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FTIR ANALYSIS OF ROOT ROT DISEASE INCIDENCE IN SUNFLOWER (*HELIANTHUS ANNUS* .L)

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ABSTRACT

A study has been made for four different sunflower varieties, namely CO-4, CO-5, HYCO-2 and TCSH-1 which were grown in trial fields with three different manure treatments viz. control (T1), chemical fertilizer (T2) and organic manure (T3). From the root rot disease roots, the compound identified and quantified from the four sunflower varieties by FT-IR analysis and the spectra were recorded for all the samples in the range of 4000 – 400 cm^{-1}. The marked reduction in intensities of the absorption peaks in the spectra of organic manure treated when compare to control and chemical fertilizer. Also an attempt has been made to correlate the extinction coefficient (K) values with chance in sunflower root rot disease treated fields.

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INTRODUCTION

Oil seeds play an important role in Indian agriculture as tool and industrial commodity. India is the largest production of oilseeds in the world in terms of output and second in terms of area. Among the oil seed crops, sunflower is an all season crop (Sivamurugan *et al.*,2003). It is well recognized that the disease constitutes a major constrains in increasing the yield level of sunflower crop. Macrophonina phaseolina infection as sunflower was first reported from srilanka 1927. Later, it was reported from other sunflower growing countries of the world Uruguay, Australia, Yugoslavia in 1966. Argenina and Senegal 1967. Hungary, 1970, USA, 1971. India, 1973. France, 1976. Egypt, 1980and Pakistan, 1982 (Bhutk 1995). Macrophomina phaseolina causal agent of charcoal rot is a serious threat for sunflower crop especially in the dry regions of the world (Hoes, 1905). Charcoal rot is a great economic importance in arid areas of the world. Severity of the disease is characterized by drought and high temperature. However high losses have been reported on arid ability of low relative humidity and high atmospheric temperature at flowering stage of the crop (Dhingre and Silclair, 1973; Tiklonor, 1976 and Salik, 2007). Fourier transform infrared spectroscopy (FTIR) is a powerful technique for studying molecular structures.

According to many researchers, they apply FTIR in their studies. In environmental studies, (Ibrahim and Abd-El-Aal, 2008; Ibrahim, 2008 and Ibrahim, 2009). Biological studies (Ibrahim, 2010), biopolymers; such as cellulose (Ibrahim and Osman, 2009; Chitosan Ibrahim, 2009); aquatic plants (Ibrahim and AlFifi, 2010; Ibrahim, 2010). Many researchers have reported the occurrence of this fungal disease in crops to be associated with the changes in organic constitutions (Hirano, 1998 and Sharma, 1983). In the present study, FT-IR is used to identify and quantify the organic constituents in different treated fields sunflower root rot disease.

MATERIALS AND METHODS

In the present study, four varieties of sunflower, namely CO-4, CO-5, HYCO-2 and TCSH-1 were obtained from oil seed research center (TNAU), Coimbatore, Tamilnadu, India. All the varieties were grown in kharif season and the soil location is red. In the fields three manure treatments such as control T1 (without any manure treatment), chemical fertilizer T2 (NPK) and organic manure treatment T3 (farme yard manure and neem cake). All the roots samples are powder well, oven dried to remove the moisture content. The oven dried roots are ground well into a fine power by using agate mortar. The Infra spectra were recorded in BRUKER IFS 66V model FT-IR spectrometer in the region 4000 – 400 $cm^{-1.}$

RESULT AND DISCUSSIONS

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 sunflower root rot disease which different treated field roots are presented in Fig. 1-4. In FTIR spectrum corresponding to all the sunflower root rot

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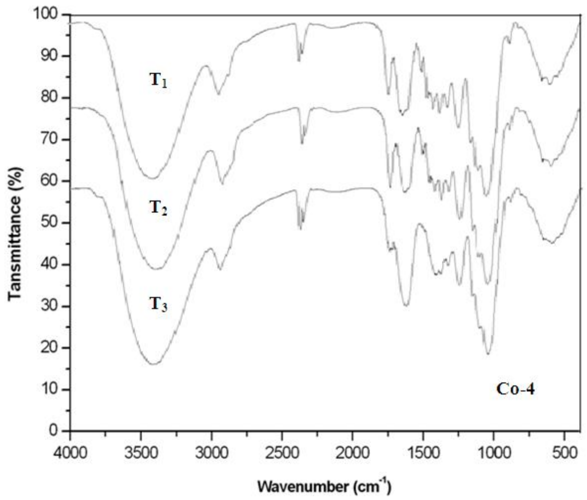

Fig.1 FTIR spectra of sunflower root (Co-4 variety)

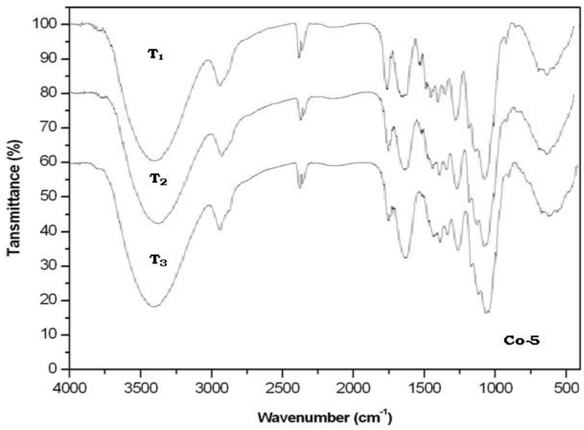

Fig.2 FTIR spectra of sunflower root (Co-5 variety**)**

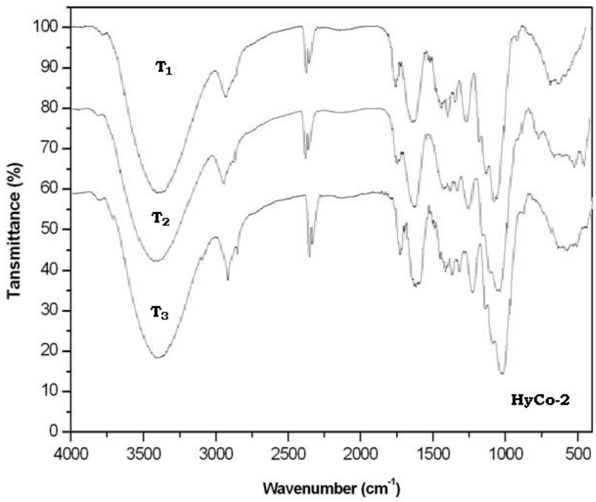

Fig.3 FTIR spectra of sunflower root (HyCo-2 variety)

disease roots of absorption bands around at 3399 cm^{-1}, 2925 cm^{-1}, 1735 cm^{-1},1638 cm^{-1},1510 cm^{-1}, 1425 cm^{-1}, 1376 cm^{-1}, 1248 cm^{-1}, 1051 cm^{-1}, 606 cm^{-1}.

A strong broad absorption band around 3399 cm–1 found in all samples may be due to the presence of hydrogen bond N–H stretching, characteristic of amino acids (Hirano Rao CNR, 1963).

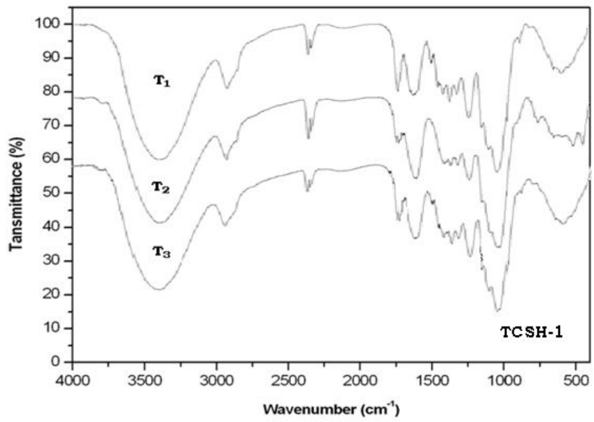

Fig.4 FTIR spectra of sunflower root (TCSH-1 variety)

The absorption band around 2925 cm^{-1}, corresponding to C–H stretching of the CH_2 groups, indicates the presence of various amino acids, this band may also be characterized for the presence of aliphatic C-H groups in these compounds (Bellamy, 1975; Ramamoorthy, 2007 and Sivakesava, 2000). The absorption band at 1735 cm^{-1}, characteristic of C=O stretching, indicates the carbonyl groups (Guillen, 2000). Bands 1638 cm^{-1} and 1510 cm^{-1} coupled with the presence of the band around 3399 cm^{-1} may be taken as indication of the presence of amino acids (Guillen, 2000). The absorption band appears at 1425 cm^{-1} due to the aromatic ring C–C stretching. The absorption bands 1248cm^{-1} and 1051 cm^{-1} are due to the stretching vibration of C–O group of esters and phenols (valchoes, 2006). The band 606 cm^{-1} belongs to C–C ring bending coumarone structure (Buchi). This quantitative infrared analysis of different treated field sunflower root rot disease roots reveals the different presence of amino acids, esters, ethers and phenols probably in different amounts.

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 3399 cm^{-1}, 2720 cm^{-1}, 1759 cm^{-1}, 1425 cm^{-1}, 1057 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:

$$K = DA/m \ cm^2/gm$$

Where,

D – Optical density of the absorption band, log (Io/I); A – area of the pellet (cm2); m – mass of the samples in the pellet (mg)

From the table-2, it is seen that, the extinction co-efficient (k) values are lower in organic manure (T3) when compare to control (T1) and chemical fertilizer (T2) treatment samples. The increase extinction co-efficient (k) values may be increase due to (macrophominal) diketopiperazine content. This shows that organic manure (T3)-effectively possesses antifungal activity compare to chemical fertilizer (T2) and control (T1), similar results have been reported (Ravi, 2002 and Gokulakumar, 2008).

Table 1 FTIR absorption wave number and their corresponding tentative assignment for Sunflower root samples

Varieties of Sunflower Root												Tentative assignments
Co-4			Co-5			HYCo-2			TCSH-1			
T_1 cm^{-1}	T_2 cm^{-1}	T_3 cm^{-1}	T_1 cm^{-1}	T_2 cm^{-1}	T_3 cm^{-1}	T_1 cm^{-1}	T_2 cm^{-1}	T_3 cm^{-1}	T_1 cm^{-1}	T_2 cm^{-1}	T_3 cm^{-1}	
3399	3389	3398	3398	3369	3394	3400	3401	3400	3399	3399	3400	Banded O-H/N-H stretching
2925	2920	2919	2921	2923	2921	2921	2919	2917	2925	2925	2921	C-H stretching (symmetric/ asymmetric) Aliphatic
1749	1739	1740	1736	1736	1735	1736	1735	1739	1749	1735	1740	Keton, ester carbonyl group
1638	1639	1637	1629	1618	1618	1618	1618	1630	1638	1629	1637	C=O stretching phenyl ring amino acid-I
1510	1502	1508	1510	1508	1508	1510	1511	1511	1510	1510	1511	N-H deformation
1425	1425	1425	1425	1425	1420	1425	1420	1425	1425	1425	1425	C-C stretching
1376	1377	1376	1377	1376	1376	1378	1377	1378	1376	1377	1378	CH3(asym/deformation)
1248	1248	1248	1249	1249	1250	1249	1249	1248	1248	1249	1248	C-O stretching
1051	1051	1051	1049	1053	1054	1054	1054	1035	1051	1051	1054	C-O stretching
617	609	617	606	617	618	608	608	608	606	608	617	CC bending

Table 2 Extinction Coefficient (k) of prominent various absorption bands of different treated sunflower root rot disease

Absorption bands cm^{-1}	Extinction Coefficient, K(cm^2/gm)											
	Co-4			Co-5			HYCo-2			TCSH-1		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
3399	62	52	45	60	53	45	60	51	48	63	53	45
2720	137	130	114	135	131	116	137	130	113	136	130	130
1759	102	97	85	103	96	86	102	98	85	103	98	102
1425	197	141	123	185	143	130	190	143	125	196	140	124
1057	55	50	40	57	50	42	55	50	40	51	51	51
606	124	116	100	120	115	100	122	114	107	125	115	98

CONCLUSION

FT-IR spectra of the sunflower 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 (T1) and also in chemical fertilizer (T2) than in the organic manure (T3) treated roots. This indicates the higher level of diketopiperazine (macrophominal) in T1 and T2 samples and the lower level of diketopiperazine in T3 samples. Here, organic treated field samples are indicate the less disease proliferation of root rot disease when compare other two treatment chemical fertilizer T2 and control T1. It has been found that the proper management of the soil with T3 treatment may lead to a reduction in root rot disease incidence.

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