

Study of Nutrient Stresses in Plants by FTIR Spectroscopy

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Abstract

The FT-IR (Fourier transform infrared spectroscopy) is a powerful technique for the determination of vibrational structure of polyatomic molecules. The plant leaf is the laboratory of the plant, where different types of pigments are synthesized. The FT-IR spectroscopy helps in determining the structure of pigments in the plant leaves. It also helps in obtaining the bond strength and number of bonds having particular strength. Using this technique the distance between neighboring atoms and orientation of bonds between neighboring atoms can be obtained. The detailed study of the FT-IR spectroscopy helps in the investigation of biochemistry and the morphology of the plant leaves. We have studied the FTIR spectra of N, P, K and NPK deficient plant Helianthus annuus L. (Sunflower).

Keywords: Photosynthesis; FTIR; Helianthus annuus L (Sunflower).

Introduction

Photosynthesis is the process in which the plant leaves absorb light energy and convert it into chemical energy. The process takes place in the cell containing chloroplast, where the carbohydrates are built up from simple inorganic materials like carbon dioxide and water in the presence of light. The chlorophyll is an essential component for the process of photosynthesis, and occurs in chloroplast as green pigment in all photosynthetic plant tissues. It acts as a catalyst in the photosynthesis process [1 - 2]. The chlorophyll-a and chlorophyll-b are major pigments in higher plants. The chlorophyll-a and chlorophyll-b molecules have cyclic tetrapyrrolic structure, consisting metalloporphyrins with a cyclic ring containing a magnesium metal atom at its center the phytol chain of chlorophyll molecule extends from one of the pyrrole rings. The empirical formula of chlorophyll molecule is $C_{55}H_{72}O_5N_4Mg$. In order to study the mechanism of biological system the molecular structure of pigments must be determined. The molecular structure may be investigated by using various spectroscopic techniques. Many of the workers employing the Fourier Transform Infrared Spectra in the study of plant pigments [3-5].

Experimental Work

Material and Methods

The plants selected for the study are procured from the nursery at Ale (Pune). The plants are grown in green house chamber supplying essential nutrients N, P and K through the fertilizers. The plants are well watered and nourished. While growing plants same environmental condition is maintained. The

species selected for the study are Sunflower (*Helianthus annuus* L.). The above mentioned five plants are divided into five groups, each consisting of one plant of a species. The five groups are provided different fertilizers as listed below.

Group I - all the three essential nutrients P, N and K are supplied.

Group II - the N and K nutrients are supplied and have a P deficiency.

Group III - the P and K nutrients are supplied and have an N deficiency.

Group IV - the N and P nutrients are supplied and have a K deficiency.

Group V - the N, P and K deficiency plant.

Sample preparation

The plants are grown for two months after cultivation providing nutrients regularly. The fully-grown and healthy leaf detached from each plant and these leafs were placed separately in polythene bags. Then these leafs are shade dried in a clear environment to avoid the contamination for ten days and oven dried at 60 °C for four hours, to remove the moisture content. The oven dried leafs were ground into a fine powder by using an agate mortar and the FTIR spectra were recorded using FTIR spectrometer in the region 4000 - 400 cm⁻¹ by employing standard KBr pellet technique. The spectra were recorded at Department of Physics, University of Pune.

Results and Discussions

The FT-IR spectra of plant Sunflower (*Helianthus annuus*) is presented in figures 1 to 5.

Control plant: The absorption band located around 3537.77 cm⁻¹ corresponds to O-H and N-H stretching vibrations that mainly occur from chlorophyll protein and carbohydrates (Figure 1). The band around 3000 cm⁻¹ represents stretching vibrations that are mainly caused by lipids and carbohydrates. Absorption raised from C-H and C-N bending modes is located around 1200 cm⁻¹ to 1490 cm⁻¹ but may overlap within the region. The absorption bands around 1700 cm⁻¹ and 1300 cm⁻¹ correspond to C=O and H-C=O stretching vibration in free legend. These bands are shifted to 1675.84 cm⁻¹ and 1449.24 cm⁻¹ due to attachment of pyrrole structure of Mg metal complex. In controlled plant, all the bands appear to overlap at 1675.84 cm⁻¹ as strong absorption broad band due to high concentration of chlorophyll pigment. The absorption band at 1160.94 cm⁻¹ assigned to C-N stretching and presence of inorganic elements like sulfur, phosphorous, nitrates and P₂O₅ etc. The C-H stretching at 2970 cm⁻¹ attributed to CH₃ group. The presence of various vibrations in the functional group region and finger print region IR spectrum supports the complex structure of chlorophyll pigments. The strong absorption bands at 1675.84 cm⁻¹ and 1449.24 cm⁻¹ and 1160.94 cm⁻¹ show higher concentration of chlorophyll pigments in control plant [6 - 7].

P deficiency plant: The bands at 1454 cm^{-1} and 1651 cm^{-1} attributed to C=O and H-C =O groups respectively are weak and shifted to low frequencies by 6 cm^{-1} [Figure 2]. The absorption band at 1108.87 cm^{-1} attributed to C=N and possible presence of inorganic elements like P, N, S, and P_2O_5 having less intensity than controlled plant. The deficiency of nutrient P affects on the absorption bands at 1651.73 cm^{-1} , 1454.06 cm^{-1} and 1108.87 cm^{-1} .

K deficiency plant: The relatively intense bands at 3031.69 cm^{-1} and 2970 cm^{-1} are due to C-H stretching in CH_3 group of chlorophyll proteins. The band 1651.73 cm^{-1} is shifted to 1673.91 cm^{-1} and attributed to C-N vibrations in pyrrole structure, which is very weak as compared to control plant.

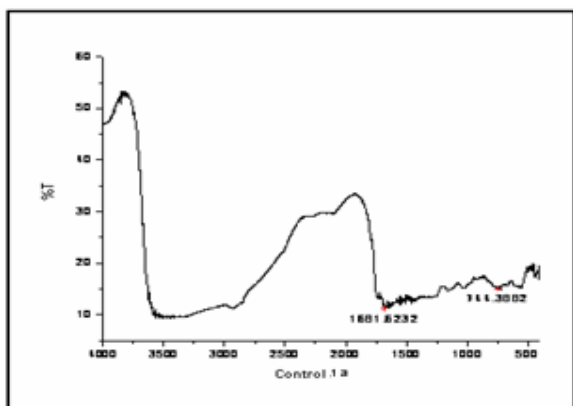


Figure 1 FT-IR spectra of plant Sunflower (*Helianthus annuus*) (control plant)

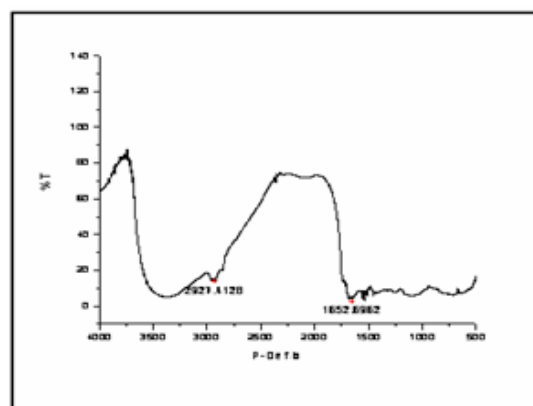


Figure 2. FT-IR spectra of P deficient plant

The other hyperfine bands are seen between 1600 cm^{-1} and 1445 cm^{-1} attributed to C-N vibrations in metal complex. The 1085.58 cm^{-1} band attributed to C=N, stretching vibration shifted to 1097.69 cm^{-1} . The K deficiency shows the broad absorption band at 1438.12 cm^{-1} shifted to less intense 1455 cm^{-1} band and nearly four hyperfine bands in the range 1600 to 1500 cm^{-1} .

N deficiency plant: The intense absorption band attributed to C-N stretching in Mg-porphyrin structure of chlorophyll pigment at 1651.73 cm^{-1} is shifted to 1665.23 cm^{-1} by 14 cm^{-1} in high frequency region and its intensity is considerably decreased as compared to controlled plant [Figure 4]. Other sharp hyperfine bands are also seen between 1600 cm^{-1} and 1445 cm^{-1} . The absorption band at 1160.94 cm^{-1} indicates the presence of inorganic element like phosphorus in the protein linked with chlorophyll, which is intense one. The sharp intense band at 717.39 cm^{-1} is attributed to O-H bending in plane and C-H out of plane bending. The N deficiency affects the absorption in the region 1600 cm^{-1} and 1445 cm^{-1} . This region attributed to Mg-porphyrin in chlorophyll clearly indicates the need of nitrogen in synthesis of chlorophyll.

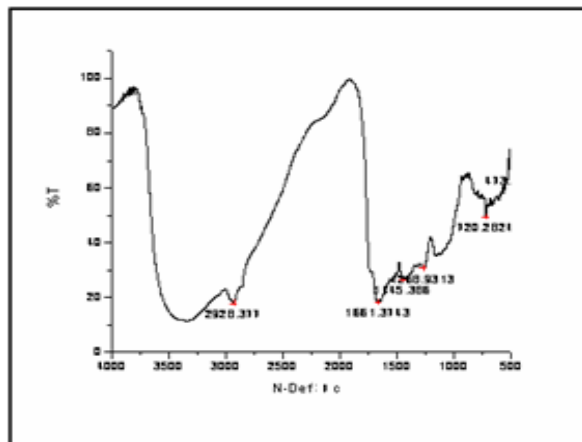


Figure 3. FT-IR spectra of K deficient plant

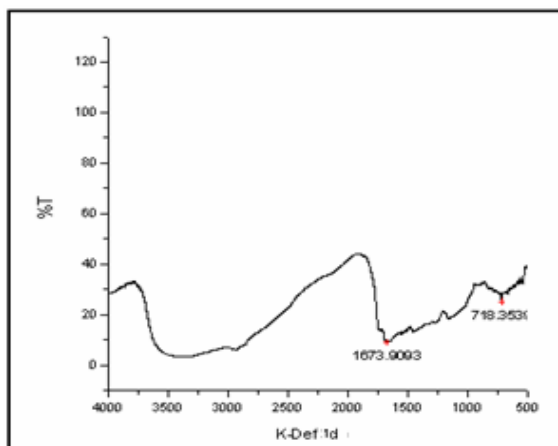


Figure 4. FT-IR spectra of N deficient plant

NPK deficiency plant: The absorption bands at 1675.84 cm^{-1} and 1452.14 cm^{-1} attributed to C=O and H-C=O group though intense but decreased in width as compared to control plant [Figure 5].

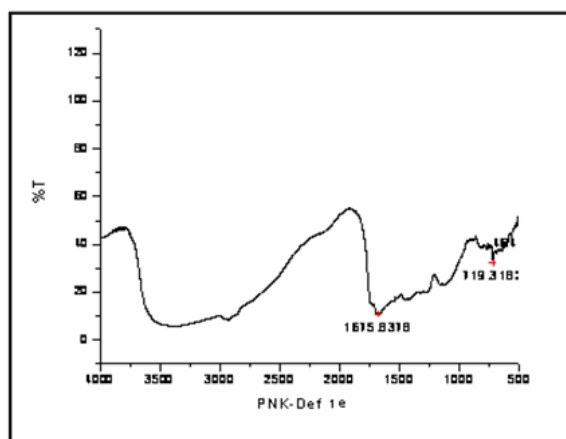


Figure 5. FT-IR spectra of NPK deficient plant

The absorption band at 1158.04 cm^{-1} is also intense and 719.318 cm^{-1} band as attributed to O-H bending is also intense. But the band at 1644.93 cm^{-1} is very weak. This assigned to the effect of N, P, and K stress on the IR spectra of the chlorophyll pigments of plant sunflower [8 -10].

Conclusions

It is concluded that FT-IR spectroscopy can be used to determine the structure of the chlorophyll like macromolecules. The FT-IR spectra can lead to the conclusion that particular pigment can be synthesized by supplying nutrients. The spectra recorded show the formation of chlorophyll is different for different plants. The spectra for particular species of plants have same pattern. The plant species is changed. Fewer change in IR pattern leads to the conclusion that the different plant species have different pigment



concentration. The spectral analysis shows that fertilizer given to the plants increase synthesis of the chlorophyll. Therefore, the FTIR spectroscopy is useful in the study of nutrient stresses. The vibrational bond strength of particular pigment in molecule can be obtained from FT-IR spectra. From the statistical study it is very clear that the different stresses in the plants may be very well studied using FT-IR spectroscopy.

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References

- [1] McCann Mc Hammouri M. Wilson R, P. Belton, and K. Roberts, "Fourier transform infrared microscopy is a new way to look at plant cell wall," *Plant Physio.*, vol. 100 pp. 1940 – 1947, 1992.
- [2] Devlin, and W. Whitham, "Plant Physiology," CBS Publishers & Distributors, New Delhi.
- [3] K. M. Smith, Ed. "Porphyrin and metal porphyrins," Amsterdam 1975.
- [4] K. M. Smith, "In porphyrin and metals phyrins Ed." Elsevier Amsterdam pp. 29 - 58 1975.
- [5] P. Giffiths, and J. A. Haseh, "Fourier transform infrared spectroscopy," New York John Wiley & Sons, 1986.
- [6] Sene CFB McCann Mc, R. H. Wilson, R. Grinter, "Fourier transform Raman and C an investigation of five higher plant cell wall and their components," *Plant Physio.*, pp. 1623 – 1631, 1994.
- [7] W. K. Surewiz, H. H. Mantsch, and D. Capman, "Determination of protein secondary structure by Fourier transform infrared spectroscopy" *An Artrial Assessment Biochemistry*, vol. 32, pp. 389 - 394, 1993.
- [8] W. K. Surewiz, and H. H. Mantsch, "New insight into protein secondary structure from resolution", *Enchanced Infrared spectra Biochemistry*, vol. 32, pp. 389 - 394, 1993.
- [9] J. Yang, and H. E. Yen, "Early slat stress effects on the changes in chemical composition in leaves office plant and arabidopsis a Fourier transform infrared spectroscopy study," *Plant Physio.*, vol. 1302, pp. 1032 – 1042, 2002.
- [10] K. V. Berezin, and V. V. Nechaev, "Calculation of the IR spectrum of ethyl chlorophyllide by the method of density functional," *J. App. Spect.*, vol. 70, pp. 632 - 635, 2003.