



## Influence of magnesium, molybdenum and sulfur sprays on the yield, fruit and oil properties of Coratina olive trees

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### Abstract

This experiment was conducted in an olive orchard in the 2018 and 2019 seasons, located in El-Khatatba region, El-Behira Governorate, Egypt, in order to study the effects of spraying trees with ammonium molybdate ( $[\text{NH}_4]_2\text{MoO}_4$ ), magnesium sulphate ( $\text{MgSO}_4$ ) and potassium thiosulfate ( $\text{K}_2\text{S}_2\text{O}_3$ ), either individually or in combinations, on leaf mineral content, the quality characteristics of Coratina olives, yield, oil content and properties. The treatments were applied twice in September and October of each season. In both seasons of the experiment, spraying trees with any of the fertilizer compounds under study had a positive effect, compared to control, on the yield, physical characteristics of the fruits, the leaves' minerals content, as well as the fruit oil content, and maintaining its quality as an extra virgin oil. Results extracted from this study, indicated that the potassium thiosulfate and ammonium molybdate together led to obtaining the highest yield of the trees compared to the untreated ones in both seasons, while spraying the trees with potassium thiosulfate, ammonium molybdate and magnesium sulphate, combined together, led to obtaining the highest percentage of oil from the fruits compared to the control trees in both seasons.

**Keywords:** Coratina; olive; oil quality; olive oil;  $[\text{NH}_4]_2\text{MoO}_4$ ;  $\text{MgSO}_4$ ;  $\text{K}_2\text{S}_2\text{O}_3$ .

### 1. Introduction

Olive (*Olea europaea* L.) is an industrial plant that has an economic importance, since it covered over 12 million hectare worldwide, with a production of more than 2 million tons [1], especially that cultivated by the countries in the Mediterranean Basin (~75% of world's total production of olive oil), which has cold, wet winters and hot, dry summers [2]. Besides the economic importance of olive-tree cultivation, the benefits of its products for human health have been revealed.

Olives are grown for the purpose of extracting olive oil for human use and for processing into table olives. The main oil in the Mediterranean diet is virgin olive oil. It is valued for its organoleptic and nutritional qualities, and its high quantity of natural antioxidants like phenols, tocopherols, and pigments makes it oxidation-resistant (chlorophylls and carotenoids). Because of its high oleic acid content, olive oil is classified as monounsaturated fatty acid oil, which is beneficial to one's health because it lowers the level of harmful low-density lipoprotein (LDL) cholesterol in the bloodstream, while increasing the level of

beneficial high-density lipoprotein (HDL) cholesterol [3].

Virgin olive oil is significant due to its use without refining, which distinguishes it from other edible vegetable oils. It is characterized by unique sensory, nutritive, and oxidation stability [4 - 6]. The composition of virgin olive oils is mainly constituted by triacylglycerols and minor compounds (0.5–2%), which is called the unsaponifiable or non-glycerol fraction [7, 8]. The main minor compounds such as polyphenols, tocopherols and pigments are the most important antioxidants compounds since they delay the oxidation of fatty acids [9].

Phenolic compounds affect olive oil stability and contribute to its sensorial and nutritional characteristics. These compounds are the most abundant antioxidants in virgin olive oil [6, 10, 11]. Tocopherols, which include vitamin E, can act as antioxidants via singlet oxygen quenchers or a chain-breaking electron donor mechanism [6, 12]. Chlorophyll and carotenoids are the major pigments of olive oil, which give the characteristic and desirable green-yellow color to olive oil. Chlorophyll pigments

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act as photosensitizers, promoting oxidation while antioxidant activity in the dark has been reported [13, 14]. Carotenoids, as single oxygen quenchers, protect oils from photo-oxidation.

Olive agriculture has transitioned from traditional, vast, widely spread, rain-fed orchards to intensive, closely spaced, and irrigated orchards in the last few decades, resulting in increasing oil production [15]. In order to keep up with this increase, attention must be paid to enhance the productivity of trees and supplying the necessary elements to improve growth and increase productivity at the appropriate times.

Nutrient foliar spray is generally effective in meeting plant requirements and has a high efficiency, particularly in nutrient-fixing soils or arid zones where a shortage of water, especially during the summer when rainfall is minimal, severely reduces soil nutrient absorption [16].

Thiosulfate potassium fertilizers are clear liquids that provide a source of sulfur and potassium. Potassium is an essential macro-element required in large amounts for normal plant growth and development in addition to being involved in many physiological processes. Potassium cation plays a major role in enzyme activation, protein synthesis, stomatal function, stabilization of internal pH, photosynthesis, turgor-related processes and transport of metabolites [17, 18]. Potassium being responsible for increasing sugar content might promote translocation of newly synthesized photosynthates causing a beneficial effect on the mobilization of stored metabolites [19].

Sulfur has a great significance for the structure of proteins and functioning of enzymes and plays an important role in the defense of plants against stresses and pests. Sulfur metabolites such as glutathione provide protection for the plants against oxidative stress, heavy metals and xenobiotics. Plant species are varying largely in sulfur requirement, adequate and balanced sulfur nutrition is crucial for their production, quality and health. The assimilation of sulfur and nitrogen are strongly interrelated and sulfur deficiency in the plants can be diagnosed by the nitrogen to sulfur ratio of plant tissue [20].

A previous study reported that potassium thiosulfate at 3 % significantly increased fruit length and fruit width (cm), fruit weight (g), yield (Kg/tree), total soluble solids [21].

Molybdenum importance for appropriate plant functioning and growth is inconsistent by the most plants in respect to the total quantity that is obligatory for them. Molybdenum is a micronutrient that is directly involved in the metabolic functions of nitrogen in the plant. The transition of molybdenum metal in molybdate form is essential for the plants as a number of enzymes use it to catalyze most important reactions in nitrogen acclimatization, the synthesis of the phytohormone, degradation of the purine and the

detoxification of the sulfite. There are more than known 50 different enzymes that need Mo, whether direct or indirect impacts on plant growth and development, primarily phytohormones and the N-metabolism involving processes. On the other hand, in the synthesis of Abscisic acid (ABA), uniquely molybdenum cofactor (Moco) is involved there on the level of ABA Moco effect is highly vital and ultimately by the response in the stress and the stomatal control, it has a very important role in the rate of transpiration and water relations. The practices that are involved in the fertilization of Mo optimization in crops, has a very important scope in discovering and improving these practices where the legumes are fixing the N or  $\text{No}_3^-$  is primarily source of available N [22].

Magnesium foliar application plays an important role in the biochemical and physiological cycle of plants [23], for example, in the initiation of starch metabolism, protein synthesis, and electricity transfer. Magnesium acts as an impetus in the oxidation and reduction responses within plant tissues, and enhances plant resistance to drought [24]. Magnesium deficit during the plant development lowers yield and reduces quality [25].

The aim of this study is to investigate the effect of foliar application with magnesium sulphate (Mg), potassium thiosulfate (KTS) and ammonium molybdate (Mo), on vegetative growth, yield, fruit quality, oil physicochemical properties, fatty acids composition, phenolic components, organoleptic characteristics, bioactive compounds and leaf mineral contents of Coratina olives.

## 2. Experimental

### 2.1. Plant material

This experiment was carried out during 2018 and 2019 seasons on 40 years old Coratina olive trees in a private orchard located in El Behera Governorate, Egypt. Trees were almost similar in vigor and planted at  $6.5 \times 6.5$  meters, grown on a sandy soil under drip irrigation system. Horticultural practices that suitable for trees' age and the area weather conditions were applied regularly and evenly to all trees under study.

### 2.2. Treatments

Foliar applications of potassium thiosulfate ( $\text{K}_2\text{S}_2\text{O}_3$ ) at 1%, ammonium molybdate ( $[\text{NH}_4]_2\text{MoO}_4$ ) at 0.02% and magnesium sulphate ( $\text{MgSO}_4$ ) at 0.5%, were sprayed individually or in combinations. Trees were sprayed twice a year, in September and October. Eight treatments were arranged in three replicates on one tree plot as follows:

- 1- (Control): Water spray only

- 2- (KTS): ( $K_2S_2O_3$ ) at 1%
- 3- (Mg): ( $MgSO_4$ ) at 0.5%
- 4- (Mo): ( $[NH_4]_2MoO_4$ ) at 0.02%
- 5- (KTS + Mg): ( $K_2S_2O_3$ ) at 1% + ( $MgSO_4$ ) at 0.5%
- 6- (KTS + Mo): ( $K_2S_2O_3$ ) at 1% + ( $[NH_4]_2MoO_4$ ) at 0.02%
- 7- (Mg + Mo): ( $MgSO_4$ ) at 0.5% + ( $[NH_4]_2MoO_4$ ) at 0.02%
- 8- (KTS + Mo + Mg): ( $K_2S_2O_3$ ) at 1% + ( $[NH_4]_2MoO_4$ ) at 0.02% + ( $MgSO_4$ ) at 0.5%

### 2.3. Measurements

In late December 2018 and 2019 (harvest time), the experiment trees were separately harvested and the following measurements were carried out as follows:

#### 2.3.1. Leaf mineral content

Leaf samples were picked from each replicate tree then washed and dried at 70°C till a constant weight to determine nitrogen (N), phosphorus (P), and potassium (K) as percentage of dry weight basis due to the method described by Cottenie *et al.* [26]. Calcium (Ca) and magnesium (Mg) were determined following the criteria described by Moody *et al.* [27].

#### 2.3.2. Yield and fruit quality

Fruits of each replicate (tree) were separately harvested, weighed and the yield as kg/tree were estimated, then samples of 30 fruits from each replicate (tree) were picked randomly around the tree to determine the following parameters: Average fruit weight (g), fruit length (cm) and fruit diameter (cm) as described by A.O.A.C. [28].

#### 2.3.3. Oil extraction and its characteristics

After harvesting, about 5 Kg of Coratina olive fruits from each tree were separately washed, crushed and packed in cheese cloth then pressed using a laboratory hydraulic after press (carver). The pressure was 12.000 lb/in<sup>2</sup> for 30 min/ each sample which was reached gradually. The obtained juice was collected and left in a separator funnel until complete separation. The upper layer was separated and centrifuged. The extracted oil was dried using anhydrous sodium sulfate, and stored in dark glass bottles at -5°C till analysis.

- **Total lipid content:** It was determined as a percentage on dry matter weight in the oil samples according to the methods of A.O.A.C. [29].
- **Quality parameters:** Free fatty acids % (FFAs), peroxide value and unsaponifiable matter (%) were carried out following the analytical methods described by A.O.A.C. [29]. UV absorption characteristics, K232nm

(conjugated dienes) and K270nm (conjugated trienes),  $\Delta K$  [ $\Delta K = k_{270} - (k_{266-4} + (k_{274+4})/2)$ ] were measured according to IOC [30].

- **Fatty acids profile:** The fatty acids methyl esters in EVOOs were prepared using transesterification with cold methanolic solution of potassium hydroxide. The fatty acid methyl esters were identified by GC-capillary column according to the methods of IOC [30].
- **Total phenolic content:** They were extracted three times with 10 ml of a methanol/water mixture (60: 40 V/V). The pooled extracts were washed with 10 ml of n-hexane and solvents were removed with a rotary evaporator (Buchi, Switzerland). Total phenols (TP) content of the methanolic extract of olive oil were colorimetrically determined using the Folin-Ciocalteu method [31].
- **Phenolic compounds:** They were determined by HPLC according to the method described by Peres *et al.* [32].
- **Total pigments:** Chlorophyll and carotenoids in EVOOs were determined calorimetrically as the method previously described by Minguez-Mosquera *et al.* [33].
- **Organoleptic test:** The organoleptic test of extra virgin olive oil was performed according to the method described by IOC [30].

### 2.4. Statistical analysis

Randomized complete block design (RCBD) was the experiment layout with three replicates for each treatment; one tree was represented as a replicate. The obtained data were subjected to analysis of variance (ANOVA) [34]; the least significant differences (LSD) at probability of 5% were applied to separate the means using Co-Stat 4.11 software.

## 3. Results and discussion

### 3.1. Olive leaves mineral content

Results in Table (1) show the leaves' various mineral contents under different fertilization treatments as the following: Concerning N content, results clear that the highest significant N value (2.11%) in the first season was measured when the trees were sprayed with Mo, while it was the highest (2.25%) with KTS+Mo in the second season. The lowest significant values (1.78 and 1.82%) were

recorded with the control in both seasons, successively.

As for P content, results show that the highest P values (0.212 and 0.215%) were measured with KTS+Mo spray in the two seasons, respectively. Whereas the lowest significant P values (0.179 and 0.172%) were detected with the control trees in both seasons, by order.

Considering K content, the highest significant K values (1.61 and 1.66%) were measured due to KTS+Mo spray in both seasons, successively. However, the lowest K content (1.22 and 1.24%) was recorded with the control trees leaves in the two seasons, respectively.

Regarding leaves Ca content, different treatments did not affect it significantly. However, the highest value (1.67%) was measured with Mo in both seasons, while the lowest value (1.54%) was detected with the control and KTS+Mg treatments in the first and second seasons.

Concerning leaves Mg content, it could be seen that Mg solely and KTS+Mg treatments had raised its content to the maximum significant value (2.31%) in the first season, while Mg+Mo and KTS+Mg treatments did in the second season (2.19 and 2.18%) by order. However, the untreated trees (control) gave the minimum significant values (1.33 and 1.44%) in the two seasons, successively.

From the obtained results, it is clear that the combined treatments especially that included KTS+Mo+Mg rose both of N, P, K and Mg in olive leaves. In this concern, Saykhul *et al.* [35] reported that the application of  $K_2SO_4$  led to a significantly higher N concentration in the leaves and increased K concentration in olive cv. Chondrolia Chalkidikis. On the other hand, Swami *et al.* [36] reported that the nutrient uptake status of stover, seed and total N, P, K, Fe, Zn, Mn, Cu and Mo of Pigeon pea was improved due to the application of RDF + ammonium molybdate through foliar application.

### 3.2. Fruit physical characteristics and yield

As for average fruit weight, results in Table (2) show a significant increase with the combination of KTS+ Mo+ Mg in both seasons, where it recorded 3.721 and 3.704 g in the two seasons, respectively. Whereas, KTS solely and the control treatments recorded lower significant values of fruit weight and gave 2.403 and 2.279 g in the first season and 2.418 and 2.258 g in the second one, successively.

On the other hand, a significant increase in fruit length (2.37 and 2.35 cm) was revealed when the trees treated with KTS+Mo+Mg and Mo+Mg combinations, subsequently in the first season, while KTS+Mo+Mg and KTS+Mo combinations respectively scored the tallest significant fruits (2.37 and 2.32 cm) in the second season,. However, the

shortest fruits (2.07, 2.01 and 2.00 cm in the first season and 2.09, 2.06 and 1.96 cm in the second season) were obtained with KTS, control and KTS+Mg treatments without significant differences among them.

Considering fruit diameter, the highest values (1.70 and 1.67 cm) and (1.69 and 1.68 cm) in the two seasons, respectively were measured with KTS+Mo+Mg and Mg solely treatments. Whereas, the lowest values of fruit diameter (1.47, 1.45 and 1.44 cm in the first season) and (1.45, 1.44 and 1.43 cm in the second one) were obtained due to KTS+Mg, control and KTS solely treatments successively without significant differences among them.

As for the yield, the heaviest significant yield/ tree (84.6 kg) was scored by KTS+Mo combination in the first season, while the heaviest yield in the second season (85 and 81.6 kg) was recorded with KTS+Mo and Mo alone treatments, respectively in the second season. On the contrary, the lightest significant yield in the first season (64.42 kg) was weighed with the control; while in the second season the lowest values (66.67 and 65 kg) were weighed with KTS+Mg and the control, successively without significant difference between them.

From the previous results mentioned in Table (1), it is clear that the combined treatments especially that included KTS, Mo and Mg raised both of N, P, K and Mg in olive leaves, which may corresponded with the positive effect on the yield and fruit physical properties presented in Table (2), since increasing these nutrients have a significant effect on productivity and fruit quality of many fruit crops. In this concern, Abou El-Wafa *et al.* [21] reported that potassium thiosulfate at 3 % significantly increased fruit length and fruit width (cm), fruit weight (g), yield (Kg/tree) of pomegranate trees. Our obtained results confirmed with previous results mentioned that spraying olive trees with potassium and magnesium led to increase the fruit yield per trees, and the fruit physical characteristics, also the oil accumulation percentage in the fruits, with maintaining its characteristic and quality as extra-virgin olive oil, compared to the control [37]. On the other hand, Swami *et al.* [36] reported that various growth and yield parameters like plant height, number of branches, number of pods, seed yield and stover yield of Pigeon pea was increased due to application of RDF+ ammonium molybdate through foliar application.

### 3.3. Olive oil Percentage and quality parameters

Results in Table (3) show the effect of the tested treatments on fruit oil content and the oil quality parameters; free fatty acids% (FFAs) as oleic acid, peroxide value (PV) (meq  $O_2$  Kg<sup>-1</sup> oil) and ultraviolet (UV) absorbency of the oils obtained from Coratina olive fruits in 2018 and 2019 seasons. Acidity, as oleic

acid and peroxide value ranged between 0.10-0.14%, 0.13-0.18% and 2.50-2.63, 2.74-2.90 meq O<sub>2</sub> Kg<sup>-1</sup> oil in 2018 and 2019 seasons, respectively. The highest value for acidity and peroxide value were 0.18% and 2.90 meq O<sub>2</sub> Kg<sup>-1</sup> oil, respectively which were lower than the quality reference stated by IOC [30]; 0.8% and ≤ 20 meq O<sub>2</sub> Kg<sup>-1</sup> for acidity and peroxide value respectively [30]. There was a slight increase in FFAs values and PV in the second season, these changes may be attributed to the extraction process and climate conditions. On the other hand, these changes were not significant in both seasons. Consequently, all the oil samples obtained were classified as extra virgin olive oils, and these results are in agreement with that mentioned by El Antari *et al.* [38]. On the other hand, K232 and K270 mainly indicate the conjugated dienes and trienes, absorption bands between 200 and 300 nm, K232 and K270 were used to determine the purity of olive oil. These frequencies are associated with conjugated diene and triene systems. A high-quality extra virgin olive oil will have low absorption in this area. Three metrics, K232, K270 and Δk, can be used to evaluate the purity of olive oil [39]. Results in Table (3) show that K232 and K270 values for all fertilizing treatments were lower than the control value with no significant differences in the two successive seasons. However, ΔK at the first season (2018) had the lowest value (-0.0084) with KTS+Mo+Mg treatment followed by KTS+Mg treatment (-0.0072), KTS+Mo treatment (-0.0063) and KTS treatment (-0.0055). Results were significantly differenced when compared to the control. At 2019 season, there was a slight increase in ΔK, since KTS+Mo+Mg treatment had the lowest value of ΔK (-0.0068) followed by KTS+Mg treatment (-0.0054), KTS treatment (-0.0049) and KTS+Mo treatment (-0.0047) with a significant difference when compared to the control. The values of K232 and K270 extinction coefficients for all oils complied with the limits for "Extra" virgin olive oil K232 of <2.50, K268 of <0.22 and ΔK ≥ 0.01) as reported by IOC [30]. Thus the low K232 and K268 nm values mentioned correspond to good olive oil quality. These results are in harmony with those obtained by Solinas *et al.* [40]. The values may be impacted by the levels of accessible minerals in foliar fertilizer, which allow for the production of oils rich in beneficial phytochemicals, particularly polyphenols, which protect the oil from oxidation and deterioration.

It is clear from Table (3) that all treatments significantly increased the oil content (on fresh weight basis) than the control sample in both seasons. The maximum oil content was obtained by spraying trees with KTS+Mo+Mg and Mg (28.83 and 24.75 % in the first season and 24.99 and 24.95 % in the second one, respectively). While, the control trees had the lowest oil content (20.02 and 20.9 %) in both seasons, respectively. All treatments considerably increased oil content on a dry weight compared to the control.

According to Nissim *et al.* [41], the oil content in Coratina olive fruits ranged between 40% - 46.3% and 35.97% - 46.48% on a dry weight basis, while it was 18.5% on a fresh weight basis [42]. The increase in total photo-assimilates (e.g., lipids) and trans-located assimilates to the sink as a result of applying magnesium, which may control the biosynthesis of oil by 42 enzymes during the main metabolic pathway, and that could be attributed to the progress in oil content over the two years of the study, due to fertilizer applied at a suitable time and throughout the vital stages of the olive tree's development. Indeed, the accumulation of oil in the olive is a process that influenced by the amount of carbohydrates presented in the fruits and old leaves [43]. All parameters were not substantially different between treatments, except oil content, which was shown to be considerably varied between the two seasons.

### 3.4. Olive oil minor components and sensory attributes

Table (4) shows the minor components of olive oils extracted from different fertilizing treatments, such as total polyphenol contents, un-saponifiable matters% (UNSAF %), chlorophyll and carotenoids (in ppm) contents and organoleptic attributes (fruity, bitter and pungent). The bioactive components of extra virgin olive oil are the result of a number of variables acting before extraction (such as variety, environmental, climatic, soil and cultivation conditions, olive ripeness and olive health) and during olive oil extraction and storage [14]. The content of phenolic compounds is an important parameter in the evaluation of extra virgin olive oil quality due to its correlation with oxidative stability and sensorial quality [6]. Results in Table (4) shows that total phenols content ranged between 824.9-981.4 ppm and 788.4-980.8 ppm in the two successive seasons (2018 and 2019), respectively, which were in line with the results reported by Manai-Djebali, *et al.* [44] in five different extra virgin olive oils (253–1400 ppm). All treatments had shown high significant differences of total phenol content compared with control treatment in the two successive seasons except for molybdenum (Mo) which was lower than control treatment in the first season 2018. KTS+Mg+Mo foliar application has the highest phenolic content (955.3 ppm in 2018 and 979.7 ppm in 2019) in the two successive seasons. Extra virgin olive oil contains a considerable amount of phenolic compounds that are responsible for its peculiar taste and for its high stability. Recent findings demonstrate that olive oil phenolic compounds are powerful antioxidants, either in vitro or in vivo, and possess other potent biological activities that could partially account for the observed healthful effects of the Mediterranean diet [45].

**Table 1: Effect of different fertilizing treatments on leaf mineral content in 2018 and 2019 seasons**

Treatment	N (%)		P (%)		K (%)		Ca (%)		Mg (%)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	1.78	1.82	0.179	0.172	1.22	1.24	1.54	1.55	1.33	1.44
KTS	1.84	1.84	0.185	0.198	1.29	1.32	1.66	1.61	1.62	1.53
Mg	1.83	1.97	0.192	0.199	1.34	1.33	1.62	1.62	2.31	2.13
Mo	2.11	2.23	0.200	0.211	1.57	1.52	1.67	1.67	1.84	1.88
KTS+Mg	1.87	2.11	0.194	0.197	1.47	1.44	1.54	1.57	2.31	2.18
KTS+Mo	1.95	2.25	0.212	0.215	1.61	1.66	1.63	1.62	1.81	1.78
Mg+Mo	1.94	2.00	0.199	0.195	1.50	1.57	1.55	1.57	2.11	2.19
KTS+Mo+Mg	1.89	1.94	0.210	0.211	1.60	1.62	1.61	1.66	1.84	1.94
LSD (0.05)	0.19	0.17	0.020	0.021	0.13	0.15	N.S	N.S	0.19	0.21

KTS: potassium thiosulfate , Mg: magnesium sulphate, Mo: ammonium molybdate

**Table 2: Effect of different fertilizing treatments on fruit physical characteristics and yield/tree in 2018 and 2019 seasons**

Treatment	Fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)		Yield/ tree (Kg)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	2.279	2.258	2.01	1.96	1.45	1.44	64.4	65.0
KTS	2.403	2.418	2.07	2.06	1.44	1.43	70.0	71.7
Mg	3.221	3.198	2.16	2.23	1.67	1.68	74.1	73.0
Mo	2.989	2.922	2.21	2.22	1.57	1.52	80.6	81.6
KTS + Mg	2.468	2.481	2.00	2.09	1.47	1.45	68.3	66.6
KTS + Mo	3.239	3.560	2.25	2.32	1.61	1.63	84.6	85.0
Mg + Mo	3.379	3.173	2.35	2.27	1.62	1.61	78.3	75.0
KTS + Mo + Mg	3.721	3.704	2.37	2.37	1.70	1.69	73.3	76.6
LSD (0.05)	0.176	0.168	0.13	0.12	0.14	0.11	3.1	3.4

KTS: potassium thiosulfate , Mg: magnesium sulphate, Mo: ammonium molybdate

**Table 3 : Effect of different fertilizing treatments on oil content of Coratina olive fruits and quality parameters of the obtained oils in 2018 and 2019 seasons**

Treatment	FFA% as oleic acid		PV (meqO <sub>2</sub> kg <sup>-1</sup> oil)		K232		K270		ΔK		Oil %	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Cont	0.14	0.18	2.50	2.90	1.612	1.732	0.073	0.091	-0.0026	-0.0013	20.02	20.90
KTS	0.10	0.14	2.56	2.86	1.152	1.205	0.064	0.082	-0.0055	-0.0049	23.13	23.25
Mg	0.14	0.16	2.57	2.80	1.622	1.635	0.061	0.070	-0.0039	-0.0027	24.75	24.95
Mo	0.13	0.15	2.63	2.74	1.361	1.547	0.053	0.078	-0.0039	-0.0030	23.97	23.71
KTS + Mg	0.11	0.13	2.62	2.88	1.597	1.717	0.056	0.079	-0.0072	-0.0054	24.45	24.66
KTS + Mo	0.13	0.16	2.62	2.75	1.414	1.409	0.060	0.069	-0.0063	-0.0047	24.30	24.55
Mg + Mo	0.12	0.15	2.60	2.74	1.370	1.588	0.055	0.059	-0.0038	-0.0028	24.34	24.53
KTS + Mo + Mg	0.14	0.18	2.54	2.77	1.172	1.275	0.041	0.059	-0.0084	-0.0068	24.83	24.99
LSD (0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	-0.0025	-0.0021	1.19	1.47

KTS: potassium thiosulfate , Mg: magnesium sulphate, Mo: ammonium molybdate

**Table 4: Effect of different fertilizing treatments on minor components and sensory attributes of Coratina olive oil in 2018 and 2019 seasons**

Treatment	Total phenols				Chlorophyll		Carotenoids		Fruity		Bitterness		Pungency	
	Content (mg/kg as caffeic acid)		UNSAF %		content (mg/kg)		content (mg/kg)							
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	824.9	793.9	1.01	1.10	2.80	2.99	1.93	1.95	3.50	3.57	2.50	3.00	1.87	1.75
KTS	861.0	872.0	1.15	1.25	2.85	3.58	1.94	1.98	4.51	4.71	2.35	2.85	1.50	1.75
Mg	972.4	950.2	1.28	1.30	3.31	3.68	2.39	2.50	4.67	4.93	2.83	3.50	1.83	2.33
Mo	786.6	788.4	1.13	1.19	3.01	3.35	2.18	2.33	4.28	4.37	2.41	3.63	1.60	2.33
KTS+Mg	923.4	954.7	1.26	1.29	3.08	3.53	2.50	2.61	4.75	5.10	2.43	2.53	2.08	2.35
KTS+Mo	981.4	980.8	1.30	1.33	3.17	3.42	2.44	2.53	4.50	4.75	2.54	3.13	1.83	2.08
Mg+Mo	890.2	921.9	1.17	1.23	3.02	3.55	2.22	2.65	4.53	4.83	2.29	2.63	1.91	2.35
KTS+Mo+Mg	955.3	979.7	1.35	1.40	3.31	3.69	2.68	2.90	4.82	5.24	2.58	3.67	2.04	2.42
LSD (0.05)	18.9	32.7	N.S	N.S	N.S	N.S	0.31	0.21	N.S	N.S	N.S	N.S	N.S	N.S

KTS: potassium thiosulfate, Mg: magnesium sulphate, Mo: ammonium molybdate

The unsaponifiable fraction that represents about 2% of olive oil is composed of a very large number of minor compounds, very important for the flavor and the nutritional properties of EVOOs [46]. Our results in Table (4) reveal that UNSAP% ranged from 1.01–1.35% and 1.10–1.40% in the two successive seasons, respectively. The highest percentage was found due to KTS+Mo+Mg treatment in the two successive seasons. The differences between all foliar applications were not significant. The main carotenoids and chlorophylls in olive oil are lutein and pheophytin, respectively. They are mainly responsible for the color of virgin olive oil, ranging from yellow-green to greenish-gold, as well as oxidative stability due to their antioxidant nature in the dark and pro-oxidant activity in the light [44]. Results in Table (4) clarify that the chlorophyll content is ranged from 2.80–3.31 mg/kg and 2.99–3.69 mg/kg in the two consecutive seasons of 2018 and 2019, respectively. There were no significant differences among all treatments. KTS+Mo+Mg in the two seasons had the highest chlorophyll and carotenoids contents, where the carotenoids content as shown in Table (4) ranged from 1.93–2.68 mg/kg and 1.95–2.90 mg/kg, respectively. Also, all treatments were higher than those in control treatment in the two seasons. Our results agreed with those obtained by Manai-Djebali *et al.* [44] who found that the chlorophyll and carotenoid content ranged from 1.2–6.2 mg/kg and 1.3–3.8 mg/kg, respectively, for different types of extra virgin olive oils.

Polyphenol values have changed between seasons, and that may be due to climate and variety influences. Additionally, during the oxidation process, the antioxidant activity of these compounds, particularly in the early stages of carotenoids and tocopherols, may

be oxidized, resulting in a loss of vitamin activity and color, as well as nutritional value [47]. Total pigment content in olive oils is an important quality parameter because it correlates with color, which is a fundamental attribute for determining olive oil quality. Pigments are also involved in the mechanisms of autoxidation and photooxidation [33]. It is found that chlorophyll acts as a prooxidant when exposed to light, whereas beta-carotene reduces lipid oxidation through its light-filtering effect. Phenols are recognized as antioxidant compounds and their presence in olive oils has been related to their general properties of improving stability, nutritional value and sensory properties [48].

Results in Table (4) show the effect of different foliar applications on the sensory attributes of Coratina olive oils. Sensory analysis is one of the most important aspects of olive oil classification and value determination. Human sensory evaluation is much more accurate (100 times) for olive oil than laboratory equipment for certain characteristics. The first and primary objective of sensory evaluation for olive oil is to determine if oils contain one or more of the defects that commonly occur in oils from improper fruit storage, handling, pest infestation, oil storage, or processing problems, and the second objective is to describe the positive characteristics of the oil; its intensity of olive-fruity character. Bitterness and pungency are often present in olive oil, especially when newly made. These regulations lead to the classification of oil as extra virgin (EVOO), virgin (VOO), or Lampante olive oil [30]. Results in Table (4) indicate the influence of foliar fertilization with magnesium (Mg), sulfur potassium thiosulfate (KTS), molybdenum (Mo), and their mixture on the organoleptic attributes of olive oil in both seasons

(2018 and 2019). In 2018 season it is observed that olive trees treated with KTS+Mg+Mo, KTS+Mg, and Mg gave the highest fruity attribute (4.82, 4.75 and 4.67) of olive oil respectively, followed by those treated with Mg+Mo, KTS, KTS+Mo and Mo respectively, while the control treatment gave the lowest fruity attribute (3.50) in 2018 season. Also, in 2019 season, foliar fertilization with KTS+Mg+Mo, KTS+Mg, and Mg gave the highest positive properties (fruity attribute) (5.24, 5.10 and 4.93) of olive oil, respectively, while in contrast the control treatment gave the lowest fruity attribute (3.57). The defect qualities of olive oils were equivalent to nil in both fertilized (foliar) and untreated (control) samples over the two seasons. When compared to the control sample, the results in Table (4) show that bitterness and pungency were positive attributes that detected by tasters, there were no significant differences between their values among all treatments. In addition, there was no detectable defect in the oil samples under the study. Our results reveal that all olive oils in our study were classified as extra virgin olive oil based on sensory analysis and the limits of IOC standards [30] (fruity  $\geq$  0.00 and defects = 0.00). All the favorable qualities of olive oil were increased as a result of foliar fertilization. This result can be attributed to that the olive oil under investigation having the highest level of total polyphenol, specifically oleuropein (which is responsible for the bitterness feature), as shown in Table 4. Extra virgin olive oil contains considerable amounts of phenolic components, e.g., hydroxytyrosol and oleuropein, which are responsible for its peculiar taste, flavor, and high stability [35].

### 3.5. Olive oil fatty acids composition

Oil chromatographic analysis revealed a wide range of fatty acids, ranging from C16 to C22, as typical of olive oil. Fatty acids profile is essential in determining the quality of olive oil. Results in Table (5) reveal that palmitic acid (C16:0) was the predominant Saturated Fatty Acids (SFA) ranged from (12.33-13.06%) and (13.00-14.02%) in 2018 and 2019 seasons respectively. The palmitic acid percentage was decreased in samples resulting from spraying different treatments compared with the control sample (untreated). This means that different foliar fertilizations decreased palmitic acid. Oleic acid (C18:1) is the major unsaturated fatty acid in olive oil, it ranged between (71.51-71.98%) and (70.70 - 72.33%) in the two successive seasons. The highest contents of oleic acid were found due to KTS+Mg+Mo in 2018 and KTS+Mg+Mo in 2019, respectively. Linoleic acid (18:2) content was higher in the first season of the experiment, while, in contrast with the control, all fertilizing treatments decreased the percentage of linoleic acid in the second. Meanwhile,

linolenic acid (C18:3) content was found to be less than 1% in all treatments. TUSFA was increased as a result of all foliar applications in the two successive seasons in the contrary with TSFA, which was decreased in all foliar applications. Also, MUSFA/PUSFA ratio was increased as a result of most foliar applications in the two successive seasons where, increasing this ratio, led to an increment of the oxidative stability of oil. The ratio of total saturated fatty acids to the total unsaturated fatty acids (T.SFA/T.USFA) is related to the oil's oxidation stability, whereas the oleic/linoleic acid ratio (C18:1/C18:2) has a beneficial effect on the oil's taste [49]. The third factor is the ratio of linoleic acid to palmitic acid (C18:2/C16:0), which indicates the degree of oxidative deterioration of oils; particularly frying oils [50]. Generally, results in Table (5) indicate that the ratio of C18:1/C18:2, C18:2/C16:0 and T.SFA/T.USFA, of olive oil were affected by different foliar treatments in both seasons (2018 and 2019). From the results obtained in 2018 season, it is clear that foliar application of KTS, Mo and Mg+Mo show a slight increase in C18:1/C18:2 ratio, but KTS treatment caused a slight decrease in C18:2/C16:0 ratio compared with the control samples. The fatty acids composition of all olive oil samples under study was in the range of IOC trade standard for EVOO [30].

### 3.6. Olive oil phenolic compounds concentration

Phenolic compounds of olive oil have been as major interest to the researchers due to their positive effects on both human health and preservation of olive oil. Results in Table (6) show the influence of different foliar fertilization on the phenolic compounds of Coratina olive oil. In comparison with the other phenolic compounds in control and the treated samples during two seasons, it could be noticed that, sixteen phenolic compounds were detected in all olive oil samples, since catechol, oleuropein, and 3-oh tyrosol compounds recorded the highest levels, followed by benzoic, caffeic, pyrogallol, chlorogenic, p-oh-benzoic, coumarin, and gallic compounds. It could be observed that most phenolic compounds were increased as a result of foliar applications, especially KTS+Mo+Mg and KTS+Mg treatments, this increase was higher in the second season than the first one. 3-OH tyrosol compound had the greatest content comparing with the other phenolic compounds where, it ranged between 155.9-194.6 ppm and 150.4-184.0 ppm in the two successive seasons. The greatest content of 3-OH tyrosol was found due to KTS+Mo+Mg treatment in the two seasons. The highest concentration of oleuropein compound was detected by KTS+Mo+Mg and KTS+Mg, since it ranged between 144.6-161.0 ppm and 140.9-155.3 ppm in the two successive seasons. The involvement



of nutrients in foliar fertilizer to improve biosynthesis of phenolic compounds in the plants could explain the increase in phenolic compounds in the olive oil as a result of foliar fertilization under investigation. According to the above discussion, these effects are mostly attributable to the main secoiridoid derivatives such as oleuropein, oleocanthal and oleacein, as well

as simple phenols hydroxytyrosol and tyrosol, as per the results obtained by Shaker *et al.* [51], who found that identifying some bioactive derivatives (oleocanthal, oleokoronol, and oleoresin) of phenolic compounds is evidence of the quality of olive oil.

**Table 5: Effect of different fertilizing treatments on relative percentage of fatty acids composition of Coratina olive oil in 2018 and 2019 seasons**

Treatment FA%	Control		KTS		Mg		Mo		KTS+Mg		KTS+Mo		Mg+Mo		KTS+Mo+Mg	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
C <sub>16:0</sub>	13.06	14.02	12.33	13.80	13.00	13.17	12.93	13.10	12.45	13.24	12.75	13.49	12.69	13.27	12.54	13.00
C <sub>16:1</sub>	0.43	0.55	0.40	0.51	0.41	0.63	0.42	0.60	0.42	0.69	0.41	0.61	0.38	0.68	0.39	0.70
C <sub>17:0</sub>	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05
C <sub>17:1</sub>	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
C <sub>18:0</sub>	1.98	1.79	2.03	1.73	2.04	1.74	2.02	1.71	2.05	1.75	2.03	1.70	2.02	1.67	1.95	1.71
C <sub>18:1</sub>	71.51	70.70	71.73	72.01	71.70	72.28	71.65	71.66	71.80	72.25	71.75	72.05	71.88	72.00	71.98	72.33
C <sub>18:2</sub>	10.98	10.9	11.51	10.00	10.88	10.13	10.90	9.99	11.17	10.03	11.05	10.18	11.00	10.35	11.07	10.17
C <sub>18:3</sub>	0.87	0.90	0.83	0.71	0.84	0.87	0.85	0.82	0.81	0.83	0.82	0.82	0.84	0.84	0.85	0.87
C <sub>20:0</sub>	0.43	0.51	0.44	0.55	0.47	0.48	0.46	0.45	0.47	0.46	0.44	0.45	0.44	0.48	0.47	0.49
C <sub>20:1</sub>	0.49	0.40	0.50	0.45	0.50	0.46	0.51	0.44	0.53	0.50	0.51	0.46	0.51	0.47	0.50	0.48
C <sub>22:0</sub>	0.11	0.13	0.12	0.13	0.13	0.12	0.14	0.11	0.14	0.13	0.12	0.12	0.12	0.12	0.12	0.13
TSFA	15.63	16.48	14.97	16.25	15.69	15.56	15.60	15.42	15.16	15.63	15.39	15.81	15.32	15.59	15.14	15.38
TUSFA	84.36	83.52	85.03	83.75	84.4	84.44	84.4	83.58	84.8	84.37	84.61	84.19	84.68	84.41	84.86	84.62
MUSFA	72.50	71.72	72.69	73.04	72.68	73.44	72.65	72.77	72.82	73.51	72.74	73.19	72.84	73.22	72.94	73.58
PUSFA	11.85	11.80	12.34	10.71	11.72	11.00	11.75	10.81	11.98	10.86	11.87	11.00	11.84	11.19	11.92	11.04
USFA/SFA	5.40	5.07	5.68	5.15	5.37	5.42	5.41	5.42	5.59	5.39	5.49	5.32	5.52	5.41	5.60	5.50
MUSFA/PUSFA	6.11	6.08	5.89	6.82	6.20	6.67	6.18	6.73	6.07	6.76	6.12	6.65	6.15	6.54	6.11	6.66
18:1/18:2	6.51	6.48	6.23	7.20	6.59	7.13	6.57	7.17	6.42	7.20	6.49	7.07	6.53	6.95	6.50	7.11
18:1/16:0	0.84	0.77	0.93	0.72	0.83	0.77	0.84	0.76	0.89	0.75	0.86	0.75	0.86	0.78	0.88	0.78

KTS: potassium thiosulfate, Mg: magnesium sulphate, Mo: ammonium molybdate

**Table 6: Effect of different fertilizing treatments on phenolic compounds concentration (ppm) of Coratina olive oil in 2018 and 2019 seasons**

Treatment Compounds	Control		KTS		Mg		Mo		KTS+Mg		KTS+Mo		Mg+Mo		KTS+Mo+Mg	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
3-OH Tyrosol	109.5	147.0	143.2	153.7	131.3	156.0	125.8	130.8	150.4	184.0	128.0	131.5	130.43	144.0	155.9	194.6
Oleuropein	99.4	120.2	124.6	135.04	121.9	140.36	117.4	120.7	140.9	155.3	120.3	126.3	119.0	130.4	144.6	161.0
Catechol	91.00	78.0	119.2	117.5	135.00	120.0	100.43	111.5	144.2	152.9	103.4	115.2	122.1	120.1	156.7	160.4
Pyrogallol	77.60	83.0	83.9	89.9	90.7	90.1	80.9	85.3	96.9	97.01	84.1	85.9	73.1	90.5	98.5	100.1
Coumarin	35.80	26.2	44.3	35.2	39.5	30.01	38.7	32.1	51.6	50.2	39.02	35.3	40.4	36.2	41.8	55.3
Caffeic	32.7	63.4	54.7	54.1	41.01	64.4	40.5	49.6	44.1	47.4	40.2	51.0	42.6	59.9	58.9	66.2
P-OH-benzoic	27.9	19.3	25.7	27	30.5	27.12	28.8	29.1	33.7	38.1	31.3	30.1	27.7	31.0	35.7	33.8
Benzoic	24.6	35.0	36.8	24.2	42.1	22.01	37.1	21.6	27.3	32.7	37.7	35.9	33.7	35.5	46.1	50.0
Gallic	18.7	27.9	17.3	34.1	20.2	37.9	21.4	25.0	24.8	30.6	21.7	28.7	19.4	37.4	27.01	33.3
chlorogenic	14.5	7.50	15.5	11.9	13.9	15.8	13.0	14.2	16.5	15.5	12.67	12.9	14.8	15.3	13.6	16.7
Vanillic	7.75	5.34	4.85	4.50	10.4	11.6	5.00	5.2	13.5	15.0	7.7	10.8	8.8	5.8	9.9	17.2

Caffeine	3.60	1.30	0.78	0.66	1.54	2.3	1.00	1.1	1.77	1.84	1.21	1.6	1.07	1.2	1.8	2.5
4-NH <sub>2</sub> benzoic	2.48	1.91	1.20	2.33	1.32	1.5	1.11	1.5	1.00	1.32	1.03	1.06	1.25	2.7	1.12	1.3
Ferulic	1.23	0.92	0.85	0.55	1.87	2.03	1.03	1.00	1.26	1.5	0.88	1.31	0.89	1.09	1.25	1.20
Salicylic	1.34	1.91	2.57	3.14	2.67	3.00	1.65	1.85	2.66	3.01	1.78	2.71	2.00	3.00	2.87	3.1
Catechein	1.09	3.29	0.83	3.62	1.30	2.5	0.99	1.8	1.54	2.55	1.05	1.36	0.90	2.2	1.59	2.00

KTS: potassium thiosulfate , Mg: magnesium sulphate, Mo: ammonium molybdate

#### 4. Conclusion

Through the previously presented results, it could be concluded that the use of any treatments in this study has a positive effect on the physical characteristics of the fruits, leaf mineral content, as well as the yield of the trees, also the percentage of oil in the fruits, and maintaining its quality characteristics (organoleptic properties, purity, total polyphenol, overall quality index and major phenolic compounds) as an extra virgin olive oil (EVOO), comparing to the untreated trees (control) in both seasons.

As Coratina olives are dedicated to the production of oil, calculating the amount of oil (oil yield) produced from each treatment is an important thing to study. The oil yield for each treatment can be calculated as the result of multiplying the amount of the fruit yield of the tree with the percentage of oil extracted from the fruits, as shown in Fig (1). As presented, it can be noted that KTS+Mo treatment recorded the highest oil yield in the two seasons of the experiment (20:21 kg oil/tree), that achieved a significant increase in oil yield by about 65% over the untreated trees (control), which recorded 13:14 kg oil/tree in both seasons.

KTS+Mg+Mo treatment, led to the best quality metrics and high total phenol content when compared to the other treatments in the two seasons of the study.

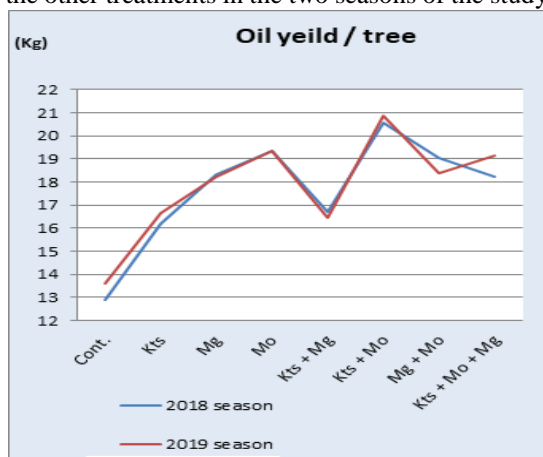


Fig. 1. Oil yield / tree

#### 5. Conflicts of interest

“There are no conflicts to declare”.

#### 6. Formatting of funding sources

“There are no funding sources”.

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