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# Possibility of using Infrared Absorption (FT-IR) Spectroscopy in Studying Effect of Fertilization with Ammonium Sulphate $(NH_4)_2SO_4$ on Nitrogen, Chlorophyll and Starch Contents of Manzanillo Olive Leaves.

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**ABSTRACT**

Fourier Transform Infrared (FT-IR) was used to study the effect of fertilization with ammonium sulphate $(NH_4)_2SO_4$ on nitrogen, chlorophyll and starch contents in Manzanillo olive leaves. Ammonium sulphate was applied at rate of 1kg/tree in each of February, May, June and August respectively. Studying FT-IR spectra of leaves were recorded at the beginning of growing season in March, at full bloom in May, at final fruit set in July and before harvest in August. Careful examination of the spectra of leaves revealed that the treatment of the soil with ammonium sulphate fertilizer causes no observable changes in their spectral features of the spectra apart from slight changes in the intensities of the absorption bands. To evaluate the changes in the spectral features on quantitative bases, the absorbance of the peaks at 2921 $cm^{-1}$ (C-H) methyl group, 1658 $cm^{-1}$ (N-H) amides, 1155 $cm^{-1}$ (C-N-C) chlorophyll (porphyrin ring) and 1049 $cm^{-1}$ (C-O) starch were determined and the absorbances ratios A1658$cm^{-1}$ / A2921 $cm^{-1}$, A1155$cm^{-1}$ / A2921$cm^{-1}$ and A1049$cm^{-1}$ / A2921$cm^{-1}$ were calculated to track the amount of change in the contents of nitrogen, chlorophyll and starch as a result of fertilization with ammonium sulphate during the growing season. Examination of the spectra of samples show that the ratio A1658$cm^{-1}$ / A2921 $cm^{-1}$ (Nitrogen content) given the highest rate of change in March. The samples taken in May indicated that increasing all absorption ratios compared to the samples taken in March. The samples which taken in July showed that the rates of changes in absorption ratios at 1658 $cm^{-1}$ /2921 $cm^{-1}$ (Nitrogen content) and 1155 $cm^{-1}$ / 2921 $cm^{-1}$(Chlorophyll) were decrease compared to the samples taken in May, while the ratio 1049 $cm^{-1}$ /2921$cm^{-1}$(Starch) increase with increasing the rate of nitrogen fertilization. The samples taken in August showed that the rate of increase in the absorption ratios in 1658 $cm^{-1}$ /2921 $cm^{-1}$ and 1155 $cm^{-1}$ /2921 $cm^{-1}$ have no significant changes a bit for the sample taken in July. Whereas, the rate of increasing the ratio of 1049 $cm^{-1}$ /2921 $cm^{-1}$ (Starch) decreased with increasing the rate of nitrogen fertilization.

**Keywords:** Olive, Manzanillo, Ammonium sulphate, Nitrogen, Chlorophyll, Starch, FT-IR, Infrared.

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## INTRODUCTION

Fourier Transform Infrared (FT-IR) absorption spectroscopy is certainly one of the most important analytical techniques. The FT-IR spectroscopy in identifying the molecular structure of materials organic and inorganic in the three forms solid, liquid and gas. The FT-IR technique can be affectively used to determine the chlorophyll, protein, starch, sugar and water in leaves [1,2] in the field. Fourier Transform Infrared (FT-IR) spectroscopy is the most sensitive technique in detecting the structure of organic molecules. The FT-IR spectrum gives characteristic absorption band of function groups of any substance. Each group has a characteristic absorption band at a certain wave number, so that any change in the structure of substance can be detected [3,4.5].

The objective of this study is to investigate the possibility of using infrared absorption spectroscopy in studying effect of fertilization with ammonium sulphate $(NH_4)_2SO_4$ on nitrogen, chlorophyll and starch contents of Manzanillo olive leaves.

## MATERIALS AND METHODS

This study was carried out during two successive seasons 2009 and 2010 on ten-years-old Manzanillo olive trees, planted at 5 X 5 m and grown in sandy soil in a private orchard located at Cairo–Alex desert road, Egypt. Trees were of normal growth, uniformed in vigour and received the same horticultural practices. Ammonium sulphate is added as soil applications at rate of 1kg/tree in each of February, May, June and August respectively.

### Infrared measurements:

### Soil analysis

Soil samples were taken randomly from two depths (0-30cm and 30-60cm) and dried in an oven at $50C^0$ for 48 hours then grounded in mortar and passed through 180-mesh sieve to remove the large structure. Two mg of the sample mixtured with 198 mg of pure KBr (potassium bromide) and pressed in hydraulic press (at 40 KN.) to form a transparent disk, diameter 13 mm approximately, then were measured by infrared spectrophotometer [6].

### Leaves samples

Twenty leaves from the middle portion at one year old shoot each treated tree were taken at the beginning of the growing season in March [sample No. 1] and in full bloom in May [sample No. 2], in final fruit set in July [sample No. 3] and before harvest in August [sample No. 4]. The samples were dried in an oven at $50C^0$ for 2-3 h then grounded by mortar and passed through 125-mesh sieves. Two mg of the sample was mixed with 198 mg of pure KBr (potassium bromide) to give 1% concentration. The mixing was carried out for suitable time in an agate mortar. The mixture was pressed in special mold under hydraulic press (at 40 KN.) to form a transparent disk, diameter 13 mm approximately. Spectra leaves were determined by infrared spectrophotometer.

### Infrared spectrophotometer

Jasco FT/IR-430 Fourier Transform Infrared Spectrometer was used for recording the IR spectra. Spectral range of 400-4000$cm^{-1}$ and the obtained spectrum was automatically recorded on a computer. For quantitative measurements, the absorbance ratios (amount of infrared absorbance by the various bands in the molecule) were calculated using soft ware Jasco by the Beer-Lambert's law. Where there is a direct correlation between the absorbance and the number of molecules absorbent material to radiate or consented. Then absorbance was measured using the base line method as the vertical distance from the maximum absorption to the base line connecting the two wings of the band (Fig. 1). Absorbency (C-H) methyl group, (N-H) nitrogen, (C-N-C) chlorophyll (porphyrin ring) and (C-O) starch were measured to determine absorbance ratio to track the amount of change in nitrogen, chlorophyll molecule and starch as a result of application with ammonium sulphate during the growing season.

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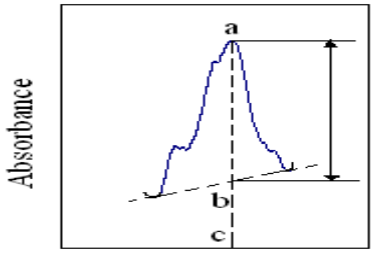


**Fig 1: The base line method.**

The aim of the experiment is to study the effect of ammonium sulphate on nitrogen, chlorophyll molecule and starch contents of Manzanillo olive leaves by FT-IR spectroscopy technique.

## RESULTS AND DISCUSSION

FT-IR spectroscopy was used to study the effect of fertilization with ammonium sulphate $(NH_4)_2SO_4$ on nitrogen, chlorophyll and starch contents in Manzanillo olive leaves.

The obtained result showed that infrared absorption spectra of the samples taken from the soil under study was measured and turns out to examine the absorption spectrum of these samples it is sandy in nature and consist mostly of ($SiO_2$) silicon oxide as well as the proportion of carbonate compounds.

Infrared absorption spectrum of ammonium sulphate fertilizers was measured and careful examination of the spectrum showed that presence of characteristic bands of the sulfate group at wave number (1113 and 617 $cm^{-1}$) and bands of amides at (3388 and 1615 $cm^{-1}$). Infrared spectra of leaves were recorded after fertilization and at different periods i.e. at the beginning of growing season in March [sample No. 1], at full bloom in May [sample No. 2], at final fruit set in July [sample No. 3] and before harvest in August [sample No. 4] Fig. 2.

Careful examination of the spectra leaves samples shows that the dates of the different fertilization not change greatly in the spectral features of the leaves except for slight changes in the intensity of absorption bands. To evaluate the changes on a quantitative base:

1. Infrared absorption bands were defined to identify the contents of nitrogen, chlorophyll molecule and starch as the following:

a. C-H absorption of the methyl group (not affected by fertilization) appeared at the frequency 2921 $cm^{-1}$.
b. N-H absorption of nitrogen appeared at the frequency 1658$cm^{-1}$.
c. C-N-C absorption of the porphyrin ring (which is the basis of the chlorophyll molecule structure) appeared at the frequency 1155$cm^{-1}$.
d. C-O absorption of starch appeared at frequency 1049 $cm^{-1}$.

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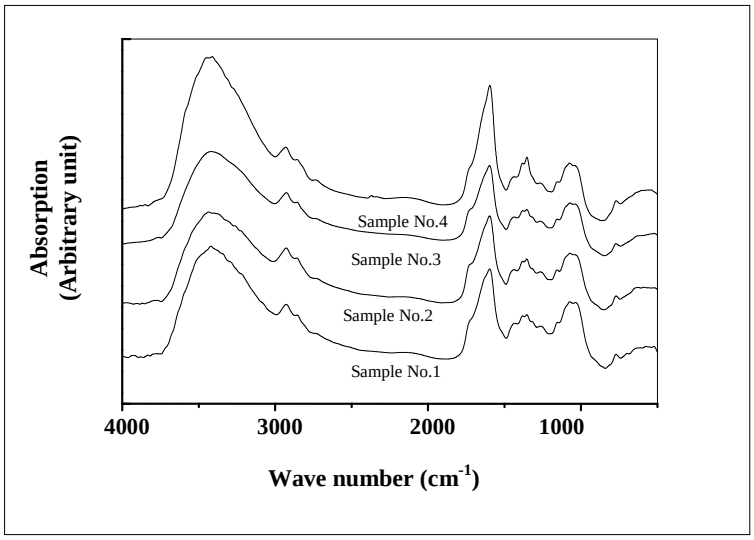


**Fig 2: FT-IR spectra of leaves during the growing season.**

2. Determined absorbance, where there is a direct relationship between the absorbance and concentration of absorbent material to IR radiation (Beer's law). The absorbance intensity will measured using base line method in which the line tangent to the ends of the band absorption and then measured the vertical distance from the top (the highest point of absorption of the infrared) to the base-line to measured absorbency of C-H, N-H, C-N-C, and C-O to track the amount of change in the contents of nitrogen, chlorophyll molecule and starch as a result of fertilization with ammonium sulphate during the growing season Table 1 & Fig. 3 and the results shows that:

a. Examining the sample taken in March showed that all absorption ratios were affected with ammonium sulfate fertilization and the ratio 1658 $cm^{-1}$/2921$cm^{-1}$ (Nitrogen content) given the highest rate of change.
b. Spectral analysis of samples taken at full bloom in May indicated to increasing all absorption ratios with increase the amount of nitrogen fertilizer compared to the sample taken in March.
c. Sample taken at final fruit set in July showed that the rates of changes in absorption ratios 1658 $cm^{-1}$ /2921 $cm^{-1}$ (Nitrogen content) and 1155 $cm^{-1}$ / 2921 $cm^{-1}$ ( chlorophyll) were decrease compared to the sample taken in May, while the ratio 1049 $cm^{-1}$ /2921$cm^{-1}$(starch) increase with increasing rates of nitrogen fertilization.
d. Sample taken before harvest in August showed that the rate of increase in the absorption ratio in 1658 $cm^{-1}$ /2921 $cm^{-1}$ and 1155 $cm^{-1}$ /2921 $cm^{-1}$ have no significant changes a bit for the sample taken in July and the highest value of absorbance ratio was obtained with application of ammonium sulphate at the rate of 1kg/tree in each of February, May, June and August. Whereas, rate of increase in the ratio of 1049 $cm^{-1}$ /2921 $cm^{-1}$ decreased with increase in nitrogen fertilization. These results can be explained that this stage is considered as the fruit maturity in which the fruit reached to maximum size, so all nutrients in the tree are directed to the fruit and this explain the decrease in the absorbance ratio A1049 $cm^{-1}$ /A2921 $cm^{-1}$ (starch).

**Table 1: Effect of fertilization with ammonium sulphate on absorbance ratios A1658$cm^{-1}$ /A2921$cm^{-1}$, A1155$cm^{-1}$/A2921$cm^{-1}$and A1049 $cm^{-1}$/A2921 $cm^{-1}$ during the growing season**

| Measuring date | Absorbance ratio A1658 $cm^{-1}$/ A2921 $cm^{-1}$ (Nitrogen) | Absorbance ratio A1155 $cm^{-1}$/ A2921 $cm^{-1}$ (chlorophyll) | Absorbance ratio A1049 $cm^{-1}$/ A2921 $cm^{-1}$ (starch) |
|---|---|---|---|
| March | 2.9 | 1.8 | 2.3 |
| May | 3.6 | 2.1 | 3.08 |
| July | 3.46 | 1.98 | 3.4 |
| August | 3.4 | 1.96 | 2.8 |

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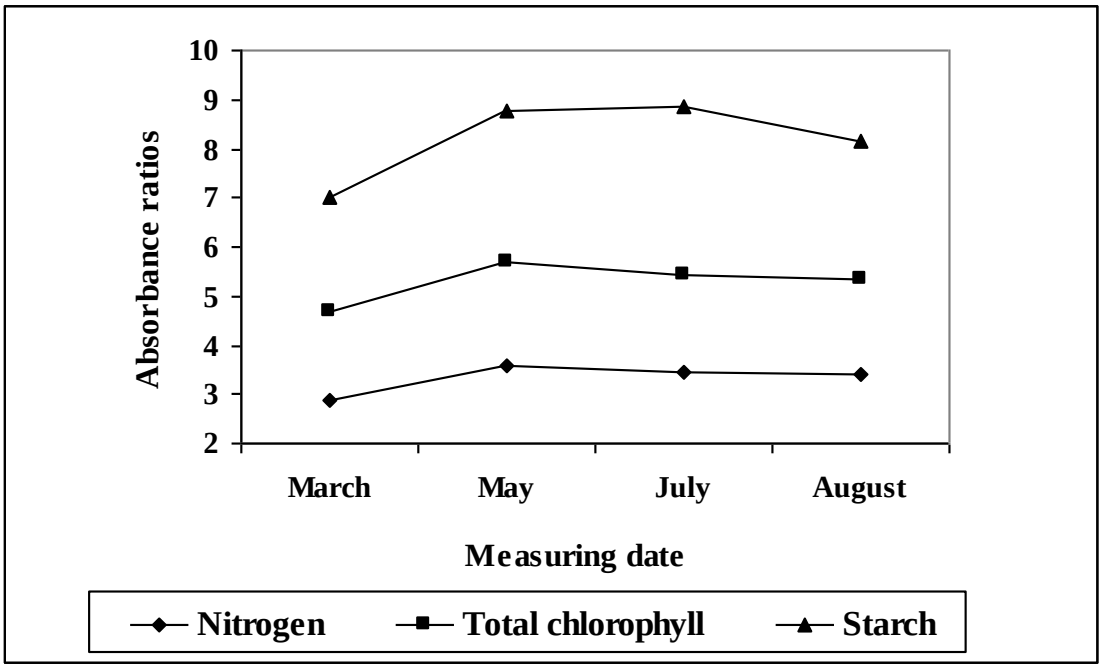


**Fig 3: The rate of change in absorbance ratios A1658cm$^{-1}$/A2921cm$^{-1}$, A1155cm$^{-1}$/A2921cm$^{-1}$and A1049 cm$^{-1}$/A2921 cm$^{-1}$ during the growing season as a result of fertilization with ammonium sulphate.**

## CONCLUSION

FT-IR spectroscopy can be used in identifying the contents of nitrogen, chlorophyll and starch of Manzanillo olive leaves as a new technique.

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