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Studies on The Effect of Humates and Microbial Humates on Fruit Quality of Picual and Kalamata Olive Trees

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ABSTRACT

This study was carried out during the two seasons 2014, 2015 on olive trees Picual and Kalamata cv. The trees were 10 years old growing in sandy soil at a private orchard in Ismailia governorate, Egypt. This investigation was performed to study the effect of humates and microbial Humates on olive trees Picual and Kalamata cv. humates and microbial humates (0, 1 and 2 %) was used as foliar application once time at 70 % final fruit size. At the end of the season, Fruit quality: average fruit weight, volume, fruit shape index and pulp\pit ratio also fruit chemical characterizes: fruit moisture content and oil percentage were recorded. It is clear from data obtained that humates and microbial humates improved Kalamata and Picual fruit quality (Fruit weight, volume, Pulp / Pit ratio and Shape index). Highest oil percentage and lowest moisture content in Kalamata fruit were obtained from olive trees sprayed with microbial humate at high concentration (2%) main while in Picual cv. fruit characterized by highest oil percentage and lowest moisture content was obtained from trees sprayed with humate at high concentration (2%).

Keywords: Olive, Picual cv., Kalamata cv., fruit quality, fruit oil percentage, humates and microbial humates.

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INTRODUCTION

Olive (*Olea europaea* L) is one of the oldest plants cultivated by man in the world. The native home of olive is considered to be Asia Minor, where it can spread to North Africa and Europe countries. The world area under this crop is reached 10 million hectares in 2008 from which 853.1 thousand tonnes oil is produced annually. The ten largest producing countries, which are all located in the Mediterranean region produces 95% of the olive in the world according to the F.A.O. The commercial and wild olive plantation is found into belts around the world between 30-45° North and South of equator. Since Egypt is located in this region. Olive conceded evergreen trees which have spread in the newly reclaimed lands. It is preferred by its supereminence in the desert region, especially under rain- fed and drip water condition, and by its ability to tolerance the salinity and droughts.

Humates are now recognized as one of the most productive inputs in sustainable agriculture. They consist of humic acid and fulvic acid combined with humates crude (prehistoric plant matter) which derive these powerful natural acids. Humates are the residues forests and other plant material, which have been preserved and compressed during the geological disturbances. Often found in the form of brown coals called Leonardite or Lignite, these residues plants contain two powerful biological acids, which have been proven to have a profound influence on the plant growth and yield. Humates are mineral salts of fulvic acid or humic acid within any humic substance there are a great number of complex molecules of humate . Humates's beneficial effects on the plant growth may be relevant to improve the efficiency of fertilizers, microbial stimulants, pest, disease and frost management and working as the hormonal stimulation.

Potassium humate improves the soil ability to hold more water, keeps the soil temperature for plant growth and increase the soil aeration and soil workability by enhancing the structure in the top soil. Potassium humate may be used as the not expensive source for potassium without causing soil pollution that could affect the water systems for our food supply. Research and experiments have shown that the natural mixture of potassium humate is very useful for the system to produce of bio-organic. It was reported by many of the researchers that the K- humate application enhanced the plant growth, nutrient uptake, yield and quality as well as (Böhme and ThiLua, 1997; Padem and Ocal, 1999; Hoang and Böhme, 2001; Türkmen *et al.*, 2005; Zaky *et al.*, 2006 and Karakurt *et al.*, 2009).

Fulvic acid is the low molecular substances that deriving from humic substances which are smaller in the molecular size than humic acids and often used as complexing factors for attracting the nutrient solutions in foliar applications for improving the plant uptake. Foliar application of fulvic acid had a positive effect on the growth due to that these molecules could readily penetrate into the leaf cuticle and plasma membrane. Fulvic acid has the capacity to sensitize plasma membrane to spread the ions into the cells and loading the ions into the conducting tissues. fulvic acid molecule consists of carboxylic groups (COOH), which issued its H atom to bind with cationic species such as the zinc, iron, copper, calcium etc. However, this binding is relatively weak. the fulvic acid also stimulates the production of plant's own auxin and improving the capacity of the plasma membrane to the sense of the other growth hormones such as Cytokinins and GA₃.The research on tomato plants revealed that plants treated with fulvic acid had significant beneficial effects on roots and stem weight, surpassing the benefits of those plants treated with humic acid. (Sladky 1959)on tomato plants observed that plants treated with fulvic acid had significant beneficial effects on roots and stem weight, surpassing the benefits of the plants that treated with humic acid. McCarlyet *al.* (1985) found that applying tomato plants with a foliar fulvic acid at (50 mg/l) caused a significantly increased on yield, stem length, fresh weight, dry stem weight and root weight as compared with control.

Hence, the present study aimed to evaluate fruit quality of Picual and Kalamata olive trees as a function of the using humates and microbial Humates.

MATERIALS AND METHODS

This study was conducted during two successive seasons, 2014 and 2015, on 10 years old olive trees Picual and Kalamata cv. really in vigour, age and size were selected for sprays treatments. Trees were grown in a private orchard in Ismailia governorate, Egypt. The trees spaced 5 x 5 meter (168 trees\ acre) in a sandy soil (Table 1) under drip irrigation system (consisted of two lateral lines per row, separated by 1.0 m). The trees were received the same cultural practices that are recommended. The farm is depending on well in irrigation

(Table 2). Humates and microbial Humates (0, 1 and 2 %) were used as foliar application once time at 70 % of final fruit size, this means that the spraying period will be during the second fruit development stage which approves August month according to fruit growth curve of Picual and Kalamata olive trees cultivated in Ismailia governorate, Egypt (Laila Haggag *et. al.*, 2013). A complete randomized block design was adopted. Six treatments were applied in three replicates. All of the 18 trees conducted in this study were vigorous and similar in growth and canopy.

Table 1: Chemical characteristics of sandy soil used for the present study.

parameters	Surface sample	30 cm depth	60 cm depth
pH	8.02	8.70	8.11
EC(dSm ⁻¹)	3.80	0.80	1.70
Soluble cations (meq\l)			
Ca ⁺⁺	6.00	2.50	3.00
Mg ⁺⁺	4.00	1.50	1.50
Na ⁺	28.60	4.40	12.90
K ⁺	0.12	0.14	0.78
Soluble anions (meq\l)			
CO ₃ ⁼	-	-	-
HCO ₃ ⁻	4.40	2.40	2.00
Cl ⁻	27.20	5.00	13.00
SO ₄ ⁼	7.12	1.14	3.18

Table 2: Chemical characteristics of water weal used for the present study

parameters	values
pH	7.49
EC(dSm ⁻¹)	4.40
Soluble cations (meq\l)	
Ca ⁺⁺	7.50
Mg ⁺⁺	5.00
Na ⁺	33.10
K ⁺	0.16
Soluble anions (meq\l)	
CO ₃ ⁼	-
HCO ₃ ⁻	1.60
Cl ⁻	40.00
SO ₄ ⁼	4.16

Composting:

To ensure uniformity, more surface area to microbial attack and rapid decomposition, the different plant materials were shredded to small pieces before being composted. The shredded plants were enriched with a chemical accelerator composed of 20-kg ammonium sulphate, 10-kg superphosphate, 50-kg calcium carbonate and 100-kg soil as microbial composting starter per ton dried plant material and wetted by both sheep’s ruminal fluid at the rate of 10% of solid mass as complementary microbial composting starter and irrigating water at the rate of 50% of solid mass water holding capacity before being incubated at room temperature until maturity *Saber et al.*, 2011(a& b).

Total Humates and falvates preparation (compost extract):

3kg (dry weight basis) Last prepared compost at natural moisture content was placed into polyethylene bottle (20 L) and 6 L of 0.2 M KOH solution were added and the bottle Stoppard tightly then left for 72 h at room temperature. The dark-colored supernatant liquors were collected from preparing experimental treatments.

Source of a bio-fertilizer (microbial Humates):

A bio-fertilizer (a liquid mixture of different appropriate aqueous media of growth promoting bacteria of non-symbiotic N₂-fixing bacteria *Azospirillum brasilense*, *Azotobacter chroococcum*, phosphate dissolving bacteria *Bacillus megatherium* and *Pseudomonas fluorescense*) at counts of about 10⁶ CFU/ ml of each, were provided by Department of Agricultural Microbiology, National Research Centre that used as bio-fertilizer application (Saber *et al.* 2011 [a] and Abdelraouf *et al.* 2013).

Fruit Quality:

In both seasons samples at harvest time of 100 random mature fruits per tree were used for the determination of fruit physical Properties average fruit size (volume), weight, shape index (length\ diameter and pulp\pit ratio and Fruit chemical characteristics(Fruit moisture percentage and Oil percentage in fruit fresh and dry) of olive trees Picual and kalamata cv.

Fruit physical characteristics:

These include fruit weight, volume, pit weight and flesh/ pit ratio. Fruit samples were collected at mid-Sept.

1- Fruit weight:

It was determined by weighing the samples (100 fruits) by ordinary balance with 0.01 gm sensitivity and average weight per fruit was calculated.

2- Fruit volume:

It was measured by water displacement method.

3- Pulp / Pit ratio:

Values were calculated by dividing the weight of the flesh over the weight of the pit (Pit weight was determined by weighing the sample (100 pits) and average weight of pit was calculated).

4- Shape index: length\diameter.**Fruit chemical characteristics:****1- Fruit moisture %:**

It was determined by drying the flesh in an oven at 60-80°C until a constant weight (A.O.A.C., 1975). Fruit moisture %: moisture percentage of fruit in the previous fruit samples was estimated, samples were dried at 60-80 ° C in electrical air oven until constant weight, the fruit moisture percentage was calculated according to A.O.A.C., (1995).

2-Fruit Oil percentage:

Fruit oil content was determined in fresh and dry weight by means of the Soxhlett fat extraction apparatus using Hexan of 60-80°C boiling point as described by (A.O.A.C. 1975).

Data Analysis:

The obtained data during the two seasons of the study was statistically analyzed by variance method; differences between means were compared using Duncan's multiple range tests at 0.05 level according to (Duncan, 1955).

RESULTS

Weight of Kalamata fruit:

Data in Table (3) cleared that fruit weight of Kalamata cv. was slightly affected by low concentration of humate or microbial humate compared with control in the first season, but spraying microbial humate at low concentration (1%) showed that fruit weight significantly increased compared with a high concentration (2%). In the second all treatments increased fruit weight compared with control. Highest fruit weight was recorded from fruit weight was recorded from fruits taking from trees sprayed with 1% microbial humate compared with other treatments.

Volume of Kalamata fruit:

Data in Table (3) proved that Volume of fruit gave more or less similar values but in the second season all treatments increased fruit volume except spraying trees with humate treatment at low concentration (1%), the increment in fruit volume lacked significant.

Kalamata fruit Shape index (length\ diameter):

From the results in Table (3) it seems that shape index (length\ diameter) was varied from season to another where no constant treatment was observed in the first and second season compared with control.

Table (3): Fruit Weight, Volume and shape index of Kalamata cv. in response to foliar application with various concentrations of humate and microbial humate.

Treatment		Fruit Weight (gm)		Fruit Volume (cm ³)		Fruit shape index	
		2014	2015	2014	2015	2014	2015
Control		7.00 ab	5.63 c	6.85 a	5.87 c	0.96 bc	1.55 ab
Humate	1%	7.11 ab	6.23 b	6.93 a	6.13 bc	0.89 c	1.61 a
	2%	6.87 b	6.34 b	6.85 a	6.27 b	1.01 ab	1.54 ab
Microbial Humate	1%	7.39 a	7.01 a	6.37 a	6.90 a	1.09 a	1.52 b
	2%	6.79 b	6.23 b	6.50 a	6.67 a	0.90 c	1.54 ab
Means of compounds	Control	7.00 A	5.63 B	6.11 B	5.87 B	0.96 A	1.55 A
	Humate	6.99 A	6.07 A	6.72 A	6.09 B	0.96 A	1.57 A
	Microbial Humate	7.06 A	6.29 A	6.57 A	6.48 A	0.98 A	1.54 A
Means of concentration	1%	7.17 A	6.29 A	6.41 A	6.30 A	0.99 A	1.57 A
	2%	6.89 B	6.07 A	6.19 A	6.26 A	0.96 A	1.54 A

Mean in each column with similar letter(s) are not significantly different at 5 % level.

Kalamata fruit Pulp pit ratio:

It is clear from Table (4) that Pulp pit ratio was affected significantly in both seasons by different treatments. Highest Pulp pit ratio was recorded due to spraying trees with humate at low concentration (%).

Table (4): Fruit Pulp/Pit ratio of Kalamata cv. in response to foliar application with various concentrations of humate and microbial humate

Treatment		Pulp		Pit		Pulp/Pit ratio	
		2014	2015	2014	2015	2014	2015
Control		5.89 b	4.72 c	0.96 a	0.91 a	6.12 bc	5.19 d
Humate	1%	6.22 a	5.44 b	0.89 a	0.79 b	7.01 a	6.88 a
	2%	5.85 b	5.39 b	1.01 a	0.96 a	5.80 c	5.64 c
Microbial Humate	1%	6.30 a	5.29 b	1.09 a	0.94 a	5.81 c	5.63 c
	2%	5.89 b	6.05 a	0.90 a	0.96 a	6.57 ab	6.29 b
Means of compounds	Control	5.89 A	4.72 B	0.96 A	0.91 A	6.12 A	5.19 C
	Humate	6.03 A	5.42 A	0.95 A	0.87 A	6.31 A	5.9 A
	Microbial Humate	6.10 A	5.67 A	0.99 A	0.95 A	6.16 A	5.71 B

Means of concentration	1%	6.26 A	5.36 A	0.99 A	0.86 A	6.41 A	6.26 A
	2%	5.87 B	5.72 A	0.96 A	0.96 A	6.19 A	5.97 B

Mean in each column with similar letter(s) are not significantly different at 5 % level.

Kalamata fruit Moisture percentage:

It is clear from data in Table (5) that all treatments significantly decreased Kalamata olive fruits moisture content in both seasons. The highest concentration used the lowest moisture content obtained; this trained was clearly noticed in microbial humate treatment.

Oil percentage in fresh and dry weight of Kalamata fruit:

Results in Table (5) clearly showed that oil percentage in fruit dry weight was influenced not only by humate source but also by its concentrations. Where highest oil percentage in Kalamata fruit dry weight was recorded from fruits sprayed with microbial humate at the high concentration. Data in Table (5) cleared that oil percentage in Kalamata fruit fresh weight followed the same trend

Table (5): Fruit moisture content, Oil percentage in fruit fresh and dry weight of Kalamata cv. in response to foliar application with various concentrations of humate and microbial humate.

Treatment		Fruit Moisture content (%)		Oil in fruit dry weight (%)		Oil in fruit fresh weight (%)	
		2013	2014	2013	2014	2013	2014
Control		62.71 a	63.18 a	41.20 c	43.03 c	15.36 d	15.56 d
Humate	1%	54.95 c	61.32 b	43.68 b	42.83 c	19.68 b	16.57 c
	2%	57.64 b	60.77 bc	39.60 c	43.33 c	16.77 c	17.16 b
Microbial Humate	1%	58.88 b	61.30 b	36.8 d	45.53 b	15.13 d	17.39
	2%	57.68 b	59.85 c	47.20 a	48.40 a	20.90 a	19.51 a
Means of compounds	Control	62.71 A	63.18 A	41.20 A	43.03 B	15.36 B	15.56 C
	Humate	58.43 B	61.76 A	41.49 A	43.07 B	17.27 A	16.43 B
	Microbial Humate	59.76 AB	61.44 A	41.73 A	45.66 A	17.13 A	17.49 A
Means of concentrations	1%	56.92 A	61.31 A	40.24 A	44.18 A	17.41 B	16.98 B
	2%	57.66 A	60.31 A	43.40 A	45.87 A	18.84 A	18.33 A

Mean in each column with similar letter(s) are not significantly different at 5 % level.

Weight and Volume of Picual fruit:

Data in Table (6) showed that both concentrations of microbial humate increased Picual fruit weight and Volume significantly compared with control, with no significant differences recorded between them. Meanwhile, humate sprayed at high concentration (2%) only increased fruit weight and Volume of Picual fruit compared with control.

Table (6): Fruit Weight, Volume and shape index of Picual cv. in response to foliar application with various concentrations of humate and microbial humate

Treatment		Weight (gm)		Volume (cm3)		L/D Fruit	
		2014	2015	2014	2015	2014	2015
Control		5.94 c	6.21 b	5.66 c	6.00 b	1.31c	1.30 a
Humate	1%	6.51 b	6.85 b	6.52 b	6.50 b	1.59 a	1.29 a
	2%	7.81 a	8.09 a	7.83 a	7.87 a	1.52 b	1.22 a
Microbial Humate	1%	8.08 a	8.29 a	7.87 a	7.80 a	1.52 b	1.23 a
	2%	7.97 a	8.17 a	7.67 a	7.83 a	1.56 ab	1.23a
Means of compounds	Control	5.94 C	6.21 C	5.66 C	6.00 B	1.31 A	1.3 A
	Humate	6.75 B	7.05 B	6.34 B	6.79 A	1.47 A	1.27 A

	Microbial Humate	7.33 A	7.56 A	7.07 A	7.21 A	1.46 A	1.25 A
Means of concentration	1%	7.30 B	7.47 B	7.18 B	7.15 B	1.56 A	1.26 A
	2%	7.89 A	8.23 A	7.84 A	7.85 A	1.54 A	1.23 A

Mean in each column with similar letter(s) are not significantly different at 5 % level.

Pical fruit Shape index (length\ diameter):

From the results in Table (6) it seems that Pical fruit shape index (length\ diameter) was affected by humate treatments only in the first season. In this respect all treatments increased Pical fruit shape index significantly than the control.

Pical fruit Pulp pit ratio:

It is clear from Table (7) that all treatments increased Pical fruit Pulp pit ratio compared with control. Whereas, highest Pical fruit Pulp pit ratio in both seasons was obtained from fruits taken from trees sprayed with microbial humate at high concentration (2%).

Table (7): Fruit Pulp/Pit ratio of Pical cv. in response to foliar application with various concentrations of humate and microbial humate.

Treatment		Pulp		Pit		Pulp/Pit ratio	
		2014	2015	2014	2015	2014	2015
Control		4.97 d	5.26d	0.97 b	0.95 ab	5.12 e	5.53 c
Humate	1%	5.70 c	5.88 c	0.88 b	0.82 b	6.51 c	7.21 b
	2%	6.77 b	6.88 b	1.20 a	1.17 a	5.65 d	5.89 c
Microbial Humate	1%	7.18 a	7.23 a	0.99 b	0.90 b	7.22 b	8.01 a
	2%	7.06 a	7.29 a	0.91 b	0.90 b	7.74 a	8.08 a
Means of compounds	Control	4.97 C	5.26 C	0.97 A	0.95 A	5.12 C	5.53 B
	Humate	6.23 B	6.38 B	1.04 A	1.00 A	5.96 B	6.55 B
	Microbial Humate	7.12 A	7.26 A	0.95 A	0.90 A	6.69 A	7.22 A
Means of concentration	1%	6.44 B	6.56 B	0.94 A	0.86 A	6.86 A	7.61 A
	2%	6.92 A	7.09 A	1.06 A	1.04 A	7.00 A	7.50 A

Mean in each column with similar letter(s) are not significantly different at 5 % level

Pical fruit Moisture percentage:

Data in Table (8) showed that all treatments decreased Pical fruit Moisture percentage significantly compared with control in both seasons.

Table (8): Fruit moisture content, oil percentage in fruit fresh and dry weight of Pical cv. in response to foliar application with various concentrations of humate and microbial humate.

Treatment		Moisture content (%)		Oil in dry (%)		Oil in fresh weight (%)	
		2014	2015	2014	2015	2014	2015
Control		62.18 a	58.92 a	42.48 c	45.35 d	16.06 d	18.63 c
Humate	1%	59.32 b	56.65 b	46.14 b	48.70 b	18.77 c	21.11 b
	2%	57.52 b	54.85 c	48.53 a	51.27 a	20.61 a	23.15 a
Microbial Humate	1%	59.36 b	55.02 c	43.57 c	46.40 cd	17.71 c	20.87 b
	2%	57.40 b	55.11 c	45.38 b	47.60 bc	19.30 b	21.37 b
Means of compounds	Control	62.18 A	58.92 A	42.48 C	45.35 C	16.06 C	18.63 C
	Humate	59.67 B	56.81 B	45.72 A	48.44 A	18.48 A	20.96 A
	Microbial Humate	59.67 B	56.35 B	43.81 B	46.45 B	17.69 A	20.29 A
Means of concentration	1%	59.34 A	55.84 A	44.85 B	47.55 B	18.24 B	20.99 B
	2%	57.50 A	54.98 A	46.95 A	49.43 A	19.95 A	22.26 A

Mean in each column with similar letter(s) are not significantly different at 5 % level.

Oil percentage in fresh and dry weight of Picual fruit:

Results in Table (8) clearly showed that most treatments significantly increased oil percentage in fresh and dry weight of Picual fruit compared with control. The highest oil percentage in fresh and dry weight of Picual fruit indicated by sparing Picual olive trees with 2% humate during August month when Picual fruit reached 70% of final fruit size.

DISCUSSION

Spraying humates and microbial humates during August (second fruit development stage) conceded the best choice to improve Kalamata and Picual olive fruit quality according to **Laila Haggag, et al (2012)** who reported that, fruit weight and volume increase in the third stage this increase occurred as a result of increasing the fruit moisture content, moisture content increased markedly during the early fruit development stage, followed by a very slow increase from July 7 till Aug. 6, then a sharp increase between Aug. 13 till September 3 (about 47% increase). Numerous studies in the Mediterranean region have shown that the oil percentage increases dramatically during early fruit ripening (**Salvador, 2001**). Also, **Laila Haggag et al. (2012)** reported that the Fruit oil content of Picual and Kalamata olive trees increased from September 5th till September 25th. This marked increase in fruit oil content occurred during fruit coloration and reached its maximum at the end of October (third fruit development stage), as fruits become completely colored.

Humates have become essential elements in improving yield and quality in many areas of agriculture throughout the world. Treated Picual and Kalamata trees with humate and microbial humate resulted in the increase and improve the fruit quality. This stimulatory effect may have also been related to increase plant shoot and root growth, and uptake of some nutrients (**Valdrighiet al., 1996; Padem et al., 1997; Tan, Nopamonbodi, 1979; Russo and Berlyn, 1992; Sanders et al., 1990; and Poincelot, 1993**) and the plant hormone-like activity of humic substances (**Tattini et al., 1991; Serenella et al., 2002**). Humates conceded biostimulants which been described as “non-nutritional products that may reduce fertilizer use and increase yield and resistance to water and temperature stresses (**Russo and Berlyn, 1992**). Moreover, the positive influences of humic substances on the productivity and fruit quality could also be primarily due to hormone-like activities of the humates through their participation in cell respiration, photosynthesis oxidative phosphorylation, protein synthesis, and various enzymatic reactions (**Vaughan et al., 1985; Chen and Aviad, 1990; Muscolo et al., 1996; Muscolo et al., 1999**). And also, the foliar sprays of these substances enhance the growth, and increases the quantity and quality in a number of plant species (**Brownell et al., 1987; Yildirim, 2007; Karakurt et al., 2009**) at least partially by increasing the absorption of nutrient, serving as a source of mineral plant nutrients and regulator of their release (**Chen and Aviad, 1990; Atiyeh et al., 2002**). To explain the impacts of humic substances, a number of hypotheses indicate that the formation of complex between these materials and mineral ions, their participation in the promotion of enzyme catalysis, their influence was to stimulate photosynthesis, respiration, and metabolism of nucleic acid and hormonal activity have been reported (**Dell’Agnola and Nardi, 1987; Nardi et al., 1988; Muscolo et al., 1999; Serenella et al., 2002**).

CONCLUSION

It could be concluded that humates and microbial Humates (1 and 2%) were used as foliar application once time at 70 % of final fruit size, this means that the spraying period will be during August (second fruit development stage) improved Kalamata and Picual fruit quality (Fruit weight, volume, Pulp / Pit ratio and Shape index). Highest oil percentage and lowest moisture content in Kalamata fruit was obtained from olive trees sprayed with microbial humate at high concentration (2%) main while in Picual cv. fruit characterized with highest oil percentage and lowest moisture content was obtained from trees sprayed with humate at high concentration (2%).

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