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Using Infrared Absorption Spectroscopy in Studying Nitrogen, Chlorophyll and Starch Contents in Manzenillo Olive Leaves Before Harvest Stage

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Abstract: 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-Alexandria desert road (about 50km from Cairo), Egypt. Trees were of normal growth, uniformed in vigour and received the same horticultural practices. This experiment aimed to study the effect of nitrogen fertilization dates on nitrogen, photosynthetic pigments and starch contents of Manzanillo olive leaves using FT-IR spectroscopy. Also minimize the quantity of added annually amounts by 20% and the best add-on dates during the growing season. In both seasons the experiment included five nitrogen fertilization treatments with ammonium sulphate (20.6%N) as soil applications. The highest value of absorbance ratio A1658cm^{-1}/A2921cm^{-1} (Nitrogen content), absorbance ratio A1155cm^{-1}/A2921 cm^{-1} (pyrophrine ring the basic structure of chlorophyll molecule) and absorbance ratio A1049cm^{-1}/A2921cm^{-1} (starch) were obtained as a result of ammonium sulphate application at the rate of 1kg/tree in each of February, May, June and August(3.4, 1.96 and 2.8, respectively) meanwhile the lowest value was obtained in control trees(2.3, 1.5 and 1.4, respectively).

Key words: Harvest stage · Infrared absorption spectroscopy · Manzanillo

INTRODUCTION

The two primary components of plant leave which absorbing visible and infrared are chlorophyll and water. Chlorophyll absorption primarily affected by electron transitions between 430 to 460 nm and 640 to 660 nm, while water absorption bands center at 970, 1200, 1450 and 1780 nm. Other important absorbing biochemical include proteins, starch, cellulose, nitrogen and oils absorption bands center at 1658, 1049, 2280, 1658 and 2310 nm, respectively [1, 2]. Curran *et al.* [3] reported that reflectance spectroscopy of chlorophyll, protein, starch, sugar and water in leaves is located at wavelengths throughout the visible and near infrared. Infrared (IR) absorption spectroscopy is certainly one of the most important analytical techniques. One of the great advantages of infrared absorption spectroscopy is that study any sample in any state. Infrared absorption spectroscopy in identifying the molecular structure of materials organic and inorganic in the three forms solid, liquid and gas as well as to identify mutations that occur as a result of various treatments. Infrared absorption spectroscopy is a technique based on the vibrations of the atoms of a molecule. Nitrogen availability is an important determinant of productivity. Nitrogen deficiency decreases yield and quality by limiting amino acid and chlorophyll synthesis. Visual symptoms of nitrogen stress include plant chlorosis and leaf senescence [4]. Methods used to detect N deficiencies during the growing season include chlorophyll meter readings, destructive plant sampling and soil sampling. Furthermore, diagnostic methods like plant reflectance techniques (Infrared) that take advantage of the optical properties of leaves are another possibility to assess the nitrogen status of plants in a field and to assess nitrogen fertility effects on yield. The use of effective plant reflectance techniques are rapid and would eliminate the need for extensive field sampling, assuming that the deficiency detection capability is high [5]. Infrared absorption spectroscopy is a technique based on the vibrations of the atoms of a molecule. In fact, it demonstrated recently that the IR technique can be effectively used to determine the water contents of leaves [6, 7] and soils [8], to determine the nitrogen

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concentration in potato tissues [9] and the soluble solids content in processing tomatoes [10], as well as to measure acidity, soluble solids and firmness of Jonagold apples [11]. McGlone and Kawano [12] used IR spectroscopy to determine dry-matter and soluble solids content of kiwifruit, oil content in groundnuts [13], free fatty acids content in sunflower [14] and measured the quality of intact olive cvs. (Ayvalik and Gemlik) including firmness, oil content and colour [15-17].

The main objective of the investigation is using infrared absorption spectroscopy in studying nitrogen, chlorophyll and starch content in Manzenillo olive leaves before harvest stage.

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-Alexandria desert road (about 50 Km from Cairo), Egypt. Trees were of normal growth, uniformed in vigour and received the same horticultural practices. This study aimed to investigate the effect of nitrogen fertilization dates on nitrogen, photosynthetic pigments and starch contents of Manzanillo olive leaves using FT-IR spectroscopy. Also minimize the quantity of added annually amounts by 20% and the best add-on dates before harvest stage (August). In both seasons the experiment included five nitrogen fertilization treatments with ammonium sulphate (20.6%N) as soil applications and the applied treatments involved the following as shown in Table 1.

Twenty leaves from the middle portion at one year old shoot each treated tree were taken before harvest in August. The samples were dried in an oven at 50°C for 2-3h 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.IR spectra leaves were determined by infrared spectrophotometer. Jasco FT/IR-430 Fourier Transform Infrared Spectrometer was used for recording the IR spectra. Spectra were recorded in a spectral range of 400-4000cm^{-1} and the obtained spectrum was automatically recorded on a computer. For quantitative measurements, the absorbance

Table 1: Fertilization treatments with ammonium sulphate (20.6%N) and time of application

Treatments	1	2	3	4	5 (Control)
Time of application	Amount of ammonium sulphate (kg/tree)				
January	1	0.5	1	-	2
February	-	-	-	1	-
March	1	1.5	1.5	-	-
May	1	-	1	1	-
April	-	-	-	-	-
June	-	1.5	-	1	1.5
July	-	-	-	-	-
August	1	0.5	0.5	1	1.5

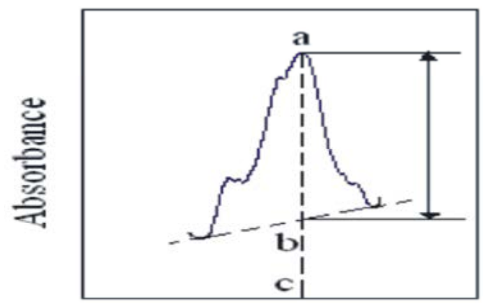

Fig. 1: The base line method.

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 radiation or concentration. 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, N-H, C-N-C and C-O were measured to determine absorbance ratio to track the amount of change in chlorophyll molecule, protein and starch as a result of application with ammonium sulphate during the growing season.

RESULTS AND DISCUSSION

FT-IR spectra of leaves before harvest in August are shown in Fig. 2 and Table 2 absorbance ratios A1658cm^{-1}/A2921cm^{-1} (Nitrogen content), A1155cm^{-1}/A2921cm^{-1} (chlorophyll) and 1049cm^{-1}/ A2921cm^{-1}(starch). The results explained that this stage is considered as the fruit maturity 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 A1049cm^{-1}/A2921cm^{-1} (starch) while nitrogen content and chlorophyll were not changed

Table 2: Effect of fertilization with ammonium sulphate on absorbance ratios A16558cm^{-1}, A1155cm^{-1}/A2921cm^{-1} and A1049cm^{-1}/A2921cm^{-1}(August).

Treatments	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)
1	3.2	1.86	2.5
2	3.3	1.90	2.6
3	3.3	1.95	2.7
4	3.4	1.96	2.8
Control	2.3	1.50	1.4

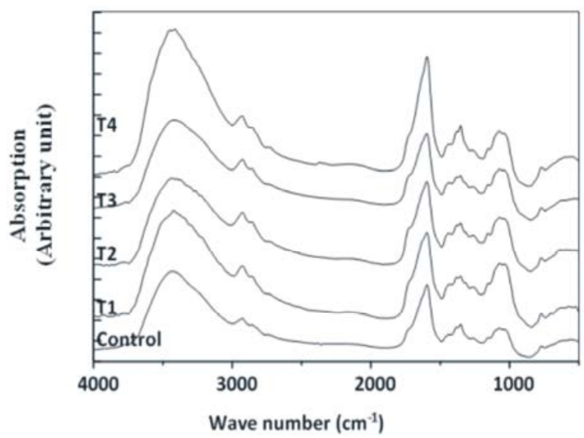

Fig. 2: FT-IR spectra of leaves before harvest in August

T1. Trees were fertilized in Jan.(1kg/tree), Mar.(1kg/tree), May (1kg/tree) and Aug.(1kg/tree).

T2. Trees were fertilized in Jan. (0.5kg/tree), Mar. (1.5kg/tree), June (1.5kg/tree) and Aug. (0.5gk/tree).

T3. Trees were fertilized in Jan. (1kg/tree), Mar. (1.5kg/tree), May (1kg/tree) and Aug. (0.5kg/tree).

T4. Trees were fertilized in Feb. (1kg/tree), May (1kg/tree), June (1kg/tree) and Aug. (1kg/tree).

T5. Control (recommended dose according to the ministry of Agriculture).

Absorbance Ratio A1658 cm^{-1}/ A2921 cm^{-1} (Nitrogen Content): The results illustrated in Table 2 and Fig. 3 show that the highest value of absorbance ratio A1658cm^{-1}/A2921cm^{-1} (Nitrogen content) was obtained with application ammonium sulphate at rate of 1kg/tree in each of February, May, June and August (3.4), meanwhile the lowest value was obtained in control trees (2.3).

Absorbance Ratio A1155 cm^{-1}/A2921 cm^{-1} (Chlorophyll): Regarding absorbance ratio A1155cm^{-1}/A2921cm^{-1}, results in Table 2 and Fig. 3 show that the highest value was recorded with

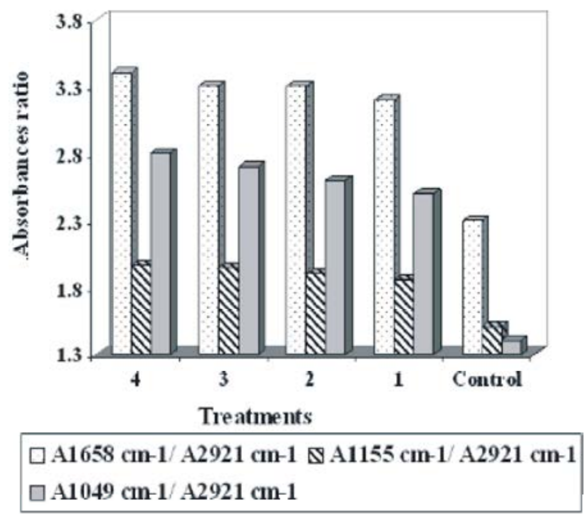

Fig. 3: Effect of fertilization with ammonium sulphate on absorbance ratios A1658cm^{-1}/A2921cm^{-1}, A1155cm^{-1}/A2921cm^{-1}, A1049cm^{-1} /A2921 cm^{-1} (August).

application ammonium sulphate at rate of 1kg/tree in each of February, May, June and August (1.96), meanwhile the lowest value was obtained in control trees (1.5).

Absorbance Ratio A1049 cm^{-1}/A2921 cm^{-1}(Starch): Concerning absorbance ratio 1049cm^{-1}/ A2921cm^{-1} (starch), results in Table 2 and Fig.3 show that the highest value was recorded with application ammonium sulphate at rate of 1kg/tree in each of February, May, June and August (2.8), meanwhile the lowest value was obtained in control trees (1.4).

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