Effect of Biostimulant and Humic Acid on Tree Growth and Fruit Characteristics of Olive (*Olea europaea* L.) cv. Arbequina .

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ABSTRACT

This study was carried out on olive tree (*Olea europaea* L.) cv. Arbequina at the orchard in Grdarasha in Erbil Directorate of Agriculture Research center/Ministry of Agriculture /Iraqi Kurdistan Region, in order to study the effects of foliar application of biostimulant fertilizer with three concentrations (0, 2, 4 ml.L^{-1}), and soil application of humic acid with three levels (0, 5, 10 m.L^{-1}). The foliar spray and soil application were used on three dates (pit hardening, one month after first application and one month after second application) during the growing season on vegetative growth parameters, yield, fruit properties and oil content was studied. Thirty-six (36) of trees used in the experiment, Four branches per tree for each experimental unit with four replications. The experiment was arranged in (RCBD), with four replications. Trees were sampled at the harvesting stage and analyzed for morphology characteristics, chemical composition, and yield production. Data were analyzed by (SAS) using Duncan's Multiple Range Test at P≤ 0.05. Results showed that foliar application (Bio) and soil application (HA) significantly increased most of the parameters of olive trees such as on leaf area, Leaf dry weight, Leaf mineral contents (N, K, Mn and Zn), Fruit size, fruit fresh weight, Total yield per tree, oil content and Acidity % as compared with control.

Keywords: Olive, cv. Arbequina, Biostimulant, Humic acid, foliar spray

تأثير حامض الهيومك ورش الأسمدة الحيوية على نمو والصفات الثمرية لاشجار الزيتون صنف Arbequina (*Olea europaea* L.)

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الخلاصة

أجريت هذه الدراسة على شجرة الزيتون (Olea europaea L.) صنف Arbequina، خلال عام 2021، لدراسة تأثير الرش الورقي لسماد المحفز الحيوي بثلاث تركيزات (0 ، 2 ، 4 مل لتر$^{-1}$)، وتطبيق حمض الهيوميك في التربة بثلاثة مستويات (0 ، 5 ، 10 مل لتر$^{-1}$). تم استخدام الرش الورقي ورش التربة على ثلاث مواعيد (مرحلة تصلب النواة ، شهر بعد التطبيق الأول وشهر بعد التطبيق الثاني) خلال موسم النمو. تمت دراسة صفات النمو الخضري والمحصول وخصائص الثمار ومحتوى الزيت. أقيمت التجربة باستخدام ((RCBD) بأربعة مكررات. تم أخذ عينات من الأشجار في مرحلة الحصاد وتحليل الخصائص المورفولوجية والتركيب الكيميائي وإنتاج الحاصل. تم تحليل البيانات بواسطة ((SASباستخدام تحليل التباين الدانكن عند مستوى

احتمال 5%, أظهرت النتائج أن إضافة الأوراق ((Bio) وتطبيق التربة (HA) أدى إلى زيادة معنوية في معظم معاملات أشجار الزيتون مثل مساحة الأوراق، وزن الأوراق الجاف، المحتوى المعدني للاوراق (N، K، Mn، Zn)، حجم الثمار، اكوزن الطري للثمار، الحاصل الكلي للشجرة، محتوى الزيت والحموضة الكلية مقارنة بعاملة المقارنة.

الكلمات الدالة : الزيتون، صنف أربيكوينا، المحفز الحيوي، الرش الورقي، حامض الهيوميك.

1. Introduction

Olive (*Olea europaea* L.) is a long-lived evergreen tree, belongs to the Oleaceae family, it is one of the most widely cultivated and economically important fruit crops for several countries. Olive tree is native to all of the Mediterranean countries and it plays an important role in the so-called Mediterranean diet (López-Cortés *et al.*, 2013). Fruits and oil are among the oldest and the most important products, the olive tree is one of the blessed trees that repeatedly mentioned in the Holy Quran for six times. The importance of olive fruit is due to heavy loading and dietary value, as the fruit is a good source of vitamins (A, B, C, D, E) and minerals such as; K, Ca, Mg and P (Ibrahim, 2005). In addition, olive oil is filled with monounsaturated fatty acids (MUFAs) and has many anti-oxidative properties as phenolic acid. It has protective effects against ailments such as coronary heart disease, various cancers and age-related cognitive decline (Erel *et al.*, 2017). The Arbequina cultivar is well-known for its ability to adapt to high density cultivation and it is an oily cultivar in which fruits are round and small, and their oils are smooth only slightly peppery and bitter (Hernández-Hernández *et al.*, 2019). Humic acid is formed by decomposition of organic matter, particularly those with a plant origin and may be found in soil, peat and coal (Moshtaghi *et al.*, 2011). Humic acid's beneficial role is associated to its direct influence on physiological and biochemical processes in plants, and its indirect effect on enhancing physical, chemical and biological characteristics of the soil (Taha and Osman, 2018). Application of humic acid frequently improved plant growth by its act in regulating carbon cycle and releasing nutrients such as; nitrogen, phosphorus and sulfur. Humic acid increases element absorption and improved soil fertility with chelating important elements (El-Razek *et al.*, 2020). Moreover, humic acid has been demonstrated to stimulate plant growth, and consequently the yield through influencing on mechanisms involved in; photosynthesis, cell respiration, water and nutrient uptake, changes in membrane permeability, enzyme activities and/or inhibition, biosynthesis of protein and nucleic acids and finally activating biomass production (Kaya *et al.*, 2020).

Biostimulant is a natural or micro-organisms relating material that applied to plants with the goal of regulating the internal physiological processes of plants and improving nutritional efficiency, and tolerance to biotic and abiotic stresses. In small concentrations, these substances are efficient, favoring the good performance of the plant's vital processes, and allowing high yields and good quality products (Van Oosten *et al.*, 2017). Many biostimulants are based on seaweed extract, amino acids (of animal and plant origin), humic and fulvic acids, peptides, enzymes, vitamins, substances with hormone-like activities (deriving from algal extracts), silicon and other minerals, antioxidants and some strains of microorganisms (Rouphael and Colla, 2020). It has been documented that biostimulants can be operated in plants at various levels, indicating the primary impacts on plant metabolic and photosynthetic activities, nutrient absorption, growth, biomass production, and yield (Puglia *et al.*, 2021). Moreover, foliar biostimulant considerably raised leaf concentrations of macro- and micronutrients, resulting in higher crop yields and quality (Tejada *et al.*, 2016). Biostimulants can take many forms depending on their application and the needs of farmers, such as liquid combinations, micro-granules, powders, and so on (Bulgari *et al.*, 2015).

The aim of this study is to investigate the foliar spray of biostimulants as (Foliastim liquid) and soil application of humic acid on some vegetative growth, olive fruit and yield characteristics, as well as quality and quantity of olive oil cv. Arbequina tree.

2. Material and Methods

This study was conducted during the growing season (April 1 to November 25, 2021) in an olive orchard located in Grdarasha, Erbil governorate, belonged to the Directorate of Agriculture Research Center, Ministry of Agriculture, Kurdistan Region-Iraq.

The age of the olive trees cv. Arbequina were 15 years, the space between trees and lines was (3×6 m), in order to study the effect of foliar spray of biostimulant fertilizer with three concentrations (0, 2, 4 $ml.L^{-1}$), and soil application of humic acid with three levels (0, 5, 10 $m.L^{-1}$). For application of the biostimulant fertilizer, few drops of tween 20 were added to the solution, for maximum absorption. Foliar spraying was done in the morning; however, humic acid was added to the soil before irrigation. The foliar spray and soil application were used at three dates (pit hardening stage, one month after first application and one month after second application). Vegetative growth parameters; through growing season on vegetative growth parameters, yield, fruit properties and oil content, were taken into consideration. The trees received the regular agricultural and horticultural practices that usually carried out in the commercial olive fields. The experiment was organized in Factorial Randomized Complete Block Design (RCBD), with four replications for each treatment, each replicate divided to 9 experimental units. No. of treatments =3X3= 9. No. of the trees in the study=9X4= 36 trees. The obtained data were analyzed statistically using SAS software program (SAS, 2005), and the means were compared Duncan's Multiple Range Test at $P\leq 0.05$(Al-Rawi and Khalafalla, 2000). From each Treatment, the following measurements were taken as study parameters:

1-Total leaf area (cm^2):

From selected fruiting branches 30 leaves were collected in each tree under treatment, used to measure the average leaf area (cm^2). All sampled leaves were scanned using the Digital Leaf Area Meter (LiDE 110, YMJ-C, Top Instrument), according to (Yang *et al.*, 2019).

2. Leaf dry weight (g):

In selected branches 60 leaves were taken, then these leaves were washed with tap water, after that washed again by (0.01 N of HCl), and rinsed by distilled water to remove any spray residues, after that the leaf samples were placed in a pre-heated oven at 70 ^{0}C for 72 hours, in order to determine dry weight (DW), according to (Gobara, 1998).

3. Leaf mineral contents (N, K, Mn and Zn):

Nitrogen percentage was estimated by the use of Microkjeldhal apparatus according to (Regni and Proietti, 2019).

Potassium was determined by the flame photometer apparatus (Horneck and Hanson, 2019). Zinc and Manganese ($mg.kg^{-1}$) on dry weight basis, were determined by the Atomic Absorption Spectrophotometer (AAS) method, according to (A.O.A.C., 1990).

4. Fruit size (cm^3):

The average size of fruit obtained by taken 60 fruits from selected branches in each tree. For determining the size of fruit, the numerical cylinder filled with water was used. The size of fruit is equivalent to the amount of displaced water.

5. Fruit weight (g):

From selected branches in each tree, 60 fruits were taken then weighted, and the average of fruit was calculated. Fruit weight was measured with scales (Kern & Sohn GmbH, D-72336 Ballngen, Germany, accuracy ± 0.001 g).

6. Total yield kg. tree^{-1}:

Careful harvesting was done early in the morning on November 25, 2021. At time, the total yield of each tree weight (kg) was taken using a field balance.

7. Oil content (%):

Oil percentage was determined in the fruit pulp on dry weight basis using soxhlet oil extraction apparatus by five steps, according to (Banat *et al.*, 2013).

8**. Acid value (%):**

It was determined according to Deffenbacker and Pocklingon, (1992)

Results:

3.1. Average leaf area (cm^2)**:**

Table (1) clearly shows that soil application of humic acid (HA) and foliar spray of biostimulant ((2 and 4 ml.L^{-1} Bio)) separately had a significant effect on average leaf area, the highest values (6.84 and 6.76 cm^2) were obtained from treatments of (10 g.L^{-1} HA and 4 ml.L^{-1} Bio), respectively. Whereas, the lowest values (5.13 and 5.37 cm^2) were recorded from control treatments (0 g.L^{-1} HA and 0 ml.L^{-1} Bio), respectively. It was also noticed that the interaction between humic acid and biostimulant significantly affected on average leaf area, the highest value (7.4 4 cm^2) was recorded from the combination between(10 g.L^{-1} HA + 4 ml.L^{-1} Bio), while the lowest value (4.12 cm^2) was obtained from control treatment (0 g.L^{-1} + 0 ml.L^{-1} Bio).

Table 1. The influence of humic acid and biostimulant on average leaf area (cm^2) of olive cv. Arbequina trees.

	Humic acid concentrations			
Biostimulant concentrations	H0 0 g.L^{-1}	H1 5 g.L^{-1}	H2 10 g.L^{-1}	Mean of biostimulant
B0 0 ml.L^{-1}	4.12 g	5.65 e	6.35 c	5.37 c
B1 2 ml.L^{-1}	5.28 f	6.60 bc	6.73 b	6.20 b
B2 4 ml.L^{-1}	5.99 d	6.86 b	7.44 a	6.76 a
Mean of humic acid	5.13 c	6.37 b	6.48 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test (P ≤ 0.05).

3.2**. Leaf dry weight** (g)**:**

Table (2) shows that each soil application of humic acid (HA) and foliar spray of biostimulant (Bio) alone significantly affected on leaf dry weight (g), especially at the level of (10 g.L^{-1} HA)- and (4 ml.L^{-1} Bio), which gave the highest values (0.09 and 0.08 g), respectively. While, the lowest value (0.07 g) was recorded from control treatment for both (0 HA and 0 Bio), respectively. Likewise, the interactions between (10 g.L^{-1} HA + 4 ml.L^{-1} Bio) obtained the highest value (0.10 g.) of leaf dry weight, whereas the lowest value (0.06 g.) was obtained from the interaction between (0 g.L^{-1} HA + 0 ml.L^{-1} Bio).

Table 2. The influence of humic acid and biostimulant on average leaf dry weight (g) of olive cv. Arbequina trees

	Humic acid concentrations			
Biostimulant concentrations	H0 0 $g.L^{-1}$	H1 5 $g.L^{-1}$	H2 10 $g.L^{-1}$	Mean of biostimulant
B0 0 ml.L-1	0.06 d	0.08 bc	0.08 bc	0.07 b
B1 2 ml.L-1	0.07 cd	0.08 bc	0.09 ab	0.08 a
B2 4 ml.L-1	0.07 cd	0.08 bc	0.10 a	0.08 a
Mean of humic acid	0.07 c	0.08 b	0.09 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

3.3. **Percentage of leaf nitrogen (N) content:**

Results presented in table (3) clarify that each of the humic acid as soil application and biostimulant as foliar spray had a significant effect on leaf nitrogen percentage, particularly at the highest concentrations (10 $g.L^{-1}$ HA) and (4$ml.L^{-1}$ Bio), which gave the highest values of leaf nitrogen percentage (2.47% and 2.46 %), respectively. While, the lowest values (2.06% and 2.09%) recorded with control treatment for each (0 $g.L^{-1}$ HA) and (0 $ml.L^{-1}$ Bio), respectively. Also, obtained results shows that the combination between both studied factors at highest rates (10 $g.L^{-1}$ HA + 4$ml.L^{-1}$ Bio) gave the maximum value (2.71%) of leaf nitrogen % compared to the combination between (0 $g.L^{-1}$ HA + 0 $ml.L^{-1}$ Bio) that gave the minimum value (1.88%).

Table 3. The influence of humic acid and biostimulant on percent leaf nitrogen content (N%) of olive cv. Arbequina trees.

	Humic acid concentrations			
Biostimulant concentrations	H0 0 $g.L^{-1}$	H1 5 $g.L^{-1}$	H2 10 $g.L^{-1}$	Mean of biostimulant
B0 0 ml.L-1	1.88 d	2.09 c	2.30 b	2.09 c
B1 2 ml.L-1	2.01 cd	2.36 b	2.39 b	2.25 b
B2 4 ml.L-1	2.28 b	2.38 b	2.71 a	2.46 a
Mean of humic acid	2.06 c	2.28 b	2.47 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

3.4, Leaf potassium content (%)

Table (4) shows that each treatment of soil application of humic acid and foliar spray of biostimulant significantly affected on leaf potassium content in olive leaves, the highest values (1.33 and 1.32%) were obtained by the highest rates of humic acid at (10 $g.L^{-1}$) and biostimulant at (4$ml.L^{-1}$), respectively. Whereas, the lowest values 1.14% and 1.16% were recorded with control treatment (0 g.L^{-1} HA and 0 ml.L^{-1} Bio), respectively. Likewise, the interactions between both studied factors had a significant effect on leaf potassium percentage, the maximum value (1.39%) recorded from the interaction between (10 g.L^{-1} HA + 4ml.L^{-1} Bio) and (10 g.L^{-1} HA + 2ml.L^{-1} Bio), while the minimum value (1.10%) was obtained from the interaction between (10 g.L^{-1} HA + 4ml.L^{-1} Bio)

3.5. Leaf manganese content (ppm)

Table (5) indicates that foliar spray of biostimulant and soil application of humic acid alone had a significant influence on leaf manganese content (ppm), the highest values (179.92 and 148.32 ppm) obtained from the highest rates of both studied factors (4ml.L^{-1} Bio and 10g.L^{-1} HA) respectively, while the lowest values (107.41 and 108.18 ppm) were recorded for control treatments of both studied factors (0 ml.L^{-1} Bio and 0 g.L^{-1} HA), respectively. Also, table (5) shows that the interaction between biostimulate and humic acid had a significant influence on manganese content in leaves, the maximum value (228.19 ppm) was obtained from the combination treatment (4ml.L^{-1} Bio + 10 g.L^{-1} HA), while the minimum value (107.13 ppm) was recorded from (0 ml.L^{-1} Bio + 0 g.L^{-1} HA).

Table 4. The influence of humic acid and biostimulant on percent leaf potassium content (K%) of olive cv. Arbequina trees.

Humic acid concentrations				
Biostimulant concentrations	H0 0 g.L^{-1}	H1 5 g.L^{-1}	H2 10 g.L^{-1}	Mean of biostimulant
B0 0 ml.L-1	1.10 d	1.18 c	1.21 c	1.16 c
B1 2 ml.L-1	1.11 d	1.30 b	1.39 a	1.27 b
B2 4 ml.L-1	1.21 c	1.36 a	1.39 a	1.32 a
Mean of humic acid	1.14 c	1.28 b	1.33 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test (P ≤ 0.05).

Table (5): The influence of humic acid and biostimulate on percentage of leaf Manganese (Mn) content (ppm) of olive cv. Arbequina trees:

Humic acid concentrations				
Biostimulant concentrations	H0 0 g.L^{-1}	H1 5 g.L^{-1}	H2 10 g.L^{-1}	Mean of Biostimulant
B0 0 ml.L-1	107.13 c	107.33 c	107.78 c	107.41 b
B1 2 ml.L-1	108.03 c	108.83 c	108.99 c	108.62 b
B2 4 ml.L-1	109.38 c	202.18 b	228.19 a	179.92 a
Mean of humic acid	108.18 c	139.45 b	148.32 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

3.6. Leaf zinc content (ppm)

The results shown in table (6) denote that each of the foliar spray of biostimulant and soil application of humic acid significantly influenced on zinc content in olive leaves (ppm), the highest values (0.16 and 0.15 ppm) were recorded from the highest concentrations of biostimulant and humic acid (4 ml.L^{-1} and 10 g.L^{-1}) respectively, whereas the lowest values (0.13 ppm and 0.14 ppm) were obtained with control treatment of biostimulant and humic acid (0 ml.L^{-1} and 0 g.L^{-1}), respectively. Likewise, the same table shows that the combination between both studied factors had a significant influence on zinc content in leaves, the maximum value (0.17 ppm) was obtained from the interaction between the highest rates (4 ml.L^{-1} Bio + 10 g.L^{-1} HA), compared to the minimum value (0.13 ppm) that recorded from control treatment (0 ml.L^{-1} Bio + 0 g.L^{-1} HA)

Table 6. The influence of humic acid and biostimulant on leaf zink content (ppm) of olive cv. Arbequina trees.

	Humic acid concentrations			
Biostimulant concentrations	H0 0 g.L^{-1}	H1 5 g.L^{-1}	H2 10 g.L^{-1}	Mean of biostimulant
B0 0 ml.L-1	0.13 e	0.13 e	0.13 e	0.13 c
B1 2 ml.L-1	0.14 d	0.14 d	0.15 c	0.14 b
B2 4 ml.L-1	0.15 c	0.16 b	0.17 a	0.16 a
Mean of humic acid	0.14 b	0.14 b	0.15 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

3.7. Average fruit size (cm^3)

Table (7) illustrates that fertilization of humic acid had a significant effect on the olive fruit size, the maximum value (1.19 cm^3) was recorded at 10 g.L^{-1}, while the minimum value (0.89 cm^3) recorded with control treatment 0 g.L^{-1}. Also, the same table clearly shows that foliar spray of biostimulant significantly affected on fruit size (cm^3), the highest value (1.15 cm^3) recorded from the highest rate of biostimulant (4 ml.L^{-1})، compared to the lowest value (0.92 cm^3) that recorded from control treatment (0 ml.L^{-1}). Concerning the interactions between the humic acid and biostimulant significantly affected on olive fruit size (cm^3), the maximum value (1.36 cm^3) recorded from the combination between the highest rates of both studied factors (10 g.L^{-1} HA + 4 ml.L^{-1} Bio) compared to the minimum value (0.84 cm^3) recorded from (0 g.L^{-1} HA + 0 ml.L^{-1} Bio).

Table 7. The influence of humic acid and biostimulant on fruit size (cm^3) of olive cv. Arbequina trees.

Humic acid concentrations				
Biostimulant concentrations	H0 0 $g.L^{-1}$	H1 5 $g.L^{-1}$	H2 10 $g.L^{-1}$	Mean of biostimulant
B0 0 ml.L-1	0.84 h	0.92 fg	0.99 e	0.92 c
B1 2 ml.L-1	0.88 fg	1.05 d	1.22 b	1.05 b
B2 4 ml.L-1	0.95 ef	1.14 c	1.36 a	1.15 a
Mean of humic acid	0.89 c	1.04 b	1.19 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

3.8. Fruit weight (g)

Table (8) explains that each the soil application of humic acid and foliar spray of biostimulate significantly affected on fruit weight (g), the highest concentrations of humic acid 10 $g.L^{-1}$ and biostimulant 4$ml.L^{-1}$, gave the highest values of fruit weight (1.14 and 1.12 g), respectively. For combinations among both factors (Humic acid + Biostimulant), results showed significant effect between them, the interaction between (10 $g.L^{-1}$ HA + 4 $ml.L^{-1}$ Bio) gave the highest value (1.22 g) compared with the lowest value (0.86 g) that recorded with (0 $g.L^{-1}$ HA + 0 $ml.L^{-1}$ Bio).

Table 8. The influence of humic acid and biostimulant on fruit fresh weight (g) of olive cv. Arbequina trees.

Humic acid concentrations				
Biostimulant concentrations	H0 0 $g.L^{-1}$	H1 5 $g.L^{-1}$	H2 10 $g.L^{-1}$	Mean of biostimulant
B0 0 ml.L-1	0.86 h	0.89 g	1.01 e	0.92 c
B1 2 ml.L-1	0.87 g	1.07 d	1.19 b	1.04 b
B2 4 ml.L-1	0.99 f	1.15 c	1.22 a	1.12 a
Mean of humic acid	0.90 c	1.04 b	1.14 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

3.9. Total yield per tree (kg)

Table (9) explains that each of the soil application of humic acid and foliar spray of biostimulant significantly increased total yield per tree (kg), the highest values (14.18 kg and 12.96 kg) were obtained from (10 $g.L^{-1}$ humic acid and 4 $ml.L^{-1}$ biostimulant), respectively, whereas, the lowest values (9.19 kg and 9.85 kg) were recorded for control treatment (0 HA and Bio), respectively.

The same table also explains that the interactions between both studied factors had a significant influence on total yield per tree (kg), the maximum value (16.22 kg) obtained from the combination treatment (10 $g.L^{-1}$ HA + 4 $ml.L^{-1}$ Bio) compared to the minimum value (8.32 kg) that recorded from (0 $g.L^{-1}$ HA + 0 $ml.L^{-1}$ Bio).

3.10. Oil content (%)

Table (10) clearly indicates that the soil application of humic acid and foliar spray of biostimulant alone and/or in combination had a positive effect and significantly increased fruit oil content (%) when compared to untreated trees, the highest values (31.74% and 32.43%) were obtained from the highest concentrations (10$g.L^{-1}$ HA and 4$ml.L^{-1}$ Bio) respectively, while the lowest values (25.15% and 25.16%) obtained from control treatment for both humic acid (0) and Biostimulant (0), respectively. Likewise, for the combinations between humic acid and biostimulant, results showed significant increased fruit oil content, the maximum value (34.97%) was recorded from the combination among (10 $g.L^{-1}$ HA + 4 $ml.L^{-1}$ Bio), while the minimum value (21.92%) was recorded from (0 $g.L^{-1}$ HA + 0 $ml.L^{-1}$ Bio).

Table 9. The influence of humic acid and biostimulant on average yield ($kg.tree^{-1}$) of olive cv. Arbequina trees.

	Humic acid concentrations			
Biostimulant concentrations	H0 0 $g.L^{-1}$	H1 5 $g.L^{-1}$	H2 10 $g.L^{-1}$	Mean of biostimulant
B0 0 ml.L-1	8.32 e	9.88 d	11.35 c	9.85 c
B1 2 ml.L-1	9.14 de	11.43 c	14.98 b	11.85 b
B2 4 ml.L-1	10.11 d	12.55 c	16.22 a	12.96 a
Mean of humic acid	9.19 c	11.29 b	14.18 a	

Values followed with the same letter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level.

Table 10. The influence of humic acid and biostimulant on oil content (%) of olive cv. Arbequina trees.

	Humic acid concentrations			
Biostimulant concentrations	H0 0 $g.L^{-1}$	H1 5 $g.L^{-1}$	H2 10 $g.L^{-1}$	Mean of biostimulant
B0 0 ml.L-1	21.92 i	25.20 g	28.37 f	25.16 c
B1 2 ml.L-1	24.06 h	30.72 d	31.87 c	28.88 b
B2 4 ml.L-1	29.48 e	32.85 b	34.97 a	32.43 a
Mean of humic acid	25.15 c	29.59 b	31.74 a	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test (P ≤ 0.05).

3.11. Acid value (Acidity%)

Table (11) illustrates that the soil application of humic acid had a positive effect and significantly decreased acid value of olive oil, the lowest value (0.92%) was recorded from 10 g.L^{-1} of humic acid, while the highest value (1.01%) was recorded from control treatment (0 g.L^{-1} HA). Also, table (11) explains that foliar spray of biostimulant significantly decreased acid value%, the lowest value obtained from 4ml.L^{-1} of biostimulnt, whereas the highest value value (0.99%) was obtained by control treatment (0 ml.L^{-1}) without the existence of a significant difference with the biostimulant treatment level of (2 ml.L^{-1}) that gave (0.97%) value. Concerning the interactions between both studied factors (humic acid + biostimulant) they had significantly affected on acid value %, the minimum value (0.85%) was obtained from (10 g.L^{-1} HA + 4 ml.L^{-1} Bio), while the maximum value (1.02%) was obtained from (0 HA + 0 Bio).

Table 11. The influence of humic acid and biostimulant on acid value (Acidity%) of olive cv. Arbequina trees.

Humic acid concentrations				
Biostimulant concentrations	H0 0 g.L^{-1}	H1 5 g.L^{-1}	H2 10 g.L^{-1}	Mean of biostimulant
B0 0 ml.L-1	1.02 a	0.97 bc	0.97 bc	0.99 a
B1 2 ml.L-1	1.01 ab	0.96 c	0.93 c	0.97 a
B2 4 ml.L-1	0.96 c	0.92 c	0.85 d	0.91 b
Mean of humic acid	1.01 a	0.95 b	0.92 c	

* Values followed by the same letter(s) are significantly not different from each other according to Duncan's Multiple Range Test (P ≤ 0.05).

Discussion

The results presented in tables (1-6) show that the soil application of humic acid significantly affected on growth characteristics such as; leaf dry weight (g), leaf area (cm^2), and leaf contents of N, K, Mn and Zn of olive trees "Arbequina" cultivar, especially at the highest level (10 g.L^{-1}). Improving of vegetative growth properties after soil application of humic acid might be due to its indirect influence on soil through increasing soil microbial activity and effectiveness of nutrient uptake by root hairs as a chelating agent, which increases nutritional status in leaves, then increasing leaf area and leaves dry weight. Abdel-Mawgoud *et al.* (2007) reported that humic acid as a soil application increased soil porosity, so it related in ventilation and improve root respiration and its penetration in soil, this can reflect an increase in vegetative growth. Also, Mayi and Saeed (2015) indicated that humic acid includes hormone like substances, which may cause an increase in endogenous levels of cytokinin, auxin and gibberellins, as well as, humic acid can increase root growth in a similar way to a cytokinins, auxins uxins (Nardi *et al.*, 2016), and due to its direct influence on biochemical and physiological processes which comes by the stimulation of enzymes and the transfer of photosynthesis products, as well as a role of division and elongation of cells in plants (Fawzy *et al.,* 2007), leading to increased vegetative growth, as a result, increased mineral content of leaves. The reason behind of increasing dry weight of leaves may be related to increasing mineral contents in the leaves. These results are in agreement with those obtained by Mayi *et al.*, (2014), who noted that vegetative growth parameters (leaf area, leaf fresh and dry weight) of two olive cultivars "Khithairy" and "Sorany", increased with increasing humic acid concentration when compared to control treatment. Also, Ihsan e*t al.* (2019) reported that humic acid significantly increased leaf area and vegetative growth of Volcamer lemon trees.

Tables (7-10)) showed that soil application of humic acid significantly increased fruit characteristics such as; fruit size (cm^3), fruit weight (g), total yield per tree and fruit oil content might be related (%), particularly at the highest rate of humic acid 10 $g.L^{-1}$, except the percentage of acidity (%), which illustrates from table (11), that decreased with increasing the level of humic acid. Increasing yield, fruit weight, fruit size and fruit oil content might be related to the positive effect of humic acid on increasing leaf area, leaf dry weight and leaf nutrient contents (N, K, Mn, and Zn), potassium increases the translocation of products (sugars), which being synthesized in leaves to the fruits through the phloem, and increase the buildup of surplus of them in the form of carbohydrates in fruits and convert a part of them into oil and increases fruit oil content. These results are in an agreement with those obtained by Abo-Gabien *et al.* (2020), who showed that the maximum value of fruit yield and fruit weight of 'Aggizi' olive cultivar were detected with applying the higher concentration of potassium humate 100 $g.tree^{-1}$ compared to lower concentrations and control (untreated) trees. Also, AL-Barwari and AL-A'araji (2020) indicated that humic acid, particularly at the highest rate (75 g HA.Tree-1), had a positive influence on the yield and fruit oil content of olive trees 'Khistawy'cultivar. In addition, (El-Razek *et al.* 2020) showed that humic acid and bio-humic significantly increased the yield (kg/tree) of olive tree "Kalamata" cultivar, also leaf nutrient contents (N, K, Zn and Mn), and increased fruit weight, fruit size and oil percentage in fresh and dry weight.

Likewise, the obtained results from the tables (1-10) showed that biostimulant significantly increased vegetative growth characteristics such as; leaf dry weight (g), leaf area (cm^2), and leaf content of N, K, Mn and Zn and fruit characteristics such as fruit size (cm^3), fruit weight (g\), total yield per tree (kg) and fruit oil content (%) of olive trees "Arbequina" cultivar, particularly at the highest level of biostimulant (4 $ml.L^{-1}$), except acidity (%) as is clarified in table (11) in which acid value was decreased with increasing the level of biostimulant. The present results regarding the effect of foliar spray with biostimulant on vegetative growth properties of olive fruits "Arbequina" cultivar, might be related to the effect of biostimulant application which had a positive effect on internal plant metabolism, due to presence of major and minor elements, and organic substances like seaweed extracts that include plant growth regulators such as cytokines, auxins and gibberellins which improved vegetative growth, leaf area, leaf dry weight and nutritional status of leaves, as a result, increased total yield, as well as improved physical and chemical properties of olive fruits. Also, foliar spray of biostimulant could supply sufficient amounts of growth promoting substances that increase cell division, cell-enlargement, eventually produces a higher yield. These findings are in agreement with those obtained by Ali *et al.* (2019a) who indicated that foliar spray of dry yeast and amino acid (used as a biostimulant) at maximum rates, gave the highest significant value in leaf area. In addition, (Regni *et al.*, 2021) showed that the aqueous extract had a beneficial influence on olive "Arbequina" cultivar, in terms of nutritional status. Moreover, (Al-Khafaji and Al-Ali, 2021) reported that foliar spray of seaweed extract at highest concentration (2.0 $g.L^{-1}$), significantly increased leaves dry matter percentage and nitrogen content in leaves. Furthermore, (Abd-Oun, 2019) indicated that the maximum rate of biostimulant

(1.25 g.L-1), gave the highest value of nitrogen, potassium, manganese and zinc in olive leaves "Khodairi" cultivar. Moreover, Zouari *et al.*, (2020a) reported that foliar spray of biostimulant on olive tree "Chemlali" cultivar had a significant increase on mineral profile of leaves for both micro and macro- nutrients, that affecting the primary and secondary metabolism synthesis in the leaves. Also, Hassan *et al.*, (2019) and Hernández-Hernández *et al.*, (2019) indicated that spraying biostimulants on olive trees, significantly increased vegetative growth and mineral contents of leaves, consequently, increased yield, fruit quality and fruit chemical characteristics including; oil percentage compared to untreated trees. Also, Ali et al. (2019b) noted that biostimulant significantly increased fruit weight, and increased fruit oil% of olive fruit "Baashiki" cultivar. In addition, each D'Amato et al. (2018) and Zouari et al. (2020b), clearly showed that foliar spray of biostimulant significantly increased fruit olive oil percentage and its quality attributes.

Furthermore, the present results showed that the interaction between soil application of humic acid and foliar spray of biostimulant, especially at the highest rates (10 $g.L^{-1}$ HA + 4 $ml.L^{-1}$ Bio) had significant effects on vegetative growth and fruit properties of "Arbequina" olive trees when compared to control. Based on the previously mentioned results, it could be concluded that application of humic acid and biostimulant improved all parameters, due to benefits on different biological processes such as: cell division and expansion, and the formation of new tissues, which has resulted in raising of fruit size and fruit weight as shown in tables (7 and 8), respectively, thus, increasing the total yield that shown in table (9). The present results are in agreement with those obtained by (Zedan, 2017; El-Shazly and Ghieth, 2019; Al-Hadethi, 2019) on olive tree. Moreover, Al-Marsoumi and Al-Hadethi, (2020) on mango, and Abourayya et al. (2020) on almond tree.

4- Conclusions

In accordance with the results of this study, it can be concluded that there is a significant improvement in the majority of parameters under study, especially when the trees had been sprayed with 4 $ml.L^{-1}$ biostimulant and/or supplied with 10 $g.L^{-1}$ humic acid. Generally, interaction between biostimulatnt and humic acid gave the best results in olive tree which significantly increased most parameter as compared with control. Application of biostimulant and humic acid. alone or in combination gave the highest yield per tree and oil content (%).

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