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Research Article

FTIR - technique for harvested seeds of chickpea and cowpea plants grown under *Rhizobium* and AM fungi inoculated condition

B. Sadhana

Assistant Professor, Department of Botany, Thiagarajar College (Autonomous), Madurai-625009, Tamil Nadu, India
*Corresponding author: *sadhanakarthik2004@yahoo.co.in*

Abstract

The FTIR (Fourier Transform infrared Spectrometry) technique applied for the detection of chemical changes in the powdered seeds of harvested chickpea and cowpea plants grown under *Rhizobium* and AM fungi inoculated condition. The FTIR spectra of both seed sample were recorded in the range of 400 cm^{-1} to 4000 cm^{-1} at a resolution of 400 cm^{-1}. A strong broad spectrum around 3360-3390 found in both seed samples may be due to the presence of hydrogen bind N-H stretching, characteristics of amino acids. In chickpea seed sample, the bands around 3423-3375.7 cm^{-1} represent O-H and N-H stretching vibrations that are mainly generated by proteins and carbohydrates. The bands between 2929.8 and 2927 cm^{-1} represent C-H stretching vibrations that are mainly generated by lipids. The bands between 1654.4-1652.2 cm^{-1} represent -C=O group of amide- I protein. The 1456.1-1412.3 cm^{-1} absorption bands contained -CH group of proteins. The absorption band between 1245.4-1245.1 representing the -P=O phosphodiester groups of nucleic acids and phospholipids. The band range of 1158.6-1018.3cm^{-1} showed the -C-O group from carbohydrates. In cowpea, the bands around 3403-3375.8 cm^{-1} represent O-H and N-H stretching vibrations that are mainly generated by proteins and carbohydrates. The bands between 2929.8 and 2928.1 cm^{-1} represent C-H stretching vibrations that are mainly generated by lipids. The bands between 1653.8-1558.0 cm^{-1} represent -C=O group of amide- I protein. The 1419.6-1405.0 cm^{-1} absorption bands contained -CH group of proteins. The absorption band between 1245.6-1242.8 representing the -P=O phosphodiester groups of nucleic acids and phospholipids. The band range of 1159.6-930.1cm^{-1} showed the -C-O group from carbohydrates. The FTIR analysis provides more detailed chemical information on the samples composition because it measures the fundamental vibrations. Thus this analysis may be used extensively in the research on plant physiology due to its virtues of simple and efficient on manipulation.

Keywords: FTIR, Bio-molecules, functional groups and Amide I proteins.

Introduction

FTIR- Fourier Transform Infrared Spectrometry can identify the structure of unknown composition or its chemical groups, and the intensity of absorption spectra associated with molecular composition or content of the chemical group (Surewicz *et al*., 1993; Mc Cann *et al*., 1992). By acquiring IR spectra from plant samples could detect the minor changes of macromolecule compound, such as carbohydrate, protein, lipid and cell wall pectin (Surewicz *et al*., 1993; Mc Cann *et al*., 1992).

Knowitall software was used to find the functional groups for preliminarily analyzing IR spectra collected. The bands around 3370 cm^{-1} represent O-H and N-H stretching vibrations that are mainly generated by proteins and carbohydrates (Wolkers *et al*., 1998). The bands between 3000 and 2800 cm^{-1} represent C-H stretching vibrations that are mainly generated by lipids (Wolkers *et al*., 1995). The proteins absorption bands mainly located between 1800 cm^{-1} and 1500 cm^{-1} contained amide-I and

amide-II bands (Surewicz *et al*., 1993; Stehfest *et al*., 2005) but overlapped with other absorption bands with in this region. The present study deal with analyzing the functional groups of bio-molecules in harvested seeds from *Rhizobium* and AM fungi inoculated chickpea and cowpea plants by using FTIR- technique.

Materials and Methods

The *Rhizobium* and AM fungi in alone and combined inoculated chickpea and cowpea plants were grown under green house condition till harvesting. The harvested seeds were used for the FTIR study. It was applied for the detection of functional groups in the powdered seeds of harvested chickpea and cowpea plants.

FTIR- sample preparation and data analysis

The seed coats of harvested chickpea and cowpea seeds were carefully removed and powdered in pestle and mortar. Each sample for FTIR analysis was prepared by mixing the fine ground powder of legume seeds with 2% KBr. The FTIR spectra of each sample were recorded in the range of 400 cm^{-1} to 4000 cm^{-1} at a resolution of 400 cm^{-1}. The spectral data of each sample was measured.

Results and Discussion

The FTIR absorption spectrum between 4000 and 400cm^{-1} was revealed on the chickpea and cowpea seed sample. The present study showed a strong broad spectrum / band around 3360-3390 found in both chickpea and cowpea samples may be due to the presence of hydrogen bind N-H stretching, characteristics of amino acids.

In chickpea, the bands around 3423-3375.7 cm^{-1} represent O-H and N-H stretching vibrations that are mainly generated by proteins and carbohydrates. The bands between 2929.8 and 2927 cm^{-1} represent C-H stretching vibrations that are mainly generated by lipids. The bands between 1654.4-1652.2 cm^{-1} represent -C=O group of amide- I protein. The 1456.1-1412.3 cm^{-1} absorption bands contained -CH group of proteins. The absorption band between 1245.4-1245.1 representing the -P=O phosphodiester groups of nucleic acids and phospholipids. The band range of 1158.6-1018.3cm^{-1} showed the -C-O group from carbohydrates (Table: 1 & Fig: 1-4).

In cowpea, the bands around 3403-3375.8 cm^{-1} represent O-H and N-H stretching vibrations that are mainly generated by proteins and carbohydrates. The bands between 2929.8 and 2928.1 cm^{-1} represent C-H stretching vibrations that are mainly generated by lipids. The bands between 1653.8-1558.0 cm^{-1} represent -C=O group of amide- I protein. The 1419.6-1405.0 cm^{-1} absorption bands contained -CH group of proteins. The absorption band between 1245.6-1242.8 representing the -P=O phosphodiester groups of nucleic acids and phospholipids. The band range of 1159.6-930.1cm^{-1} showed the -C-O group from carbohydrates (Table: 2 & Fig: 5-8).

Protein amide - I ratio were determined and chemical functional groups were identified according to published reports (Golovina *et al*., 1997; Surewicz and Mantsch, 1988; Wolker's *et al*., 1998; Yang and Yen, 2002; El-Bahy, 2005; StehFest *et al*., 2005). In plant physiology research, FTIR has been used to identify the concrete structure of certain plant secondary metabolites (Yang and Yen, 2002; Stehfest *et al*., 2005; Ivanova and Singh, 2003).

The study on the comparison of chemical composition of genetic modification (GM) crops to their conventional counterparts forms the basis of safety assessment process for GM crops (Caixia Sun *et al*., 2012). Researcher applied FTIR here for detecting the chemical and conformational changes between transgenic cotton seeds and their non-transgenic counterparts. The assignment of absorption bands and comparison in band areas of four regions from original FTIR- spectra indicated that the contents of the compounds did not differ significantly ($P>0.05$) between transgenic cotton seeds and their non-transgenic counterparts. The FTIR also confirmed fatty acid changes and revealed further carbohydrate changes in the root exudates of *Casuarina* due to the infection of *Frankia sp.* strain CcI3 (Nicholas *et al*.,2012).

In mid IR region (2000-1000 cm^{-1}) appeared large numbers of sharp peaks, indicating that the seeds have a rich chemical composition, such as carbohydrates, proteins and lipids. However, this region yielded broad and overlapped bands. At present research, the significant recovery a phenomenon was observed in biofertilizers treated legume sample, we speculated that this due to plant native physiological adjustment mechanism compared to control plant. Results

Table: 1 FTIR-Analysis of powdered chickpea seed showing the functional groups of bio-molecules

Treatments	Absorption band region (cm^{-1})	Functional groups of biomolecules
C	3396.2	-OH and –NH groups from proteins and carbohydrates
	2929.7	-CH groups from lipids and carbohydrates
	1654.2	-C=O group of amide- I protein
	1420	-CH group of proteins
	1245.4	-P=O Phospho diester groups of nucleic acids and phospholipids
	1157.3	-C-O group from carbohydrates
	1082.8	-C-O group from carbohydrates
	1022.1	-C-O group from carbohydrates
T1	3423.7	-OH and –NH groups from proteins and carbohydrates
	2929.6	-CH groups from lipids and carbohydrates
	1654.4	-C=O group of amide- I protein
	1412.3	-CH group of proteins
	1245.1	-P=O Phospho diester groups of nucleic acids and phospholipids
	1158.6	-C-O group from carbohydrates
	1081.6	-C-O group from carbohydrates
	1018.3	-C-O group from carbohydrates
T2	3397.8	-OH and –NH groups from proteins and carbohydrates
	2928.5	-CH groups from lipids and carbohydrates
	1653.9	-C=O group of amide- I protein
	1419.9	-CH group of proteins
	1245.4	-P=O Phospho diester groups of nucleic acids and phospholipids
	1158.0	-C-O group from carbohydrates
	1081.6	-C-O group from carbohydrates
	1019.8	-C-O group from carbohydrates
T3	3375.7	-OH and –NH groups from proteins and carbohydrates
	2927.0	-CH groups from lipids and carbohydrates
	1652.2	-C=O group of amide- I protein
	1456.1	-CH group of proteins
	1243.3	-P=O Phospho diester groups of nucleic acids and phospholipids
	1158.0	-C-O group from carbohydrates
	1085.4	-C-O group from carbohydrates
	1021.8	-C-O group from carbohydrates

C- Control T1-*Rhizobium* T2-AM fungi T3- *Rhizobium* + AM fungi

Table: 2 FTIR-Analysis of powdered cowpea seed showing the functional groups of biomolecules

Treatments	Absorption band region (cm^{-1})	Functional groups of bio-molecules
C	3403.7	-OH and –NH groups from proteins and carbohydrates
	2929.0	-CH groups from lipids and carbohydrates
	1653.8	-C=O group of amide- I protein
	1544.60	-NH group of amide I protein
	1405.0	-CH group of proteins
	1242.8	-P=O Phospho diester groups of nucleic acids and phospholipids
	1158.1	-C-O group from carbohydrates
	1081.1	-C-O group from carbohydrates
	1050.1	-C-O group from carbohydrates
T1	3375.8	-OH and –NH groups from proteins and carbohydrates
	2928.1	-CH groups from lipids and carbohydrates
	1653.6	-C=O group of amide- I protein
	1559.6	-C=O group of amide- I protein
	1419.6	-CH group of proteins
	1245.0	-P=O Phospho diester groups of nucleic acids and phospholipids
	1158.3	-C-O group from carbohydrates
	1081.4	-C-O group from carbohydrates
	1050.9	-C-O group from carbohydrates
	930.8	-C-O group from carbohydrates
T2	3391.8	-OH and –NH groups from proteins and carbohydrates
	2929.0	-CH groups from lipids and carbohydrates
	1652.1	-C=O group of amide- I protein
	1558.0	-NH group of amide I of protein
	1417.9	-CH group of proteins
	1245.5	-P=O Phospho diester groups of nucleic acids and phospholipids
	1159.6	-C-O group from carbohydrates
	1048.9	-C-O group from carbohydrates
	930.1	-C-O group from carbohydrates
T3	3387.1	-OH and –NH groups from proteins and carbohydrates
	2929.8	-CH groups from lipids and carbohydrates
	1652.0	-C=O group of amide- I protein
	1415.2	-CH group of proteins
	1245.6	-P=O Phospho diester groups of nucleic acids and phospholipids
	1158.1	-C-O group from carbohydrates
	1081.4	-C-O group from carbohydrates
	1048.3	-C-O group from carbohydrates

C- Control, T1-*Rhizobium* T2-AM fungi T3- *Rhizobium* + AM fungi

Fig: 1 FTIR spectrum of seed samples from control plant of chickpea

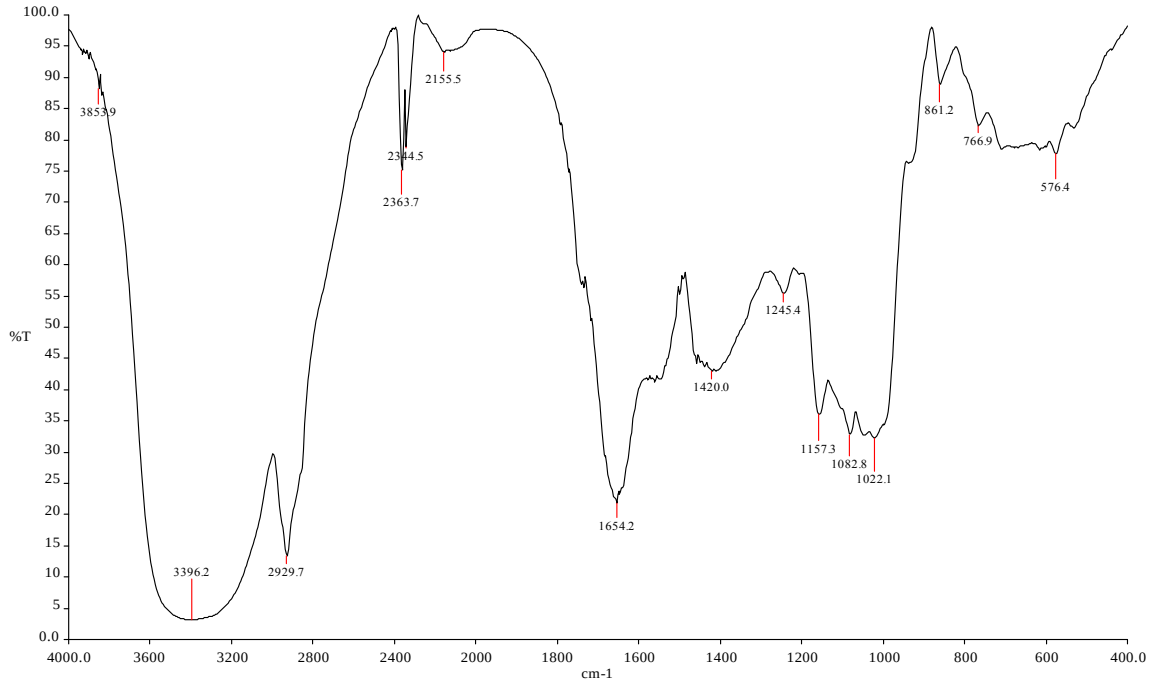


Fig: 2 FTIR spectrum of seed sample from *Rhizobium* treated chickpea plants

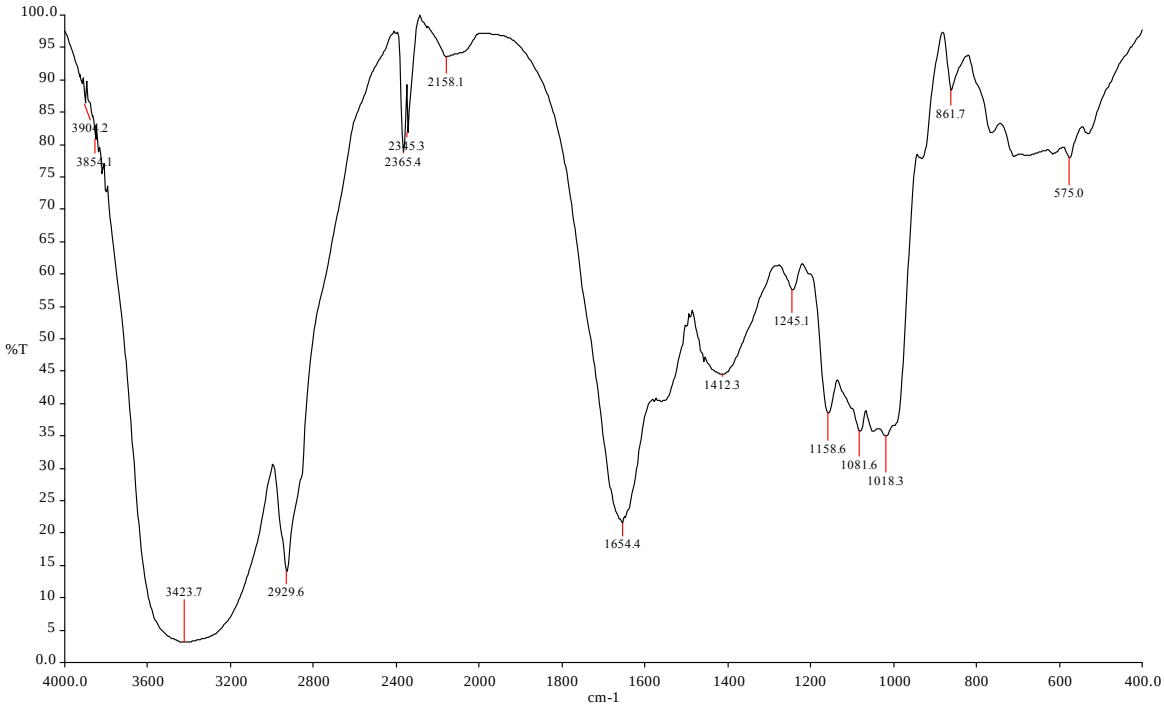


Fig: 3 FTIR spectrum of seed sample from AM fungi treated chickpea plants

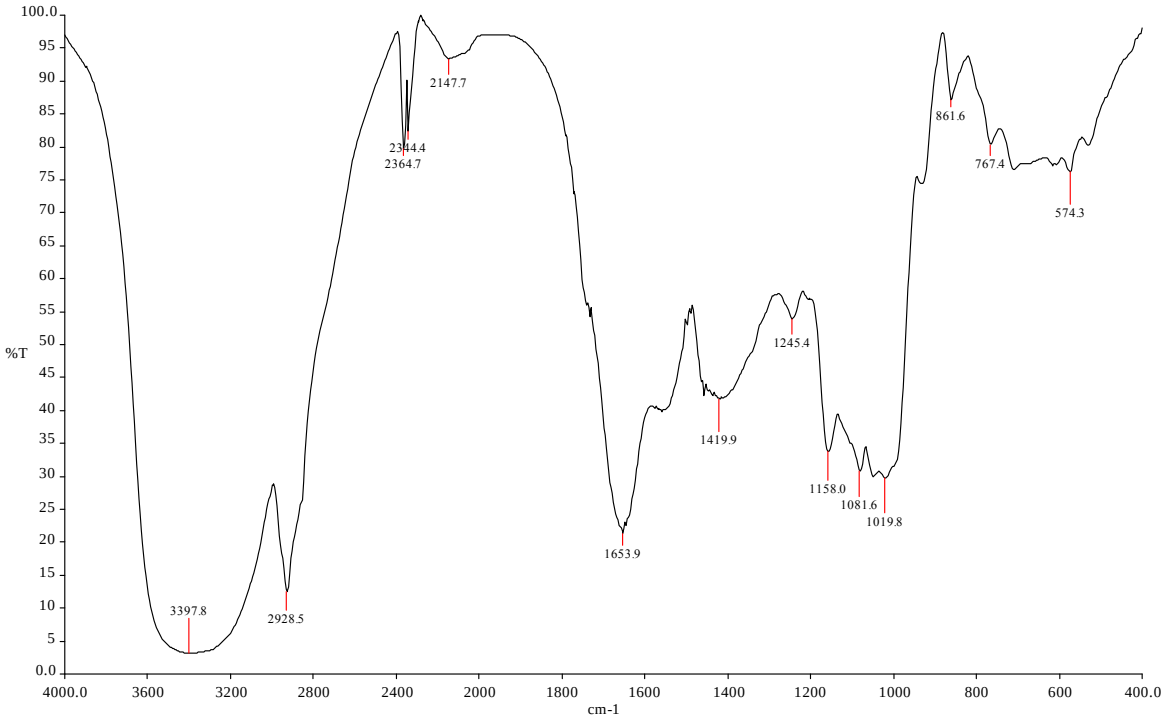


Fig: 4 FTIR spectrum of seed sample from *Rhizobium* and AM fungi treated chickpea plants

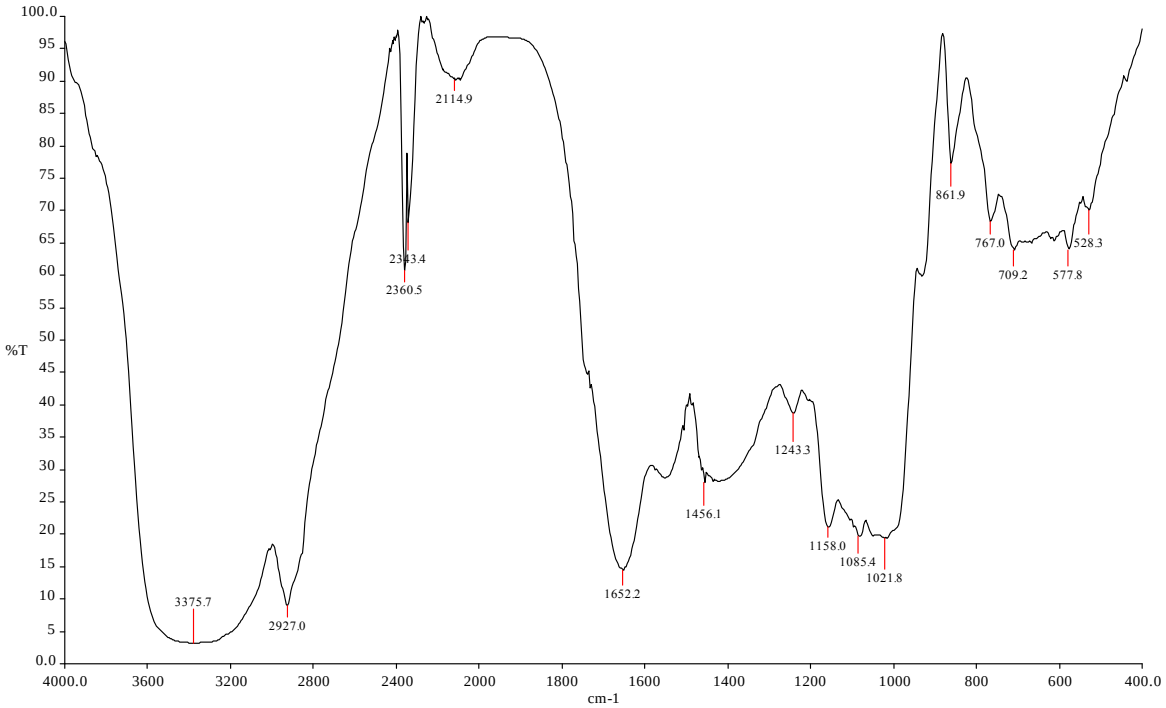


Fig: 5 FTIR spectrum of seed sample from control plant of cowpea

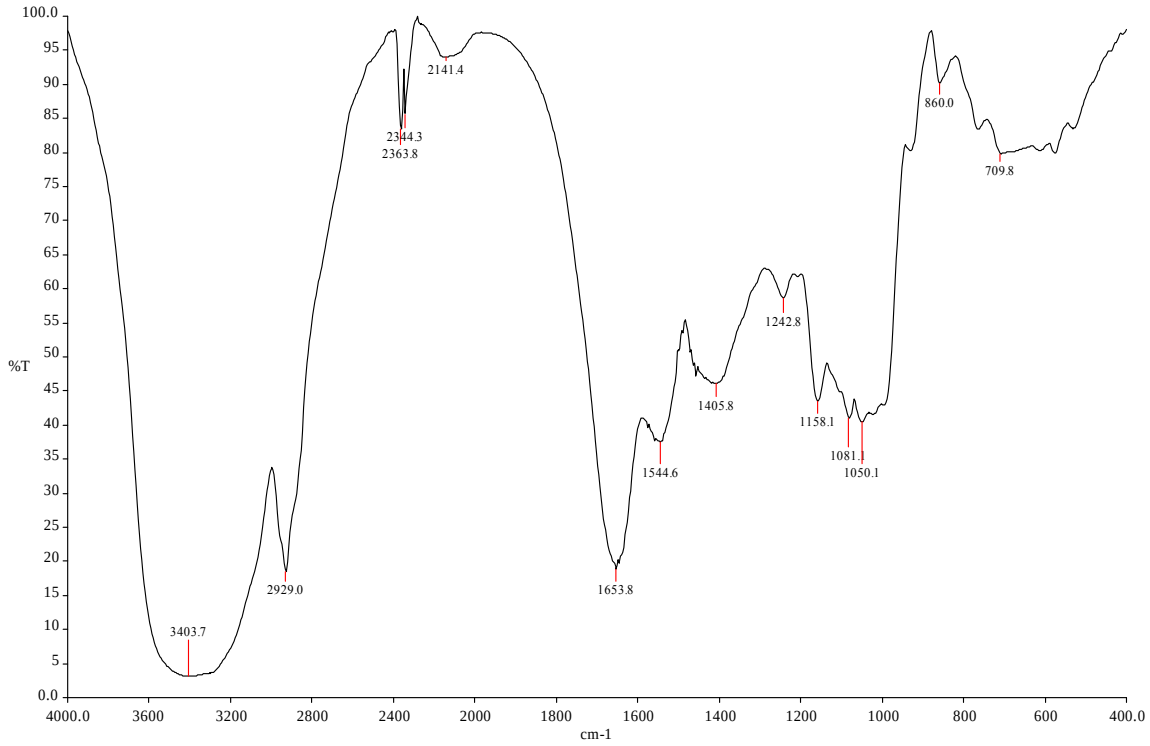


Fig: 6 FTIR spectrum of seed sample from *Rhizobium* treated cowpea plants

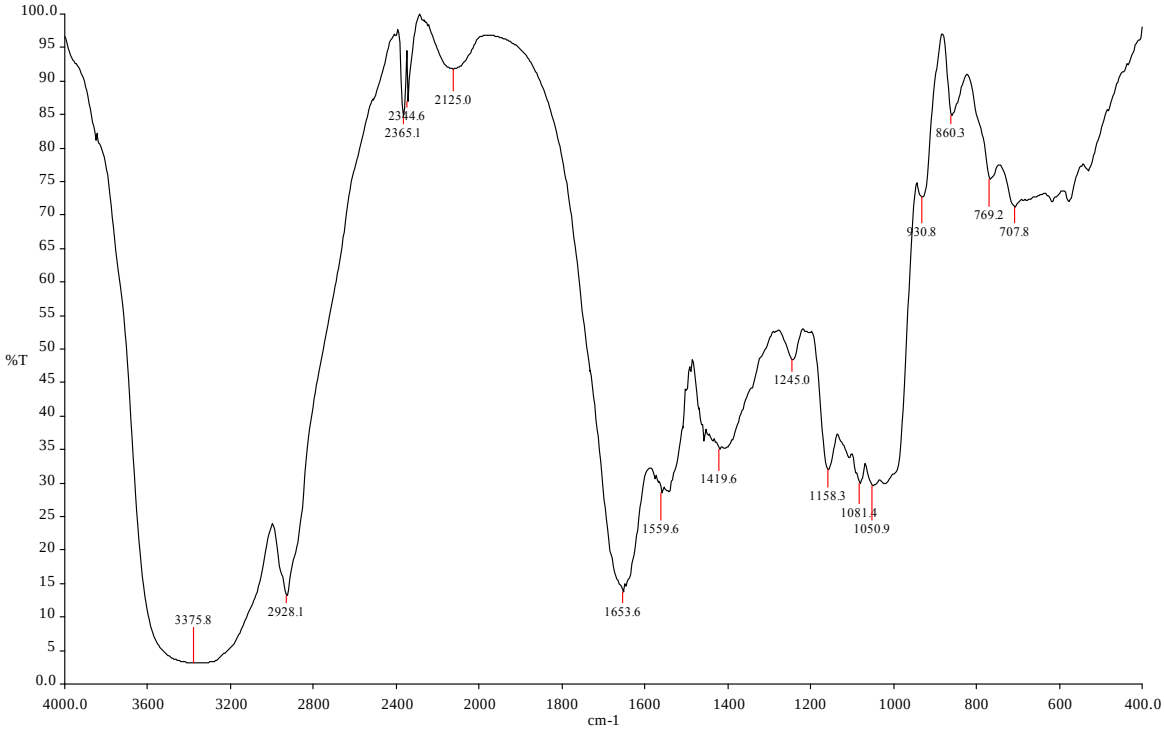


Fig: 7 FTIR spectrum of seed sample from AM fungi treated cowpea plants

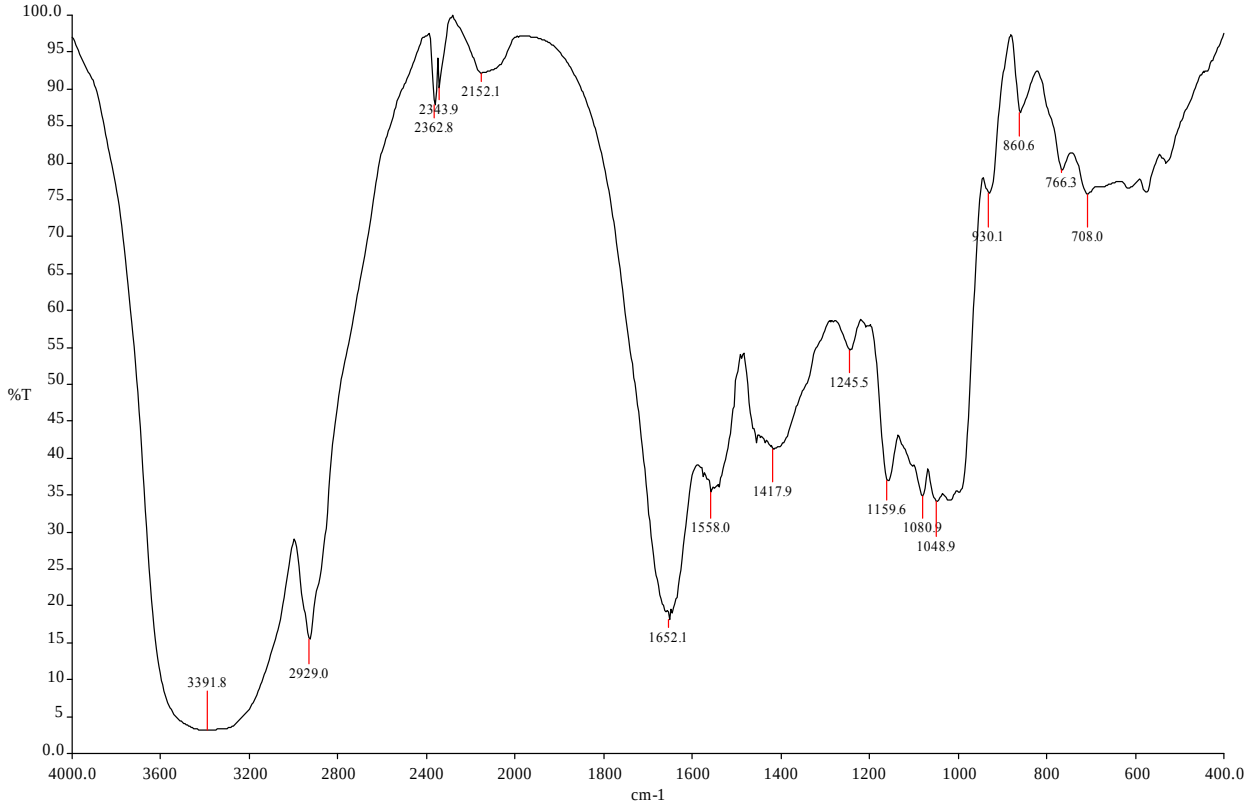
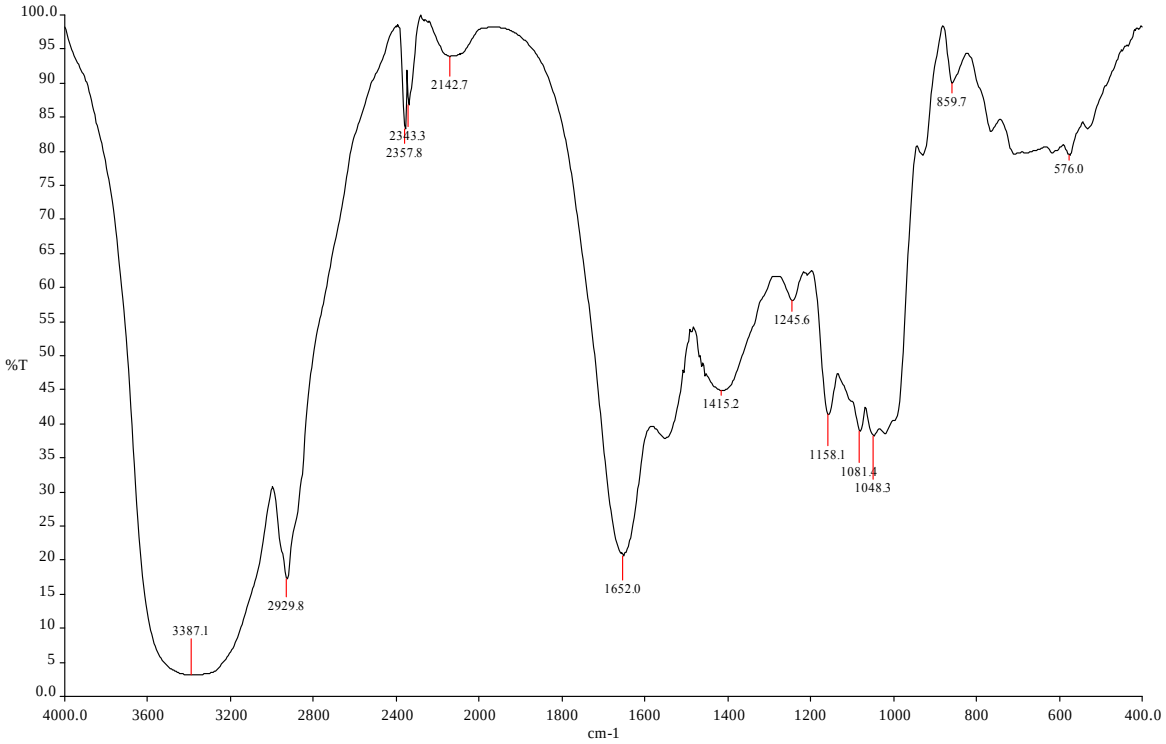


Fig: 8 FTIR spectrum of seed sample from *Rhizobium* and AM fungi treated cowpea plants



obtained in this experiment suggested that FTIR was able to detect the various functional in plants and various spectral data can be used to analyse changes of various compounds in the plants.

Conclusion

FTIR- is more quick and convenient than other techniques for detecting physiological indicators. Due to a few amount sample needed, the entire process of plants growth can be determined with this method. May be the FTIR will be used extensively in the research on plant physiology due to its virtues of simple and efficient on manipulation. The present study concluded that the application of FTIR-technique analysed the seeds of *Rhizobium* and AM fungi treated both chickpea and cowpea plants which provided more detailed functional groups of bio-molecule information on the samples because it measured the fundamental vibrations.

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