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Effect of Spraying Kalamata and Picual Olive Trees with GA_3 and $ZnSO_4$ on Fruit Oil Content and Oil Properties

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Abstract: This work was performed to study the effect of spraying Kalamata and Picual olive trees with the aqueous solution of GA_3 at (0.0, 20 and 40 ppm) or $ZnSO_4$ at (0.0, 0.5 and 1%) and their companions on fruit oil content and some oil properties. Treatments were applied when fruits reached about two third of their commercial volume shortly before the final swelling stage.

The treatment in which Kalamata trees sprayed with GA3 at 40 ppm coupled with ZnSO4 at 1% gave the highest fruit oil content comparing with the other tested ones and control. Whereas the highest fruit flesh oil percent of the Picual cultivar found in fruits of trees received the 40 ppm GA3 plus ZnSO4 at 0.5 %.The highest oil acid percentage was noticed in fruits from trees sprayed with ZnSO4 at 1%. Meanwhile the lowest peroxide value of the Kalamata cultivar was noticed in fruits from trees sprayed with ZnSO4 at 1% either alone or coupled with GA_3 at 40 ppm , while in the Picual cultivar the oil of fruits from trees received the GA3 at 20 ppm coupled with ZnSO4 either at 0.5 or 1 % showed the lowest peroxide values. Oil extracted from fruits of trees received the two high concentrations (GA_3 at 40 ppm plus $ZnSO_4$ at 1 %) showed the highest iodine value comparing with those of the other tested treatments. Concerning the change in oil quantity and quality of Picual fruits at the two harvesting dates (purple or black stage) it is clear that fruit oil content and oil properties were connected with the picking date. Fruit oil content is known to increase as the ripening stage advances this explains the higher oil content of the black stage compared with the purple one it is also interesting to note that oil properties were also related to picking stage. The purple stage is the proper harvesting stage for producing olive oil of adequate best quality.

Kew words: Olive (*Olea europaea*), Kalamata, Picual, GA_3, $ZnSO_4$, harvesting stage, fruit quality, oil content.

Introduction

Olive is one of the most important fruit trees that spread grown in Mediterranean countries and acknowledged as a valuable source of nutrients for both table and oil production. Olive trees characterized by a good ability to adapt to extremely arid conditions because of its specific leaf structure and ramified root system.

Virgin olive oil is an edible oil of great production that obtained by physical methods from the fruit it shows the sensory characteristics and nutritional properties which are the main causes of the increase of its consumption in all the world of recent years[1,2]**.** According to[3], the cultivated area of olive in the world reached

about 4,268,415 feddans producing about 20,344,343 tons about 90% of which is channeled into oil production while the remaining 10% is for table olives.

Olive oil quality is affected by the number of factors involves the geographical area of production, the cultivar was chosen, the types of Growth regulators and concentration, the period of harvesting and extraction procedure, in addition, the climatic conditions that prevalent in the year of production[4,5,6]**.** During the ripening, several metabolic processes taking place in the olives with subsequent differences in the profiles of some compounds and the impact on the physiological plant behavior and, furthermore, the chemical oil properties[7,8]**.** These changes are reflected in the oil quality grade, sensorial characteristics, oxidative stability and nutritional value of the obtained product.

The chemical properties of the oil play an important role in evaluating the oil quality. The chemical quality assurance standards, including the acidity, peroxide value, iodine value, chlorophyll and carotenoid, total phenols, unsaponifiable matter %, thiobarbituric acid value, oxidative stability and conjugated diene and triene fatty acid[9].

[10]noticed that there were increase in the fruit oil content, acidity, peroxide value with later harvesting and also[11] who notice that, the acidity increased during maturation, specifically in the black stage, which had the highest acidity percentage. Also, [12]reported that the peroxide value was increased significantly with developing in the ripening stage, while the iodine value was significantly decreased in maturity.

Gibberellins (GAs) are plant growth regulators which regulate the growth and development of higher plants and only a few of them are believed to possess biological activity. The influence of GA_3 has at the least three important roles, intensifies an organ capacity to act as a nutrient sink, the capacity to increase the IAA synthesis in plant tissues and includes acceleration synthesis of hydrolytic enzymes as amylase and other hydrolytic enzymes in aleurone cells[13,14] indicated that applying higher plants with GA_3caused elongation in the primary cells in the young tissues and growth centers.

Zn is an important microelement essential for plants because of its involvement in the synthesis of tryptophan which is the precursor of IAA. Zn is needed for the activity of different enzymes, such as oxidase, peroxidase, aldolases, transphosphorylases, dehydrogenases, isomerases, RNA and DNA polymerase[15]. Zinc used as co-factor for many enzymes, affects photosynthesis reaction, nucleic acid metabolism and protein biosynthesis[16]. The improvement in fruit quality and quantity occurred due to supplying trees with zinc could be attributed to its influence on promoting the formation and translocation of carbohydrates and enzymes of carbohydrate[17].

Therefore, this study was aimed to investigate the effect of spraying Kalamata and Picual fruits with GA_3 and $ZnSO_4$ on fruit oil and some oil properties.

Materials and Methods

This study was carried out during two successive seasons, (2012, 2013) in a private orchard located at Ismailia Governorate, Egypt. The study was conducted on forty -five mature trees of the tow olive cultivars (Kalamata and Picual). The Kalamata cultivar used for the double purposes[18] and also Picual cultivar used for table olives and for oil production[9] .The trees were about 8 years old, grown in a sandy soil at five meters apart under drip irrigation system The trees were almost similar in vigor, free from any visible pathogenic symptoms and at the same bearing phase. Trees received the ordinary agricultural practices.

Selected trees were divided into nine similar groups each of five trees; each group received one of the following treatments:

1. Control (water spray). .
2. GA_3 at 0 ppm + $ZnSO_4$ at 0.5%.
3. GA_3 at 0 ppm + $ZnSO_4$ at 1 %.
4. GA_3 at 20 ppm + ZnSO4 at 0.0 %.
5. GA_3 at 20 ppm + $ZnSO_4$ at 0.5%.
6. GA_3 at 20 ppm + $ZnSO_4$ at 1 %.

7. GA_3 at 40 ppm + $ZnSO_4$ at 0.0 %.
8. GA_3 at 40 ppm + $ZnSO_4$ at 0.5%.
9. GA_3 at 40 ppm + $ZnSO_4$ at 1%.

Therefore, each treatment was replicated five times and each replicate consisted of one tree.

Sprays were applied when fruits reached about two third of their commercial volume. Each tree was sprayed alone with the aqueous nutrient solution which was enough for thorough tree drenching (1% soluble soap was added as a wetting substance).

The measurements:

1. **Oil percentage:** It was determined according to[19]by extracting the oil from the dried flesh fruit with soxhelt apparatus using petroleum ether 60-80 º C of boiling point and expressed in percentage on dry weight basis.
2. **Acid percentage**: It was determined according to[20] and calculated as (weight of free oleic acid in 100 g of the oil).
3. **The peroxide value:** It was determined according to[21].
4. **Iodine value:** It was determined according **to[19].**

Statistical analyses:

The data were subjected to analysis of variances **(ANOVA)** according to[22] using MSTAT program. Least significant ranges (LSR) were used to compare between means of treatments according to[23] at the probability of 5%.

Results and Discussions

1- Oil percentage (on fresh weight basis)

Data concerning fruit oil percentage was shown in Table (1). It could be seen that the effect of treatments on increasing fruit oil content was limited and lake significant in most cases. However in the case of GA3 at 40 ppm the increase in Kalamata oil content was more evident reaching the significant level (about 15 & 19 %) over the 20 ppm concentration and the control respectively.

It is interesting to mention that the fruit flesh oil content seemed to increase as the maturity stage advances. This explains the higher values of Picual fruit oil content at the black stage compared with the purple stage. For instance the flesh oil percent was 16.18, 17.37 and 16.19 % for the control, 0.5% and 1% at the purple stage compared with 18.19, 19.03 and 18.07 % in the black stage. Moreover black Picual fruits from trees received GA3 at 0, 20&40 ppm showed about 9, 11 &13 % increase in flesh oil % compared with fruits at the purple stage.

Applying GA_3 at 40 ppm coupled with 0.5% $ZnSO_4$ gave the maximum flesh oil content during purple and black harvested stages (about 13% and 14%) increase over the control respectively.

The obtained results of spraying Picual olive trees with spraying ZnSO4 are in harmony with those reported by[24, 25], on olive who found that oil content in fresh weight was increased by spraying ZnSO4.

Due to accumulation of synthesized oil during maturation process and the rise in oil content at black stage might due to the decreased of moisture content during the late maturation stage[26], and also[12] reported that the increased in oil might due to that the accumulation of synthesized oil during maturation process and also the results of effect the harvesting in different stage in oil content are agree with[27] who reported that oil content increased with delaying fruit harvest at high altitude. Also [10] on olive, they reported that the oil accumulation increased until the onset of epicarp blackening, when oil no longer accumulated into the fruit.

Table (1): Effect of spraying Kalamata and Picual olive trees with GA_3 and $ZnSO_4$ on oil percentage (on fresh weight basis) (Combined analysis 2012& 2013 seasons)

$ZnSO_4$ / GA_3	0%	0.5%	1%	Mean	0%	0.5%	1%	Mean	0%	0.5%	1%	Mean
	Kalamata				Picual (at purple stage)				Picual (at black stage)			
0 ppm	17.19 d	18.19 c	18.27 c	**17.88 B**	16.25 cd	16.52 bc	16.04cde	**16.27A**	17.55cd	18.08 c	17.73cd	**17.79 B**
20 ppm	17.85 cd	18.01 cd	18.69 bc	**18.18 B**	15.54 de	17.30 b	17.18 b	**16.66 A**	17.27 cd	19.03 b	19.37 ab	**18.56 AB**
40 ppm	20.46 a	19.51 ab	20.18 a	**20.05 A**	16.73bc	18.33 a	15.32 e	**16.79A**	19.76ab	19.99 a	17.10d	**18.95A**
Mean	**18.50A**	**18.57 A**	**19.05 A**		**16.18 B**	**17.37 A**	**16.19 B**		**18.19 B**	**19.03 A**	**18.07 B**	

Means having the same letters within a column are not significantly different at 5% level.

2-Oil acidity:

The acidity of olive oil is considered a basic characteristic of the quality. The acidity is an indirect measure of a number of free acids is present in fats and oils. The high the amount of acid value, the high the deterioration or rancidity of the oils and fats are undergone deterioration or rancid[10]. Late harvest fruit will tend to have higher FFA and the I.O.C. standard for free fatty acids in extra virgin olive oil is a maximum of 0.8 % [28]

Data in Table (2) indicated that, the oil acid content of fruit Kalamata trees sprayed with the GA_3 at the two tested concentrations showed similar values to that of the untreated trees. Contrarily the oil from trees received the $ZnSO_4$ treatments gave significant lower value than that of the control. The reduction in oil acidity reached about 15 and 10 % for the 0.5 and 1% $ZnSO_4$ treatments respectively.

Concerning the interaction, it could be noticed that spraying GA_3 alone either at 20 or 40 ppm and $ZnSO_4$ at (1%) alone gave the highest oil acidity values. This increased reached about 39, 35 and 48% over the control for the three treatments respectively

As for the Picual cultivar at the purple stage, the GA_3 treatment seemed to have no effect in this respect and also, no significant increase in fruit acidity was detected when treated with $ZnSO_4$.

In black stage, there was an increase in fruit acidity reached about 22, 26 and 27 % for the 0.0, 20 and 40 ppm GA_3, respectively compared with fruits at the purple stage. In this stage, a significant increase in oil acid content was detected in the fruit of trees sprayed by GA_3 at 40 ppm alone and fruits from trees received the $ZnSO_4$ at 1%. This increased amount about 38 and 53% over the control for the two treatments respectively.

The results of spraying GA3 are in line with[9,29] who found that oil acidity was not affected by spraying GA_3.

The results of the effect of harvested stage in line with that observed by[11], who noticed that, the acidity percent increased during fruit ripening, specifically in the black stage and also agree with[30,31,32,33] they noticed that the oil acidity increased slightly as fruit ripening progress as during the olive ripening there is progressive activation of lipolytic activity and olives are more sensitive to pathogenic infection and mechanical damage, which results in oils with higher acidity values.

Table (2): Effect of spraying Kalamata and Picual olive trees with GA_3 and $ZnSO_4$ on oil acidity (Combined analysis 2012&2013 seasons)

$ZnSO_4$ / GA_3	0%	0.5%	1%	Mean	0%	0.5%	1%	Mean	0%	0.5%	1%	Mean
	Kalamata				Picual (at purple stage)				Picual (at black stage)			
0 ppm	0.31 b	0.32 b	0.46 a	**0.36 A**	0.22 b	0.26 b	0.34 a	**0.27 A**	0.26 bc	0.33ab	0.40 a	**0.33 A**
20 ppm	0.43 a	0.32 b	0.34 b	**0.36 A**	0.27 ab	0.23 b	0.20 b	**0.23 A**	0.34 b	0.28 bc	0.25 c	**0.29 A**
40 ppm	0.42 a	0.35 b	0.33 b	**0.37 A**	0.31 ab	0.24 b	0.24 b	**0.26 A**	0.36 ab	0.32 b	0.30 bc	**0.33 A**
Mean	**0.39 A**	**0.33 B**	**0.37AB**		**0.27A**	**0.24 A**	**0.26 A**		**0.32 A**	**0.31A**	**0.32A**	

Means having the same letters within a column are not significantly different at 5% level.

3-Peroxide value:

Peroxide value is used as an indicator reveal to the deterioration of enzymatic and oxidation in oil[34]. Also, It is used to monitor production problems, which occur after harvest of the fruits and during the processing[35]**.** The high levels of oil Peroxide values are a bad sign. The IOC standard is < 20 mEq O2 /kg oil[28].

Data in Table (3) noticed that, the fruits from trees received either the GA_3 or $ZnSO_4$ treatments exhibited higher peroxide value compared with that of control

In Kalamata, the GA_3 sprays have shown to increase oil peroxide value over the control. This increase reached a significant level in the two tested concentrations and the 20 ppm one showed the higher value which was about 25% over the control. However, the $ZnSO_4$ sprays tended to cause a significant decrease in oil peroxide value compared with this of the control. This reduction in oil peroxide value reached about 17 and 16 % for the 0.5 and 1% $ZnSO_4$ respectively.

Concerning the interaction, it could be seen that spraying GA_3 either at 20 or 40 ppm alone gave the highest peroxide value compared with other treatments (about 34 and 42%) over the control, respectively. While the lowest peroxide value was observed by spraying 0.5% $ZnSO_4$ alone.

Concerning the Picual cultivar It could be seen that the GA_3 at the high concentration tended to increase the oil peroxide value at the purple and black stage. This increase reached about 8 and 5% in the two stages respectively. However, the low concentration 20 ppm seemed to have a rather low effect in oil peroxide value at the two ripening stage.

Concerning the Picual fruit for trees sprayed with $ZnSO_4$ at the two tested concentration exhibited significant lower peroxide value this reduction reached about 11 and 6 % comparing with that of the control in both stages.

The data of the interaction, it could be seen that applying GA_3 either at 20 ppm or 40 ppm alone gave the highest peroxide values in the both harvested stage. On the other country, the fruits from trees received the GA_3 20 ppm coupled with $ZnSO_4$ either at 0.5 or 1% and control gave the lowest iodine values.

The results of the effect of spraying GA_3 on oil peroxide are in line with[9,29]on olive who found that the peroxide value was increased by spraying GA3 or NAA**.**

The increment in the peroxide value during fruit ripening could be due to increasing the activity of the lipoxygenase enzyme[36].

And also the results are in hormone with [10, 12, 27]**,** on olive who found increasing in the peroxide values during developing the fruit in the ripening.

Table (3): Effect of spraying Kalamata and Picual olive trees with GA_3 and $ZnSO_4$ on oil peroxides (Combined analysis 2012&2013 seasons)

$ZnSO_4$ / GA_3	0%	0.5%	1%	Mean	0%	0.5%	1%	Mean	0%	0.5%	1%	Mean
	Kalamata				Picual (at purple stage)				Picual (at black stage)			
0 ppm	4.50 c	3.96 d	4.53 c	**4.33 B**	5.37 d	5.69 cd	6.63 ab	**5.90 B**	6.59 d	7.41 bc	8.03ab	**7.34 B**
20 ppm	6.01 a	5.04 b	5.17 b	**5.41 A**	6.90 a	5.48 d	5.25 d	**5.88 B**	8.55 a	6.50 d	7.06cd	**7.37 B**
40 ppm	6.37 a	5.07 b	4.44 c	**5.29 A**	6.98 a	5.91 cd	6.20 bc	**6.36 A**	8.72 a	7.08 cd	7.34cd	**7.71 A**
Mean	**5.62 A**	**4.69 B**	**4.71 B**		**6.42 A**	**5.69 B**	**6.03AB**		**7.95 A**	**7.00 C**	**7.48 B**	

Means having the same letters within a column are not significantly different at 5% level.

4 - Iodine value:

The iodine number identify the number of milligrams iodine used to saturate the percentage of fatty acid in 100 g of the oil. Therefore, the high iodine value was observed in the oils that rich in unsaturated fatty acid and vice versa. The determination of oil iodine value noticed that the high quality with a high level of unsaturated fatty acid particularly oleic acid [12].

The data in the table (4) indicated the effect of applying the both cultivars with GA_3 and $ZnSO_4$ on the iodine value of oil.

It could be seen that the effect of treatment on oil iodine value was rather slight. This increase no significant difference between any examined treatments and control was observed. This was true in both cultivars and maturity stages. As expected the iodine value, on the other hand, differed from stage to stage, while it was decreased in the black stage. This decreased reached about 5, 7and 7% for the control, 20 ppm and 40 ppm GA3. Corresponding value is 7, 6 and 5% for the control, 0.5 and 1% ZnSO4

Concerning the interaction, it could be seen that applying Kalamata olive trees with GA_3 either at 20 or 40 ppm coupled with $ZnSO_4$ 1% caused the highest iodine value.

As for Picual cultivar, the oil extracted from fruits of trees received the two high concentrations (GA_3 at 40 ppm plus $ZnSO_4$ at 1 %) showed the highest iodine value comparing with those of the other tested treatments.

The obtained results of spraying GA_3 are in line with those reported by[9, 29,37] on olive they noticed that the oil iodine value was not affected by spraying GA_3.

The results of the effect of fruit development are agreed with[12] on olive, who found that the iodine value was significant decrease during the ripening stage of fruit (from S1 to S5).

Table (4): Effect of spraying Kalamata and Picual olive trees with GA_3 and $ZnSO_4$ on iodine value (Combined analysis 2012&2013 seasons)

$ZnSO_4$ / GA_3	0%	0.5%	1%	Mean	0%	0.5%	1%	Mean	0%	0.5%	1%	Mean
	Kalamata				Picual (at purple stage)				Picual (at black stage)			
0 ppm	77.21ab	74.92 ab	75.31 ab	**75.81 A**	79.17 ab	78.88 ab	79.04 ab	**79.03 A**	74.45ab	74.86ab	75.31ab	**74.87 A**
20 ppm	76.81ab	76.09 ab	78.90 a	**77.26 A**	82.27 ab	78.38 b	79.20 ab	**79.95 A**	75.71ab	72.93 b	74.34ab	**73.99 A**
40 ppm	74.97 ab	74.45b	78.63 ab	**76.02 A**	79.85 ab	78.77 ab	82.38 a	**80.33 A**	73.90ab	73.26 b	77.77a	**74.97 A**
Mean	**76.33 A**	**75.15 A**	**77.61 A**		**80.43 A**	**78.67 A**	**80.21 A**		**74.69 A**	**73.68 A**	**75.80A**	

Means having the same letters within a column are not significantly different at 5% level.

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