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Photosynthetic pigments and fruit quality of Manzanillo olive as affected by 6-benzyladenine and studying the chemical constituents in leaves using Fourier transform infrared spectroscopy technique

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Abstract: This study was carried out during 2013 and 2014 on ten years old Manzanillo olive trees, planted at 5 X 5 m in sandy soil in a private orchard located at 50km. of the Cairo –Alexandria desert road, Egypt. 6-benzyladenine (BA) at 0, 40, 60, 80 and 100 mg.L^{-1} were sprayed twice; at the first week of April and one month later at both seasons. Chlorophyll a, b and carotenoids were determined using visible spectroscopic techniques at wave lengths 450, 645 and 665 nm respectively and the Fourier Transform Infrared (FT-IR) spectroscopic technique also was used. This technique proved the presence of phenols, methyl group, pyrophrine ring, amines, amides bands of tissue protein and carbohydrate. Results cleared that photosynthetic pigments increased with increasing BA concentrations. This result is in good agreement in both methods of determination. Phenols, protein and carbohydrate in the leaves were increased with increasing concentration of 6- benzyladenine from 40 to 100 mg.L^{-1}.The highest yield (kg/tree), fruit weight, moisture and oil content were attained from in BA treatments of 80 and 100 mg.L^{-1}. Meanwhile 100 mg.L^{-1} treatments resulted in obtaining the highest fruit size, length, diameter and fruit flesh weight and plup / seed ratio.
Key words: Olive, Manzanillo, 6-benzyladenine, Chemical constituents, Phenols, Yield, Fruit quality, FT-IR.

Introduction

Cytokinins are a large group of plant hormones that regulate various processes of plant growth and development. Shudok [1] reported that chemical structure of cytokinin active substances has determined two groups of adenine cytokinins and urea cytokinins with similar physiological effects, it has pronounced effect of cotyledon growth and expansion and other processes. Cytokinins appeared to play an important role in the regulation of cell division, differentiation and organogenesis in developing plants, enhancement of leaf expansion, nutrient mobilization and delayed senescence and benzyladenine (BA) is one of the most active cytokinins [2]. It has been identified as a natural cytokinin in number of plants [3].

Application of BA has been reported to influence leaf senescence and leaf chlorosis [4]. BA is a synthetic cytokinnin which has the ability to hasten cell division, promote carbohydrate metabolism and create new source-sink relationships hence leading to increased sink strength and fruit size at harvest [5]. Application of BA increased mineral nutrition of both fruits and leaves [6]. Also, BA increased mineral nutrient mobilization, a phenomenon known as the cytokinin-induced nutrient mobilization [7]. The nutrients will move from the site of production and/or storage (roots and/or leaves) to the site of utilization (leaves/ fruits). The effect of BA on the increased potassium mineral content in both the leaves and fruits may have influenced the increased uptake of other minerals (N, P, Mg, Na, and Ca). Potassium plays an important role in osmo-regulation and translocation of nutrients into plant cells [8]. BA increased carbohydrate and protein content in the leaves [9].There are a lot of studies about the effects of cytokinins on the level of photosynthetic pigments [10, 11, 12, 13]. It has been reported that cytokinin generally increases the level of photosynthetic pigments. The effect of cytokinins especially benzyladenine on the plant growth, chemical constituents and fruit quality of different plants have mentioned by [14, 15, 16, 17, 18, 19].

Fourier transform infrared spectroscopy (FT-IR) is one of the most widely used methods to identify the chemical constituents and elucidate the structures of compounds [20]. FT-IR spectroscopy is a physic-chemical analytical technique that does not determine concentrations of individual metabolites but provides a snapshot of the metabolic composition of a tissue at a given time [21]. The FT-IR method measures predominantly the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic “fingerprint” of the sample [22]. Infrared spectroscopy provides a useful method for herbal analysis as well as for quantitative analysis of drugs and with the help of FT-IR continuous monitoring of the spectral baseline and simultaneous analysis of different components of the same sample is possible [23,24] and generally used to identify the functional groups [25, 26].

The main object of the present work is to study the effect of 6-benzyladenine on photosynthetic pigments and fruit quality of Manzanillo olive, using FTIR absorption spectroscopic technique for determining the functional constituents present in the leaves powder and effects of 6-benzyladenine on it.

Materials and Methods

The present work was conducted during two successive seasons 2013 and 2014 on ten years old Manzanillo olive trees, planted at 5 X 5 m in sandy soil in a private orchard locates at 50km. of the Cairo – Alexandria desert road, Egypt. Trees were of normal growth, uniform in vigour and received normal fertilization and cultural practices as scheduled in the program of the orchard. The experiment followed complete randomized block design on 15 trees as 5 treatments were applied. Each tree was considered a replicate, three replicates trees per each treatment. The trees were sprayed twice; at the first week of April and after one month from the first at both seasons with freshly prepared solutions of 6-benzyladenine (BA) 0, 40, 60, 80 and 100 mg.L^{-1} each containing 10ml (0.1 %) Tween-20 surfactant.

Photosynthetic pigments determination:

At mid. July of each season samples from the fresh leaves were grounded in mortar with 85% aqueous acetone the pigments were filtered through sintered glass filter G.4, then the filtrate was made up to a known volume 85% acetone. The optical density of the filtrate was determined using spectrophotometer at wave length 450, 645 and 665 nm for chlorophyll a, chlorophyll b and carotenoids, respectively by the formulas [27, 28]. The concentrations were measured in mg/g dry weight.

Yield and fruit quality determination:

At maturity stage (early October), fruits of each replicate tree were separately harvested, then weighted and yield as Kg/tree was estimated, then 20 fruits from each replicate tree i.e. 60 fruits from each of the applied treatments were picked randomly at harvest to determine: fruit weight (g), fruit length (cm), fruit diameter (cm), seed weight (g), fruit flesh weight (g), percentage of pulp/seed, fruit moisture percentage and fruit oil content (%) according A.O.A.C.[29].

FTIR spectrum analysis:

Fourier transform infrared spectroscopy (FTIR) of absorbent was done and transferred to Microsoft Excel by using an FTIR spectrophotometer (Model: FTIR 2000, Shimadzu, Kyoto, Japan). About 150mg KBr disks containing approximately 2% of Manzanillo olive leaf powder samples was prepared shortly before recording the FTIR spectra in the range of 400-4000cm^{-1} [30].

Statistical analysis:

All obtained data during both 2013 and 2014 experimental seasons were subjected to analysis of variances according to [31] using (SAS/STAT). Least significant difference (L.S.D) was used to compare between means of treatments according to [32] at probability of 5%.

Results and Discussion

Photosynthetic pigments:

The effect of foliar application with 6-benzyladenine treatment on photosynthetic pigments concentrations of Manzanillo leaves were presented in Figure (1).The results show that photosynthetic pigments; chlorophyll a, b, total chlorophyll (a + b) and carotenoids concentration were significantly increased by all foliar application with 6-benzyladenine in both seasons 2013 and 2014 as compared to those of control. The highest significant chlorophyll a, b, total chlorophyll (a + b) and carotenoids were obtained as a result of the concentration 100 mg.L^{-1} 6-benzyladenine (1.55, 1.22, 2.77 and 0.99mg/g) in season 2013 respectively and (1.57, 1.25, 2.82 and 0.95mg/g) in season 2014 respectively. The lowest significant chlorophyll a, b, total chlorophyll (a + b) and carotenoids resulted from 0 BA (0.89, 0.44, 1.33 and 0.70 mg/g) in season 2013 respectively and (0.85, 0.42, 1.27 and 0.75 mg/g) in season 2014 respectively. The increasing contents of photosynthetic pigments at certain concentrations of BA in may be due to the increase of cell number under the effect of BA on cell division subsequently the size and number of chloroplasts in leaves [22, 33]. Also, could indirectly be mediated by BA as a result of regulation of some metabolic events or by a direct effect of BA on the photosynthetic reactions [34].

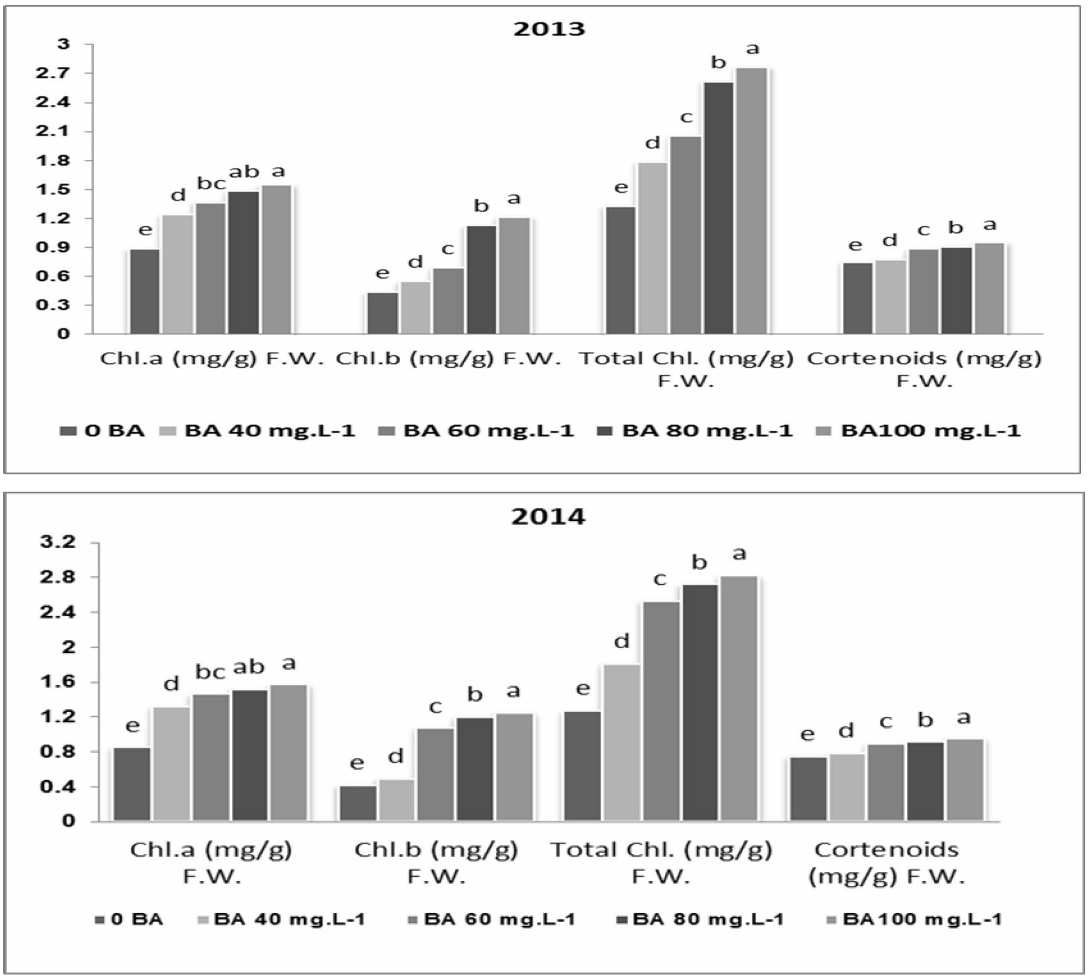

Figure 1: Effect of 6-benzylaadenine on photosynthetic pigments of Manzanillo olive leaves during 2013 and 2014 seasons.

Yield:

Table (1) cleared that the effect of spraying 6-benzyladenine on yield (kg/tree) is considered a reflection of the studied treatments on fruit productivity of the examined trees. During the two seasons of the study 80 and100 mg.L^{-1} of 6-benzyladenine applications at the first week of April and one month later resulted in the highest significant yield (20.15 & 25.18 kg/tree) and (20.15 & 25.19 kg/tree) during 2013 and 2014 respectively compared to the lowest significant yield produced from 0 BA (10.25 and10 kg/tree) during 2013 and 2014 respectively. These results are in agreement with those of [10,16 ,35] found that foliar application of benzyladenine caused significant increase yield/tree compared to the control.

Fruit physical characteristics:

Almost all the applied treatments significantly improved physical characteristics of Manzanillo olive fruits compared to those of the control trees. However there were significant differences in the enhancement effects of the applied treatments table (1, 2).

Fruit weight:

During both seasons highest significant fruit weight (4.53& 4.58g) and (4.55&4.61g) resulted from the concentration 80 and 100 mg.L^{-1} of 6-benzyladenine respectively. Meanwhile the lowest significant fruit weight was produced from 0 BA (3.82 and 3.79g) in seasons 2013 and 2014.

Fruit length:

In both seasons of the study, highest significant fruit length (2.73& 2.85cm) and (2.76 & 2.88cm) resulted from the concentration 80 and 100 mg.L^{-1} of 6-benzyladenine respectively table (1), whereas the lowest significant fruit length was resulted from 0 BA (2.05 and 2.49cm) during seasons 2013 and 2014 respectively.

Fruit diameter:

Highest significant fruit diameter (2.00 and 1.99cm) in seasons 2013 and 2014 respectively. The highest values were observed as a result of application with 100 mg.L^{-1} of 6-benzyladenine in both seasons of the study, whereas the lowest significant fruit diameter was resulted from 0 BA (1.48 and 1.49cm) during seasons 2013 and 2014 respectively.

Fruit size:

Highest significant fruit size reached (5.55 and 5.61cm^3) in seasons 2013 and 2014. The highest levels were observed as a result of application with 100 mg.L^{-1} of 6-benzyladenine in both seasons of the study table (1), whereas the lowest significant fruit size resulted from 0 BA (4.13 and 4.36cm^3) during seasons 2013 and 2014 respectively.

Seed weight:

With respect to seed weight table (2), 0 BA treatment gave the heaviest seed weight (1.15 and 1.14g) in the 1st and 2nd seasons, whereas application with 100 mg.L^{-1} of 6-benzyladenine gave the lightest seed weight (1.08 and 1.09g) in the first and second season respectively.

Fruit flesh weight:

The highest significant fruit flesh weight (3.47 and 3.52g) in the first and second season respectively was obtained with 100 mg.L^{-1} of 6-benzyladenine followed by with 80 mg.L^{-1} of 6-benzyladenine (3.05 and 3.47g) in both seasons respectively. Meanwhile the lowest fruit flesh weight was obtained from 0 BA (2.67 and 2.65g) in both two seasons respectively table (2).

Pulp/seed ratio:

In both seasons of the study foliar application of 100 mg.L^{-1} of 6-benzyladenine resulted in the highest significant pulp/seed ratio (76.26 and 76.36%) in both seasons 2013 and 2014 respectively. However the lowest significant levels of pulp/seed ratio (69.81 and 69.92 %) in 0 BA treatment during the two seasons of the study table (2).

Fruit growth after bloom is dependent in large part on photosynthesis supplied by spur leaves [36]. Cytokinins have the ability to promote carbohydrate metabolism and create new source-sink relationships, thus leading to increase fruit size and fruit dry matter [37]. The cytokinin 6-benzyladenine (BA) influenced larger fruit size or weight by increasing the number of cells per fruit through the stimulation of cell division and increasing number of cell layers and not cell expansion [33].

Fruit moisture content:

Concerning fruit moisture content at the first season, there were not significant differences between 80 and 100 mg.L^{-1} of 6-benzyladenine compared to other treatments table (2). Whereas at the second season, fruit moisture content was influenced significantly as a result of different concentrations of 6-benzyladenine treatments; it is obvious that the highest significant fruit moisture content was attained as a result of 100 mg.L^{-1} of 6-benzyladenine compared to 0 BA which recorded the lowest significant values.

Oil content:

It is obvious from table (2) that oil content was influenced significantly as a result of 6-benzyladenine treatments. The highest significant oil content was obtained from concentrations 80 and 100 mg.L^{-1} of 6-benzyladenine (18.96 &19.89%) and (18.98 & 19.91%) in both seasons 2013 and 2014 respectively, whereas the lowest significant oil content in 0 BA (13.26 and 17%) in both two seasons respectively.

The results are in harmony with those obtained by Abou Aziz et al. [10] and Abd El-Razek et al. [19] found that spraying olive trees with benzyladenine increased fruit oil content.

Table1: Effect of 6-benzyladenine on yield and fruit physical characteristics of Manzanillo olive trees during 2013 and 2014 seasons.

Treatments	Yield (Kg/ tree)		Fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)		Fruit size (cm³)	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Control	10.25d	10.00d	3.82d	3.79d	2.05d	2.49e	1.48d	1.49e	4.13d	4.36d
BA 40 ppm	12.46c	13.06c	3.99cd	3.40c	2.17c	2.55cd	1.52cd	1.56d	4.27c	4.46c
BA 60 ppm	18.73b	15.61b	4.03bc	4.45b	2.24b	2.69bc	1.66c	1.69c	4.28c	4.52b
BA 80 ppm	20.15a	25.18a	4.53a	4.58a	2.73a	2.85a	1.75b	1.78b	5.43b	5.57b
BA100 ppm	20.15a	25.19a	4.55a	4.61a	2.76a	2.88a	2.00a	1.99a	5.55a	5.61a

Means in each column with similar letters are not significantly different.

Table 2: Effect of 6-benzyladenine on fruit physical characteristics and oil content of Manzanillo olive trees during 2013 and 2014 seasons.

Treatments	Seed weight (g)		Flash weight (g)		Pulp /seed (%)		Fruit moisture content (%)		Oil content (%)	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Control	1.15a	1.14a	2.67e	2.65e	69.81d	69.92 d	68.00d	70.16e	13.26e	17.00e
BA 40 ppm	1.12a	1.13a	2.87d	2.27d	71.93c	66.76c	71.95c	75.10d	15.45d	17.85d
BA 60 ppm	1.11a	1.11a	2.92c	3.34c	72.46bc	75.06ab	75.34b	78.24c	17.72bc	17.94bc
BA 80 ppm	1.10a	1.11a	3.05b	3.47b	73.49b	75.76ab	80.10a	80.00ab	18.96a	19.89a
BA100ppm	1.08a	1.09a	3.47a	3.52a	76.26a	76.36a	80.13a	81.51a	18.98a	19.91a

Means in each column with similar letters are not significantly different.

FTIR spectrum analysis:

The FTIR spectrum was used to identify the functional groups of the chemical constituents based on the peak value and when run under IR region in the range of 4000 - 400 cm^{-1} there was a variation in the peaks in both the plant samples [38]. The leaf powder of Manzanillo olive was analyzed by the FT-IR spectrometers and the functional groups of the components in leaves powder were separated based on its peak ratio. The FT-IR

analysis of leaves powder proved the presence of phenols, methyl group, pyrophrine ring, amines, amides bands of tissue protein and carbohydrate (Table 3, Fig 2). The functional groups which are present in the samples are further described in details. O-H bond of phenols (3600-3200 cm^{-1}) group frequency and CH_2 bond of methyl (3040-2840cm^{-1}) group frequency. The peaks at (1740-1650 cm^{-1}) attributed to pyrophrine ring which considers the basic structure of chlorophyll molecule and (1600 -720 cm^{-1}) attributed to the region of amines and amides bands of tissue protein. C-O bond of carbohydrate (1200-800 cm^{-1}) group frequency.

Table 3: Assignment of FTIR absorption bands in the spectra of Manzanillo olive leaf powder.

Group Frequency cm^{-1}	Bond	Functional Group Assignment	References
3790-3780	-	Unknown	-
3600- 3200	O-H	Phenols	Raman and Pednekar [26]
3040-2840	CH_2	Methyl group	Diem[39] and Koca et al. [40]
1740-1650	C=O---H-—O --- Mg | H	Pyrophrine ring	Mitali and Baruah [41]
1600 -720	N-H C-N	Amines, amides bandsof tissue protein	Raman and Pednekar [26] and Manokaran et al.[42]
1200-800	C-O	Carbohydrate	Manokaran et al. [42] and Martín et al.[43]

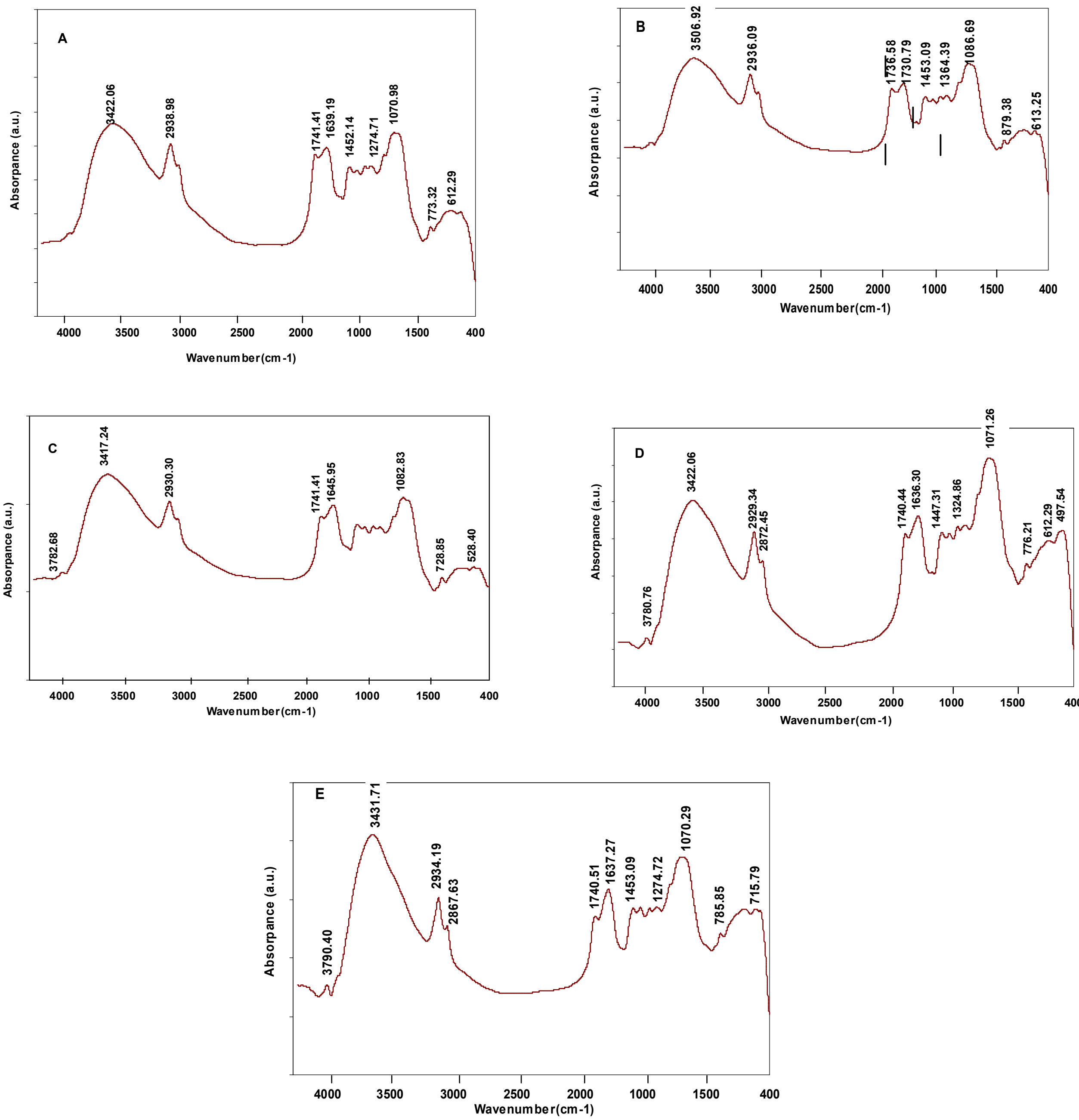

Figure 2: Fourier transforms infrared spectroscopy analysis of Manzanillo olive leaf powder treated with 6-benzyladenine. A, B, C, D and E corresponds to concentrations of 6-benzyladenine 0,40,60,80 and 100 $mg.L^{-1}$ respectively.

Careful examination of the spectra leaves samples shows that the different concentration of 6-benzyladenine not changed 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 bases the absorbance intensity were determined by using base line method. A base line was drowning as a horizontal line intersecting the two ends of the bands O-H, C=O--- H — O - - - Mg, N-H, C-N and C-O respectively.

H

The absorbance ratios of these bands were calculated and the relationship between absorbance ratios and concentration of 6-benzyladenine figure (3).

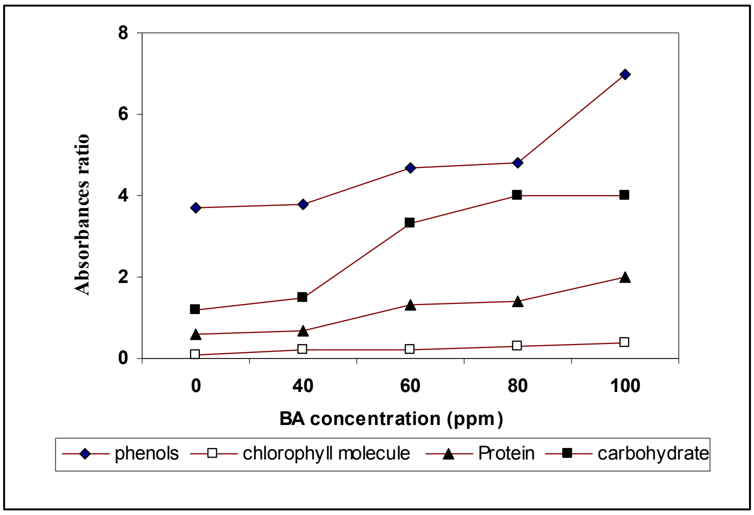

Figure 3: Relationship between absorbance ratios of phenols, chlorophyll, protein and carbohydrate and 6-benzyladenine concentrations

It is obvious from Fig (3) that the absorbance ratios of phenols, chlorophyll, protein and carbohydrate increased with increasing concentrations of 6-benzyladenine.In this respect, Gintare et al. [17] observed that benzyladenine increased leaf chlorophyll content by a strong retardation of the leaf senescence by retarding the terminal changes in chlorophyll or by preserving much of the chlorophyll. Sakine et al. [9] recorded that BA increased carbohydrate and protein content in the leaves.

Conclusion

It may be recommended the application of 6-benzyladenine 80 or 100 mg.L^{-1} at the first week of April and one month later for improving yield and fruit quality of Manzanillo olive trees. Using FT-IR spectroscopy to identify the chemical constituents of Manzanillo olive leaves as a new technique.

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