence of amino acids, esters, ethers and phenols probably in different amounts.

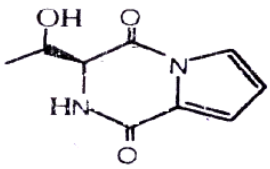
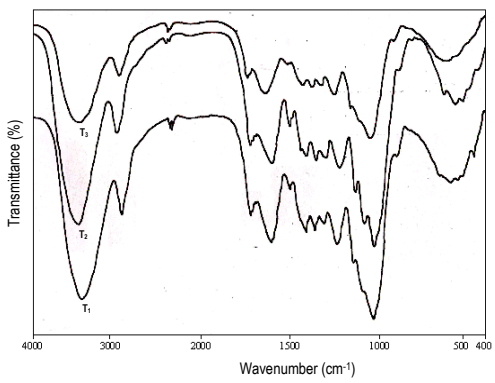


Fig. 1. Diketopiperazine (Macrophominal) from root rot caused by fungi *Macrophomina phaseolina*.

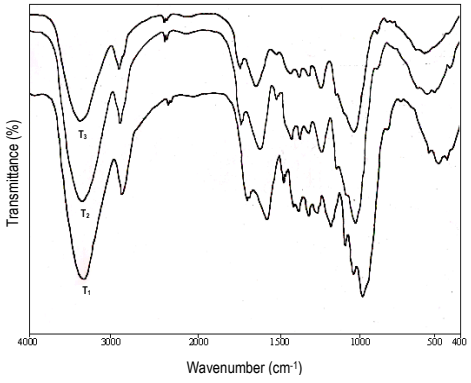
Table 1

Evaluation of the FT-IR spectrum

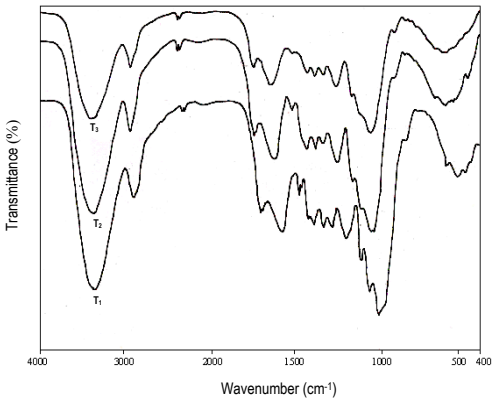
Absorption Frequency cm^{-1}	Tentative assignments
3368, 3359	Bonded O–H / NH stretching
2925	CH (Sym/Asym) aliphatic
1735	Ketones, Ester carbonyl group
1643	C = O stretching phenyl ring amino acid-1
1549	N–H deformation
1439, 1425	C–N stretching – in – plane OH bending
1376	CH_3 (asym./ deformation)
1246	C–O (stret.) Ester
1035	CO (stret.)
606	CC (bending)



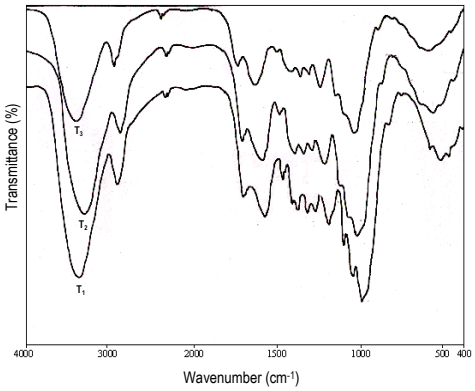
a: CO – 1



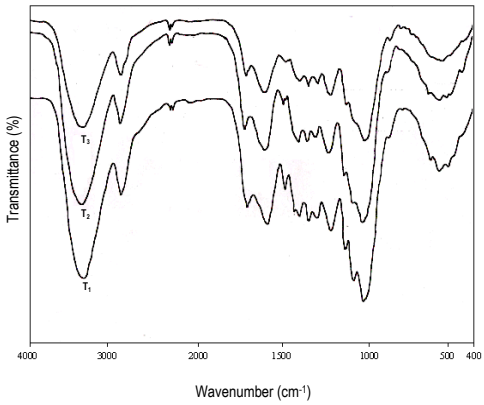
b: VRI – 1



c: VRI – 2



d: TMV – 3



e: TMV – 4

Fig. 2. FT-IR absorption spectra of different sesame varieties with root rot diseases.

It is our interest to estimate quantitatively the changes in the total organic constituents in the control, chemical fertilizer and organic manure application. For this purpose spectral extinction coefficients (K) are calculated for the absorption bands at 2924 cm^{-1}, 1735 cm^{-1}, 1643 cm^{-1}, 1425 cm^{-1}, 1035 cm^{-1} and 606 cm^{-1}.

From the characteristic chromophoric groups of amino acids, phenols and esters, the extinction coefficient (K) can be calculated using the relation:

$$K = \frac{DA}{m} \text{cm}^2/\text{mg} \tag{1}$$

where: D – optical density of the absorption band, log (I_o/I); A – area of the pellet (cm^2); m – mass of the samples in the pellet (mg).

Table 2.1

Extinction coefficient (K) values of T_1, T_2 and T_3 treated sesame root rot diseased root samples of CO-1, VRI-1 and VRI-2 varieties

Absorption band (cm^{-1})	Extinction coefficient, K (cm^2/g)								
	CO-1			VRI-1			VRI-2		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
2924	41.17	40.14	23.08	40.56	36.76	25.21	43.97	40.46	26.79
1735	58.18	26.59	21.84	33.54	29.85	16.98	38.23	35.72	16.86
1643	49.90	46.48	26.59	53.72	45.08	28.49	56.16	52.31	30.39
1425	29.27	27.30	25.42	27.04	24.86	10.59	36.09	31.51	10.81
1035	191.98	167.70	81.95	202.26	176.18	89.08	203.98	187.51	91.52
606	41.75	24.52	23.64	47.52	35.88	17.52	41.76	35.83	22.25

From Tables 2.1 and 2.2 it was observed that the extinction coefficients are higher in control (T_1) and chemical fertilizer (T_2) samples than in organic manure (T_3) treatment samples. This clearly indicates that the root rot disease is more in control and chemical fertilizer (T_2) than in organic manure (T_3). Interestingly, the root rot disease is found to be in the lowest level in the root samples collected from fields treated with organic manure (T_3) alone. This shows that organic manure treatment effectively possesses antifungal activity when compared with chemical fertilizer (T_2) and control (T_1). The present study confirms the study of earlier workers who have reported similar results about organic farming which is known to reduce the toxic residues in the foodstuff and improves the quality [4].

Table 2.2

Extinction coefficient (K) values of T_1, T_2 and T_3 treated sesame root rot diseased root samples TMV-3 and TMV-4 varieties

Absorption band (cm^{-1})	Extinction coefficient, K (cm^2/g)					
	TMV-3			TMV-4		
	T_1	T_2	T_3	T_1	T_2	T_3
2924	37.70	35.67	25.20	40.81	38.32	23.43
1735	37.23	28.49	20.22	31.26	30.15	16.95
1643	50.38	45.96	30.36	51.39	50.24	26.59
1425	23.14	20.54	14.11	34.11	28.12	21.89
1035	200.02	164.32	91.52	199.2	172.38	89.05
606	41.75	32.65	19.09	44.28	24.89	19.08

CONCLUSIONS

FT-IR spectra of the sesame roots exhibit the absorption bands of chromophoric group characteristics of phenols, amino acids and proteins. From the quantitative analysis of these organic constituents, it is found that the levels of total phenols and amino acids are higher in control (T_1) and also in chemical fertilizer (T_2) than in the organic manure (T_3) treated roots. This indicates the higher level of *diketopiperazine (macrophominal*) in T_1 and T_2 samples and the lower level of *diketopiperazine* in T_3 samples. Here it is inferred that the organic treated samples are more resistant to the root rot disease, which is evidenced by the value of the extinction coefficient calculated, obtained for T_3 treatment (organic manure).It has been found that the proper management of the soil with T_3 treatment may lead to a reduction in root rot disease incidence.

REFERENCES

1. BELLAMY, L. J., *Infrared Spectra of Complex Molecules*, Chapman Hall, London, 1975.
2. BUCHI, G., M. STEVEN, J. WEINREB, Total syntheses of aflatoxins M_1 and G_1 and an improved synthesis of aflatoxin B_1, *J. Am. Chem. Soc.*, 1971, **93(3)**, 744–753.
3. DINAKARAN, D., K. SACHITHANANTHAN, S.E. NAINAMOHAMMED, Screening of sesame genotypes against root rot pathogen, *Macrophomina phaseolina* (Tassi.) goid, *J. Plant Pathology,* 2005, **23**, 66 – 68.
4. FUJII, S., Y. OSAWA, H. SUGIMURA, Infrared spectra of Japanese coal: The absorption bands at 3030, 2920 and 1600 cm^{-1}, *Fuel,* 1970, **49**, 68–74.

5. GUILLEN, M.D., N. CABO, Some of the most significant changes in the Fourier transform infrared spectra of edible oils under oxidative conditions, *Journal of Science, Food and Agriculture,* 2000, **80**, 2028–2036.
6. HIRANO, S., N. OKAWARA, S. NARAZAKI, Near infra red detection of internally moldy nuts, *Bioscience, Biotechnology and Biochemistry,* 1998, **62**(1), 102–107.
7. JOHNSON, H.E., D. BROADHURST, R. GOODACRE, A.R. SMITH, Metabolic fingerprinting of salt-stressed tomatoes, *Phytochemistry,* 2003, **62**, 919–928.
8. Mc COMBS, C.L., N.N. WINSTEAD, Changes in sugars and amino acids of cucumber fruits infected with *Pythium aphani dermatum*, *Phytopathology,* 1964, **54**, 233–234.
9. PARVEEN, I., J.M. MOORBY, W.M. HIRST, S.M. MORRIS, M.D. RASER, Profiling of plasma and faeces by FT-IR to differentiate between heath land plant diets offered to zero-grazed sheep, *Animal Feed Science and Technology*, 2007 (in press).
10. PELLATI, FEDERICA, SAMUELE CALO, STEFANIA BENVENUTI, High-performance liquid chromatography analysis of polyacetylenes and polyenes in *Echinacea pallida* by using a monolithic reversed-phase silica column, *Journal of Chromatography A,* 2007, **56**, 1149–1153.
11. RAMAMURTHY, N., S. KANNAN, Fourier transform infrared spectroscopic analysis of a plant (*Calotropis gigantea* Linn) from an industrial village, Cuddalore Dt., Tamilnadu, India, *Romanian J. Biophys.*, 2007, **17**, 269–276.
12. RAO, C.N.R., *Chemical Application of Infrared Spectroscopy,* Academic Press, New York, 1963.
13. SHARMA, S.G., R. NARAYAN, R.N. LAL, S.S. LAL, C. CHATURVEDI, Role of phenolic compounds in resistance of maize to leaf blight caused by *Drechslera* state of *Cochlibolus heterostrophus*, *Indian Phytopathology*, 1983, **36**, 43–48.
14. SIVAKESAVA, S., J. IRUDAYARAJ, Analysis of potato chips using FTIR photoacoustic spectroscopy, *Journal of Science, Food and Agriculture*, 2000, **80**, 1805–1810.
15. VALCHOS, N., Y. AKOPELITIA, M. PSAROUDAKI, V. KONSTANTINIDOU, A. CHATZILAZAROU, E. TEGOU, Applications of Fourier transform-infrared (FTIR) spectroscopy to edible oils, *Analytica Chemica Acta*, 2006, **573/574**, 459–465.
16. VELMURUGAN, S., Spectroscopic studies on the status of redroot disease incidence and its variation in sugarcane (*Saccharum officinarum* L.), Ph.D. Thesis, 2006, Annamalai University, Annamalinagar, India.