Effect of soil application of humic acid and bio-humic on yield and fruit quality of “Kalamata” olive trees

E. Abd El-Razek*, Laila F. Haggag, Eman S. El-Hady and M. F. M. Shahin

Abstract

Background: The present study was carried out during the two successive seasons of 2016 and 2017 on “Kalamata” olive trees (Olea europaea L.), at a private orchard located on Cairo-Ismailia Km 107 desert Road, Egypt. The trees were treated with four soil applications as follows: (T1) control (water only), (T2) organic matter of 10 kg chicken manure was added in both side of the tree under drip irrigation system at the 1st week of January, (T3) organic matter + 100 cm³ humic acid (Actosol®) for each tree, (T4) organic matter + bio-humic which contain 100 cm³ Actosol® and 150 cm³ of Azotobacter chroococcum, Bacillus megaterium, and Bacillus circulans in equal doses. Humic acid and bio-humic were added 3 times at the 1st week of March (full bloom), at 1st week of May (starting fruit set stage), and at the last week of July in the third stage of fruit development (70% of final fruit size) to study the effect of humic acid and bio-humic on the yield and fruit quality of Kalamata olive trees.

Results: The result showed that all treatments improve the nutrient status (N, P, K, Ca, Mg, Fe, Zn, Mn, Cu) of the leaves, yield (kg/tree) and fruit quality, i.e., fruit weight, volume, specific gravity, dimension, and shape index, as well as fruit moisture content percentage and oil percentage in FW and DW than the control. Within all treatments, the bio-humic treatment (T4) is recommended, since it had the highest value of these parameters in comparison with the other treatments or control.

Conclusion: It could be concluded that improving yield and fruit quality attributed to the positive effect of organic matter alone or in combination with humic or bio-humic in increasing the cation exchange capacity of the soil, reducing soil pH, enhancing the root development, increasing the root/shoot ratio, and production of root hairs of olive trees which increase the active uptake for most of the nutrients in the soil. In addition, bio-humic contains three bacteria that are now considered as plant growth-promoting rhizobacteria (PGPR) that play a great role in providing trees with NPK as bio-fertilizers and increased also the mineral status. Therefore, bio-humic positive effects reflected on improving the yield and fruit quality of Kalamata olive trees. In general, organic manure in combination with bio-humic had great effects on improving the yield and fruit quality of Kalamata olive trees than using organic manure alone or use organic manure combined with humic acid.

Keywords: Olive, Humic acid, Bio-humic, Azotobacter chroococcum, Bacillus megaterium, Bacillus circulans, Yield, Fruit quality

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Olive cultivation (Olea europaea L.) has been widespread in many countries throughout the world since several hundred years due to that it is an important fruit crop for human nutrition and many food industries such as oil extracting, pickling, and fodder. All ancient civilizations of the Mediterranean basin and parts of Asia Minor (Egyptians, Phoenicians, Greeks, and Romans) contributed to the spread of olive cultivation in this region. Today, the olive oil industry occupied a huge economic sector in many countries due to that olive oil has many health benefits such as it is a non-saturated oil and has a fine aroma and pleasant taste, and due to these advantages, its production is approximately 3,144,000 tons of oil/year according to IOOC; however, Egypt produces 27,500 tons of olive oil/year (IOOC, 2019a). In Egypt, the cultivation area is approximately 73,774 ha (177,058 Feddan; 1 ha = 2.4 Fed.) which produces about 768,176 tons (FAO, 2018). Egypt is considered one of the top producers of table olives in the world and produces about 690,000 tons which presented 23.6% of the world’s table olives production (IOOC, 2019b). “Kalamata” olive cultivar is considered one of the most important commercial table olive varieties in Egypt and needs special treatments under sandy soil and other types of reclaimed soils condition, since the poor soil fertility and low water holding capacity are generally the main soil problems that influence on Kalamata yield and fruit quality.

However, many factors affected the olive tree productivity such as the nutrition statue and the environmental conditions like poor soil fertility and low water holding capacity which are generally the principal soil problems. Under these soil problems, organic matters, humate substances, and bio-fertilizers avoid these soil problems and reduce the costs of fertilization. In this regard, humic acid and bio-humic (humic acid combined with symbiotic bacteria) are bio-stimulants that enhance trees’ growth and they help to withstand harsh environments when applied in small quantities (Chen et al., 1994). Moreover, various functions were noticed when they are applied such as the positive influence on enhancing the root development, increasing the root/shoot ratio, and production of thin lateral roots of olive plants as a result to its component of heterogeneous mixture of several composite behaviors (Tattini et al., 1991). In addition, humic acid and bio-humic are composed of leonardite coal, and this organic matter stimulates both root and vegetative growth as a result to raise cation exchange capacity that activates nutrient uptake, beneficial microorganism in the soil, tolerance to different stress (drought and heat), and difficult environment conditions such as low organic matter and unavailability of soil nutrients (Russo and Berlyn, 1990, Tattini et al., 1990, Chunhua et al., 1998, Eissa et al., 2007, Ismail et al., 2007 and Haggag Laila et al., 2015a, b). Furthermore, both of humic acid and bio-humic improve the nutrient availability and nutrient uptake due to its mode of action that is similar to chelating agent performance which facilitates the availability of nutrients and also its mode of action like auxins that enhance root growth (O’Donnell, 1973, Tatini, et al., 1990 and Khattab et al., 2012).

Bio-fertilizers applied instead of the chemical fertilizers is considered a favorite target to achieve sustainability in olive production. In this respect, Azotobacter chroococcum strains used as the nitrogen biofertilizer due to that it plays a principal role in adapting the atmospheric nitrogen through its fixation in the roots. Azotobacter chroococcum can improve the soil fertility, since the aerobic bacteria that belong to the genus Azotobacter represent a diverse group of free-living diazotrophic (with the ability to use N2 as the sole nitrogen source) and these microorganisms commonly occur in the soil. The genus Azotobacter includes 6 species and Azotobacter chroococcum is the most common inhabiting in various soils over all the world (Mahato et al., 2009). In addition, Bacillus megaterium and Bacillus circulans are phosphate (P)- and potassium (K)-solubilizing bacteria, respectively, and they are used as biofertilizers due to that they may enhance mineral uptake by plants through solubilizing insoluble P and releasing K from silicate in the soil (Goldstein and Liu, 1987). A group of bacteria is now considered as plant growth-promoting rhizobacteria (PGPR), which participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling, and seedling establishment and therefore deserve particular attention for agricultural or forestry purposes (Elo et al., 2000). PGPR may colonize the rhizosphere, the surface of the root, or even superficial intercellular spaces of plants (McCully, 2001).

The aim of this research is to study the effect of organic manure in combination with humic acid or in combination with bio-humic (which consist of humic acid combined with three plant growth-promoting rhizobacteria (PGPR); Azotobacter chroococcum is used as nitrogen biofertilizer, Bacillus megaterium and Bacillus circulans which are used as phosphate (P)- and potassium (K)-solubilizing bacteria) on the yield and fruit quality of Kalamata olive trees.

Materials and methods

Plant materials

The present study was carried out during the two successive seasons of 2016 and 2017 on Kalamata olive trees (Olea europaea L.) at a private orchard located on Cairo-Ismailia Km 107 desert Road, Ismailia Governorate, Egypt. Table 1 showed the analyses of orchard well
water and Table 2 presented the analysis of the physical and chemical properties of the orchard soil. The experimental trees were about 15 years old, spaced at 5 × 6 m within deferent varieties, cultivated in sandy soil under a drip irrigation system, similar in growth vigor, and received the same horticultural practices.

The following treatments were applied:

T1 = Control (water only)
T2 = Organic matter (10 kg chicken manure was added in both sides of the tree under a drip irrigation system) at the 1st week of January
T3 = Organic matter + 100 cm³ humic acid (Actosol) for each tree
T4 = Organic matter + bio-humic (mixed of 100 cm³ Actosol and 150 cm³ of Azotobacter chroococcum, B. megaterium, and B. circulans in equal doses where humic acid and bio-humic were added 3 times at 1st week of March (full bloom), at 1st week of May (starting fruit set stage), and at the last week of July in the third stage of fruit development (70% of final fruit size).

**Bio-humic preparation**
Bio-humic consisted of humic acid plus liquid cultures of three bacteria: *Azotobacter chroococcum, Bacillus megaterium, and Bacillus circulans*. They are provided by the Unit of Biofertilizers, Faculty of Agriculture, Ain Shams University. Each organism was grown separately in batch culture to the late exponential phase of each microorganism to give a cell suspension of $5 \times 10^5$, $6 \times 10^5$, and $4 \times 10^7$ cell/ml for *Azotobacter chroococcum*, *B. megaterium*, and *B. circulans*, respectively. Cultures were mixed on site in equal doses to form 1 liter of biofertilizer. Then, each 1 liter of biofertilizer was mixed with 1 liter of humic acid.

**Yield and fruit quality**
The yield was harvested at 1st November and recorded as kilograms/tree. Fifty fruits were handpicked randomly from all sides of each tree at harvest (100% purple flesh and dark purple skin) to determine the following fruit quality: fruit weight (g), fruit volume (cm³), fruit length and diameter (cm), shape index (L/D), fruit moisture content (%), and fruit oil content in dry and fresh weight (%) according to AOAC (1990).

**Leaf mineral content**
Macro-nutrients were determined in dry leaf samples which are collected from each tree at the harvest on 1st November in both seasons. N% was measured by Micro-Kjeldahl according to Pregel (1945). Also, P% was determined as described by Champman and Parker (1961), while K% was measured according to Brown and Lillie-land (1945). Mg, Ca, Fe, Zn, Mn, and Cu were determined by the atomic adsorption spectroscopy (AAS) method according to A.O.A.C (1990).

**Statistical analysis**
Data were analyzed by analysis of variance (ANOVA), and means were compared using Duncan’s test at $p < 0.05$ to determine the significance of differences between the conducted treatments (Duncan, 1955).

**Results**
Table 3 illustrated the effect of soil application with humic acid or bio-humic (humic acid combined with three bacteria: *Azotobacter chroococcum, Bacillus megaterium, and Bacillus circulans*) on total yield, fruit weight, and volume, as well as fruit specific gravity of Kalamata olive trees. It is clear that T4 recorded the

### Table 1 Analysis of the orchard well water

| Parameter | Value  |
|-----------|--------|
| pH        | 7.49   |
| EC (dSm⁻¹) | 4.40  |

| Soluble cations (meq/l) | Ca²⁺ | Mg²⁺ | Na⁺ | K⁺ |
|-------------------------|------|------|-----|----|
| Ca²⁺                   | 7.50 | 5.00 | 33.10 | 0.16 |
| Mg²⁺                   |      |      |      |    |
| Na⁺                    |      |      |      |    |
| K⁺                     |      |      |      |    |

| Soluble anions (meq/l) | CO₃⁻ | HCO₃⁻ | Cl⁻ | SO₄²⁻ |
|------------------------|------|------|-----|-------|
| CO₃⁻                   |      |      | 40.00 | 4.16  |
| HCO₃⁻                  |      |      |      |       |
| Cl⁻                    |      |      |      |       |
| SO₄²⁻                  |      |      |      |       |

### Table 2 Analysis of physical and chemical properties of the orchard soil

| Parameters | Depth parameters of simple (cm) |
|------------|---------------------------------|
|            | Superficial sample | 30 cm | 60 cm |
| pH (2.5:1) | 8.7 | 8.02 | 8.11 |
| EC(dSm⁻¹) (1:1) | 0.8 | 3.85 | 1.75 |

| Soluble cations (meq/l) | Ca²⁺ | Mg²⁺ | Na⁺ | K⁺ |
|-------------------------|------|------|-----|----|
| Ca²⁺                   | 6.05 | 2.55 | 2.05 | 0.12 |
| Mg²⁺                   | 4.05 | 1.55 | 1.55 | 0.14 |
| Na⁺                    | 28.60| 4.45 | 12.95| 0.78 |
| K⁺                     | 0.12 | 0.14 | 0.78 | 0.14 |

| Soluble anions (meq/l) | CO₃⁻ | HCO₃⁻ | Cl⁻ | SO₄²⁻ |
|------------------------|------|------|-----|-------|
| CO₃⁻                   |      |      | 4.45| 7.12  |
| HCO₃⁻                  |      |      | 2.45| 1.14  |
| Cl⁻                    | 27.25| 5.05 | 13.05| 3.18  |
| SO₄²⁻                  | 7.12 | 1.14 | 3.18 | 1.14  |
highest value in these parameters. Concerning the yield, all treatments improved the yield than the control (T1). T4 gave the highest yield/tree in both seasons (89.67 and 83.65 kg/tree) followed by T3 (81.00 and 75.43 kg/tree). In addition, T2 came in the third order and achieved 70.67 and 60.27 kg/tree in both years, whereas the control (T1) recorded the lowest yield in both seasons (43.33 and 48.31 kg/tree). Regarding the fruit weight, all treatments produced higher weight than the control. T4 achieved the heaviest weights in both seasons (6.52 and 7.74 g) followed by T3 which had 6.22 and 7.23 g in the two studied years. T2 came also in third place (5.66 and 6.16 g), while the T1 (control) had the lowest fruit weight during this study (5.15 and 5.44 g). The same trend was found in the fruit volume as affected by treatments. Concerning the fruit specific gravity, it is clear that all treatments improve this parameter (arranged from 1.005 to 1.010 g/cm\(^3\) in the 1st season and from 1.014 to 1.032 g/cm\(^3\) in the 2nd season than the control (0.975 and 0.953 g/cm\(^3\), respectively).

The results in Table 4 showed the effect of soil application of humic acid and bio-humic on fruit dimensions and shape index of Kalamata olive trees. Data revealed that all treatments affected the fruit dimension more than the control (T1) in both years. In this regard, T4 had the highest length and width in both seasons (L = 2.96 and 2.98 cm, W = 2.08 and 2.26 cm), followed by T3 (L = 2.71 and 2.77 cm, W = 1.96 and 2.18 cm), while T2 came in the third class (L = 2.58 and 2.51 cm, W = 1.89 and 1.95 cm). The control was the last order (L = 2.08 and 2.02 cm, W = 1.52 and 1.56 cm). Concerning the shape index, T4 was different among all treatments including the control and achieved L/D = 1.42 and 1.32 in both seasons.

Table 5 presented the effect of soil application of humic acid and bio-humic on moisture content percentage and oil content percentage in dry and fresh weights of Kalamata olive trees. It is clear from the result that all treatments had higher values in these parameters than the control (T1). The highest value of moisture content percentage was achieved by T4 (58.04 and 56.17%) in both seasons, followed by T2 and T3 which had the same significant value and recorded 47.77 and 49.79% in the 1st year and 48.36 and 49.98 in the 2nd year, respectively, while the control recorded the lowest moisture content percentage in the two years (34.26 and 36.31 %). Oil percentage in dry weight (DW) increased by all treatments than the control (T1). In this regard, the quality of Kalamata olive as pickling variety improved as a result to increase the oil content percentage in dry and fresh weights. T4 had the highest oil percentage in DW (45.00 and 43.07% in both years), followed by T3 which had 41.03 and 40.33%, whereas there was no significant difference between T4 and T3. Moreover, T2 came in the second order (39.65 and 38.80% in both seasons) and was non-significant with T3. The lowest oil percentage in DW was noticed by the control (35.10 and 36.20%). The same trend was found in oil percentage in fresh weight (FW), since T4 produced the highest value in both seasons (31.56 and 32.11%), followed by T3 which had 26.08 and 25.77%, then T2 which had 22.26 and 22.10%, while the control had the smallest value (16.36 and 18.90%).

### Table 3: Effect of soil application of humic acid and bio-humic on yield, fruit weight, and volume, as well as fruit specific gravity of Kalamata olive trees

| Treatments         | Yield (kg/tree) | Fruit weight (g) | Fruit volume (cm\(^3\)) | Fruit specific gravity (g/cm\(^3\)) |
|---------------------|-----------------|------------------|--------------------------|-------------------------------------|
| 1st season          | 2nd season      | 1st season       | 2nd season               | 1st season                          | 2nd season                          | 1st season                          | 2nd season                          |
| T1 = Control        | 43.33 d         | 48.31 d          | 5.15 d                   | 5.44 d                             | 5.28 d                               | 5.71 d                               | 0.975 b                              | 0.953 b                             |
| T2 = Organic matter | 70.67 c         | 60.27 c          | 5.66 c                   | 6.16 c                             | 5.63 c                               | 6.08 c                               | 1.005 a                              | 1.014 a                             |
| T3 = Organic matter + humic acid | 81.00 b | 75.43 b          | 6.22 b                   | 7.23 b                             | 6.17 b                               | 7.03 b                               | 1.008 a                              | 1.029 a                             |
| T4 = Organic matter + bio-humic | 89.67 a | 83.65 a          | 6.52 a                   | 7.74 a                             | 6.46 a                               | 7.50 a                               | 1.010 a                              | 1.032 a                             |

Means within a column followed by different letter(s) are statistically different at 5% level.

### Table 4: Effect of soil application of humic acid and bio-humic on fruit dimensions and shape index of Kalamata olive trees

| Treatments         | Fruit length (cm) | Fruit width (cm) | Shape index (L/D) |
|---------------------|-------------------|------------------|-------------------|
| 1st season          | 2nd season        | 1st season       | 2nd season        | 1st season                          | 2nd season                          |
| T1 = Control        | 2.08 c            | 2.02 c           | 1.52 c            | 1.56 C                              | 1.36 b                              | 1.29 b                             |
| T2 = Organic matter | 2.58 b            | 2.51 b           | 1.89 b            | 1.95 b                              | 1.37 b                              | 1.29 b                             |
| T3 = T2 + humic acid | 2.71 ab        | 2.77 ab           | 1.96 ab           | 2.18 ab                             | 1.38 ab                              | 1.27 b                             |
| T4 = T2 + bio-humic | 2.96 a            | 2.98 a           | 2.08 a            | 2.26 a                              | 1.42 a                              | 1.32 a                             |

Means within a column followed by different letter(s) are statistically different at 5% level.
Therefore, T4 was the best treatment that increased the oil percentage in DW and FW among all treatments.

Table 6 cleared the effect of soil application of humic acid and bio-humic on N, P, K, Ca, and Mg leaf mineral content of Kalamata olive trees. Concerning NPK, T4 had the highest content of NPK among all treatments during the two seasons (N = 1.92 and 1.98%, P = 0.19 and 0.21%, and as K = 1.35 and 1.38%). In addition, T3 was the second order in NPK content (N = 1.46 and 1.54%, P = 0.17 and 0.19%, and K = 1.23 and 1.24%). Moreover, T2 recorded the third grade in NPK content (N = 1.10 and 1.24%, P = 0.13 and 0.15%, and K = 1.14 and 1.16%), while the control (T1) recorded the lowest content of NPK among all treatments through the two seasons of this study (N = 0.97 and 1.00%, P = 0.10 and 0.11%, and K = 0.99 and 0.96 %). Regarding Ca and Mg leaf content, all treatments increased the leaf content of these elements than the control. T4 was the highest content in Ca and Mg leaf content in the two years (Ca = 1.19 and 1.20%, Mg = 0.17 and 0.16%), followed by T3 (Ca = 0.98 and 0.96%, Mg = 0.13 and 0.14%), and then came T2 (Ca = 0.78 and 0.77%, Mg = 0.10 and 0.11%), whereas the control (T1) had the lowest Ca and Mg leaf content in the two years (Ca = 0.63 and 0.65%, Mg = 0.08 and 0.09%). T4 was the superior treatment between all treatments including the control in N, P, K, Ca, and Mg leaf content.

Table 7 showed the effect of soil application of humic acid and bio-humic on leaf microelement content of Kalamata olive trees. Fe, Mn, Zn, and Cu leaf content were increased by all treatments than the control. T4 was the promising treatment among all treatments, since it had the highest value of Fe, Mn, Zn, and Cu leaf content (61, 41, 35, and 7.56 ppm in the 1st year, respectively, and 65, 42, 37, and 7.64 ppm in the 2nd year, respectively), followed by T3 (55, 36, 30, and 6.51 ppm in the 1st year, respectively, and 57, 35, 32, and 6.73 in the 2nd year, respectively) and T2 (51, 33, 23, and 6.00 ppm in the 1st year, respectively, and 50, 30, 24, and 6.05 in the 2nd year, respectively), whereas the control (T1) gave the lowest value of leaf content (30, 23, 16, and 4.50 ppm in the 1st year, respectively, and 32, 21, 18, and 4.38 in the 2nd year, respectively).

Discussion

Olive trees’ growth, total yield, and fruit quality are affected by many environmental factors and nutritional status. In this regard, poor fertility of soil as well as low water holding capacity in general present the major soil troubles which impact on the yield and fruit quality. Therefore, the trees need organic fertilizers to improve the soil characteristics and to reduce the costs of fertilization as well as to avoid soil pollution which caused by chemical fertilization. In this research, besides adding the organic manure, humic and bio-humic not only play a great function in raising the water holding capacity by increasing cation exchange capacity but also improve the nutritional status as a result to activate nutrient uptake and beneficial microorganism due to the positive effect of humic acid and bio-humic in enhancing the root development, increasing the root/shoot ratio, and production of thin lateral roots of olive plants which enhance the active uptake for most of the nutrients in

Table 5

| Treatments               | Moisture content (%) | Oil in dry weight (%) | Oil in fresh weight (%) |
|--------------------------|----------------------|-----------------------|-------------------------|
|                          | 1st season           | 2nd season           | 1st season             | 2nd season           | 1st season                 | 2nd season |
| T1 = Control             | 34.26 c              | 36.31 c              | 35.10 c                | 36.20 c              | 16.36 c                    | 18.90 c   |
| T2 = Organic matter      | 47.77 b              | 48.36 b              | 39.65 b                | 38.80 b              | 22.26 b                    | 22.10 b   |
| T3 = T2 + humic acid     | 49.79 b              | 49.98 b              | 41.03 ab               | 40.33 ab             | 26.08 ab                   | 25.77 ab  |
| T4 = T2 + bio-humic      | 58.04 a              | 56.17 a              | 45.00 a                | 43.07 a              | 31.56 a                    | 32.11 a   |

Means within a column followed by different letter(s) are statistically different at 5% level

Table 6

| Treatments               | N (%) | P (%) | K (%) | Ca (%) | Mg (%) |
|--------------------------|-------|-------|-------|--------|--------|
|                          | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season |
| T1 = Control             | 0.97 d    | 1.00 d  | 0.10 d  | 0.11 d  | 0.99 d  | 0.96 d  | 0.63 d  | 0.65 d  | 0.08 d  | 0.09 d  |
| T2 = Organic matter      | 1.10 c    | 1.24 c  | 0.13 c  | 0.15 c  | 1.14 c  | 1.16 c  | 0.78 c  | 0.77 c  | 0.10 c  | 0.11 c  |
| T3 = T2 + humic acid     | 1.46 b    | 1.54 b  | 0.17 b  | 0.19 b  | 1.23 b  | 1.24 b  | 0.98 b  | 0.96 b  | 0.13 b  | 0.14 b  |
| T4 = T2 + bio-humic      | 1.92 a    | 1.98 a  | 0.19 a  | 0.21 a  | 1.35 a  | 1.38 a  | 1.19 a  | 1.20 a  | 0.17 a  | 0.16 a  |

Means within a column followed by different letter(s) are statistically different at 5% level
the soil. This fact was confirmed by Tattini et al. (1991) and their findings explain our results.

Bio-humic was more characterized than the humic acid with the included 3 beneficial bacteria: Azotobacter chroococcum which utilizes the atmospheric nitrogen through its fixation in the roots as well as Bacillus megaterium and Bacillus circulans that release phosphate (P) and potassium (K) from the soil to a simple form which will be able to uptake by roots. This true parallel with B. megaterium which utilizes the atmospheric nitrogen biofertilizer and contains three PGPR: Azotobacter chroococcum, Bacillus circulans that release phosphate (P) and potassium (K)-solubilizing bacteria that provided the trees with the mineral nutrient and raised the mineral leaf content. These findings were in harmony with Russo and Berlyn (1990), Tattini et al. (1990), Chunhua et al. (1998), Eissa et al. (2007), Ismail et al. (2007), and Haggag Laila et al. (2015a, b).

Table 7 Effect of soil application of humic acid and bio-humic on leaf microelements content of Kalamata olive trees

| Treatments         | Fe (ppm) | Mn (ppm) | Zn (ppm) | Cu (ppm) |
|--------------------|----------|----------|----------|----------|
| T1 = Control       | 30 c     | 23 c     | 16 d     | 4.50 c   |
| T2 = Organic matter| 51 b     | 33 b     | 23 c     | 6.00 b   |
| T3 = T2 + humic acid| 55 ab    | 36 ab    | 30 b     | 6.51 ab  |
| T4 = T2 + bio-humic| 61 a     | 41 a     | 35 a     | 7.56 a   |

Means within a column followed by different letter(s) are statistically different at 5% level.

The application of organic manure alone or combined with humic acid treatment as a result it contains three PGPR: Azotobacter chroococcum used as nitrogen biofertilizer and Bacillus megaterium and Bacillus circulans which are used as phosphate (P)- and potassium (K)-solubilizing bacteria that provided the trees with the mineral nutrient and raised the mineral leaf content. These findings were in harmony with Russo and Berlyn (1990), Tattini et al. (1990), Chunhua et al. (1998), Eissa et al. (2007), Ismail et al. (2007), and Haggag Laila et al. (2015a, b).

The improving nutrient availability can attribute to the role of humic acid and bio-humic in enhancing the nutrient uptake in the form of a chelating agent as well as that both of them may enhance root growth in a similar mode of action to auxins. This explanation is in agreement with O’Donnell (1973), Tatini et al. (1990), Tao et al. (2008), and Khattab et al. (2012).

In a general, the result proved that organic manure compound with humic acid or bio-humic had a positive influence on increasing leaf mineral status that reflected on improving yield and fruit quality, especially the treatment of bio-humic (T4) which maximized the yield and gave excellent fruit quality due to the bio-humic content of the mentioned three PGPR that play a great role in providing trees with NPK as bio-fertilizers and increased the mineral status. This is in agreement with many researchers that found the same effect on increasing seedling growth, growth vigor, and yield and fruit quality of fruit trees as a result to improve the mineral status, and they confirmed our results. This concerns with Senn and Kingman (2000), Rengrudkij and Partida (2003), Mohammed et al. (2010), Abd El-Razek et al. (2012), Khattab et al. (2012), Khattab et al. (2014), Abobatta (2015), Abdel-Hayani and Hadi (2015), Haggag Laila et al. (2015a, b), Sândor et al. (2015), Ennab (2016), Jahromi and Khankahdani, (2016), Abd El-Rheem et al. (2017), Asgharzade and Babaeian (2012), Ferrara and Brunetti (2008), Haggag Laila et al. (2013), Rahil and Jabi (2015), Shahin et al. (2015), Mosa et al. (2016), Abd El-Rhman (2017), Danyaei et al. (2017), Hidayatullah et al. (2018) and Abd El-Razek et al., (2018a, b).

Conclusion

The application of organic manure alone or combined with humic or bio-humic had positive effects on improving the total yield and fruit quality of Kalamata olive trees. However, soil application of organic manure (10 kg chicken manure was added in both sides of the tree under drip irrigation system) at the 1st week of January combined with bio-humic (mixed of 100 cm³ Actosol® and 150 cm³ of Azotobacter chroococcum, B. megaterium, and B. circulans in equal doses) added 3 times at the 1st week of March (full bloom), at the 1st week of May (starting fruit set stage), and at the last week of July in the third stage of fruit development (70% of final fruit size) is recommended to improve the nutrient status (N, P, K, Ca, Mg, Fe, Zn, Mn, Cu) and the total yield (kg/
tree) and fruit quality (fruit weight, volume, specific gravity, dimension, and shape index, as well as fruit moisture content percentage and oil percentage in FW and DW) of Kalamata olive trees. In this respect, improving the mineral status and productivity (yield and fruit quality) attributed to the positive effect of organic matter, humic and bio-humic, in improving the cation exchange capacity of the soil, reducing soil pH, enhancing the root development, increasing the root/shoot ratio, and production of thin lateral roots of olive plants. In addition, bio-humic contains three bacteria that are now considered as plant growth-promoting rhizobacteria (PGPR) that play a great role in providing trees with NPK as bio-fertilizers that increased also the mineral status. All of these effects increase the active uptake for most of the nutrients in the soil. Therefore, the organic manure in combination with bio-humic had positive effects reflected on improving the yield and fruit quality of Kalamata olive trees than using organic manure alone or combined with humic acid.

**Abbreviations**

PGPR: Plant growth-promoting rhizobacteria; FAO: Food and Agriculture Organization of the United Nation; IOOC: International Olive Oil Council; CA: California; USA: United State of America; AOAC: The Association of Official Analytical Chemists

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Author LFH designed the study, wrote the protocol, managed the field works, and reviewed the final draft of the manuscript; Author EA managed the literature searches, participated in the field application, collected the field samples for physical and chemical analyses, tabulated the field data for the statistical analyses, prepared the samples for analyses, and wrote the first draft of the manuscript. Author ESE conducted the field applications, tabulated the field data for the statistical analyses, prepared the samples for analyses, conducted the physical and chemical analyses, and performed the statistical analyses. Author MFMSH participated in the field works, collected field samples, and tabulated the data for statistical analyses. The authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

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