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Role of organic fertilizer, ascorbic and salicylic acids applications on chemical components of leaves of olive Olea europea L. cv.Khestawi

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I. Abstract

The experiment was conducted in the fabric canopy of the Department of Horticulture and Landscape Design, College of Agriculture, University of Basra, for the growing season 2024-2025 to determine the efficiency of organic fertilizer, ascorbic and salicylic acids applications on chemical components of olive leaves . A factorial experiment was designed with two factors: the first was soil application of organic fertilizer (Green Gold), rich in seaweed and amino acids, at three levels (0, 3, 6) ml.l⁻¹, and the second was spraying with ascorbic acid at concentrations (50, 100) mg.l⁻¹ and salicylic acid at concentrations (150, 300) mg.l⁻¹, in addition to the control treatment 0 mg.l⁻¹ in a Randomized Complete Block Design (R.C.B.D.) with three replicates for each treatment. Results of the study showed that the organic fertilizer soil application treatment of at two concentrations (6 ml. l⁻¹ and 3 ml. l⁻¹) was superior to the control treatment (0 ml.l⁻¹) in increasing a leaf content of total total soluble carbohydrates, vitamin C, proline and total phenols. The spraying treatment with 100 mg. l⁻¹ ascorbic acid and 300 mg. l⁻¹ salicylic acid was superior in increasing a leaf content of total total soluble carbohydrates, vitamin C, proline and total phenols, thus improving leaf chemical characteristics.

Key words *: olive , organic fertilizer, plant height, chlorophyll , ascorbic acid*

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II. Introduction

The olive tree is a perennial, dicotyledonous, evergreen fruit tree belonging to the Oleaceae family. It was first identified in 1809 by Hoffmansing, who identified 30 genera and 600 species, including the genus Olea, which comprises more than 30 known olive varieties, the most important of which are:
- The European wild olive (Olea europaea sylvestris), whose seeds always produce wild olive trees.
- The cultivated olive (Olea europaea sativa), whose seeds do not always produce cultivated trees but often wild olive trees. Therefore, we find ourselves resorting to propagating cultivated olive trees through grafting (Jamal and Al-Susu, 2009 ; Therios, 2009).

Excessive use of nitrogenous mineral fertilizers in orchards can negatively impact both the trees and the surrounding environment. It reduces the quality of produce and shortens the fruit's shelf life. Environmentally, exceeding natural levels of these fertilizers leads to groundwater pollution, increased salinization, and desertification, resulting in significant economic losses (Fadala et al., 2023). Therefore, serious consideration has turned to alternatives to mineral fertilization and organic farming, also known as clean agriculture, as a form of sustainable agriculture aimed at avoiding the excessive use of chemical fertilizers (Ati and Shatha, 2006). In this regard, the use of organic fertilizers has increased to reduce environmental pollution. Furthermore, organic fertilizers improve the physical, chemical, biological, and fertility properties of the soil due to their chelated nutrient content. Additionally, their decomposition produces numerous acidic organic acids that lower soil pH. The increased nutrient content also enhances the availability of nutrients in the soil, facilitating their absorption by plants and increasing their concentration. In the leaves, increasing the chlorophyll content of the leaves, increasing carbohydrate concentration, and improving the vegetative growth of trees (Al-Ubaidi, 2008 ; Moran, 2014) .

Seaweed extracts are organic products used as an organic source to improve the growth and production of horticultural plants. More than 15 million tons are used annually in agricultural production worldwide due to their importance in stimulating plant growth at low concentrations, leading to improved physical and chemical properties. They are environmentally friendly and do not leave a harmful effect on plants or soil. Furthermore, they contain macro- and micronutrients, amino acids, organic acids, and growth regulators such as auxins, cytokinins, hormones, vitamins, and complex sugars. They also increase plant resistance to salinity and drought (Morales – Payan and Norrie, 2010) . Additionally, seaweed extracts contribute to increased plant strength and nutrient absorption, thus enhancing disease resistance, which leads to increased plant production and improved quality (Spinelli et al., 2010).

Ascorbic acid, or vitamin C, with the chemical formula ($C_6H_8O_6$), is a sugar acid. It has physiological roles, including protecting the plant from the harmful effects of high and low temperatures, salt stress, freezing stress, and drought stress (Amin et al., 2009). It is considered an antioxidant that promotes vegetative and fruit growth in various fruit trees. The use of ascorbic acid as a foliar spray on plants has increased recently because it has an effect on plant growth similar to that of growth regulators that promote growth. In addition, it reduces stress caused by temperature and toxins, stimulates respiration and cell division, and influences flower sex, increases seed germination rates and vegetative growth, and increases plant tolerance to excess salinity (Afzal et al., 2006). Several researchers have pointed to the role of ascorbic acid in promoting photosynthesis by observing a strong relationship between leaf area, increased vegetative growth, and the ascorbic acid content of seedlings (Ahmed and Morsy, 2001).

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Salicylic acid with the chemical formula ($C_6H_4(OH)CO_2H$), is a phenolic derivative widely distributed in plants. Salicylic acid plays important physiological roles in plant growth and development, ion absorption, and has an effect on stomatal movement and ethylene synthesis. It has an effect opposite to the growth inhibitor abscisic acid (ABA), which is responsible for plant aging. It also accelerates the formation of chlorophyll and carotene pigments, speeds up carbon synthesis, and increases the activity of some important enzymes and flowering (Hayat et al., 2010 ; Khan et al., 2012) .

The current study aims to improve the chemical components of olive leaves cv. Khestawi via application the organic fertilizer , ascorbic and salicylic acid .

III. Materials and methods

Olive plants cv. Khestawi were brought from a commercial nursery on 15/9/ 2024. They were similar in volume and age. The plants were transferred to the greenhouse of the College of Agriculture, University of Basra, and placed in larger pots with a well-drained soil mixture of peat moss and sand at a ratio of 1:3.

The experiment included the organic fertilizer soil application consisting of (seaweed and humic acid) at three concentrations (0, 3, 6) $ml.l^{-1}$ and foliar spraying with ascorbic acid at the two concentrations(50, 100) mg. l^{-1} and salicylic acid at the two concentrations (150, 300) mg. l^{-1} , in addition to the control treatment with distilled water. Drops of soap were added to reduce the surface tension of the water and to ensure complete wetting of the leaves. The soil addition of the organic fertilizer and the foliar spray treatments were carried out five times in November, December, March, April and May.

The experiment was designed as a factorial experiment with two factors: soil application of organic fertilizer at three levels and spraying with ascorbic and salicylic acid at five levels using a Randomized Complete Block Design (R.C.B.D.) with three replicates for each treatment (3×5×3). Thus, the number of experimental units was 45 experimental units, while the total number of plants was 90, at a rate of 2 plants for each experimental unit. The mean values were compared by using Revised Least significant difference (RLSD) at probability level 0.05 (Al-Rawi and Khalaf Allah, 2000).

The studied parameters included total soluble carbohydrates (mg $100g^{-1}$) which were estimated by using the Phenol- Sulfuric acid Colorimetric Method Modification as described by Dubois et al. (1956) , vitamin C (mg 100 g^{-1} fresh weight) determined according to A.O.A.C. (1992) , proline (mmol g^{-1}) which were estimated according to the method of Bates et al. (1973) using ninhydrin, phosphoric acid, and glacial acetic acid with toluene and total phenols (%) which were estimated according to Folin-Denis method mentioned in Dalali and Al-Hakim (1987).

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IV. Results and Discussion

1. **Total Soluble Carbohydrates (mg 100g⁻¹)**

Table (13) shows the effect of soil application of organic fertilizer and spraying with ascorbic and salicylic acids on the concentration of total soluble carbohydrates in the leaves of the Khastawi olive cultivar. The highest concentration of total soluble carbohydrates was observed in the treatment with 6 ml L⁻¹ organic fertilizer, reaching 17.02 mg100g⁻¹, with a significant difference compared to the other treatments. The treatment with 3 ml L⁻¹ organic fertilizer also significantly outperformed the control treatment, which recorded the lowest value of 7.78 mg 100g⁻¹. The table also shows the significant effect of spray treatments on total soluble carbohydrate concentration. The highest total soluble carbohydrate concentration, 16.34 mg 100g, was recorded with salicylic acid spray at a concentration of 300 mg L⁻¹, with no significant differences with salicylic acid spray at a concentration of 150 mg L⁻¹. The lowest total soluble carbohydrate concentration was recorded with ascorbic acid spray at a concentration of 100 mg L⁻¹, yielding 9.54 mg 100g⁻¹, with no significant differences with ascorbic acid treatment at a concentration of 50 mg L⁻¹ and the control treatment .

The interaction between organic fertilizer and spray treatments was significant, with the highest concentration of total soluble carbohydrates in the treatment of soil application of organic fertilizer at a concentration of 6 ml L⁻¹ and spraying with salicylic acid at a concentration of 300 mg L⁻¹, which reached 24.95 mg 100 g⁻¹, while the lowest concentration of total soluble carbohydrates was in the treatment of organic fertilizer at a concentration of 0 ml L⁻¹ and spraying with salicylic acid at a concentration of 0 mg L⁻¹, which reached 5.79 mg 100 g⁻¹.

Table 1 The effect of adding organic fertilizer and spraying treatments and the interaction between them on the leaf content of total soluble carbohydrates (mg 100g⁻¹)

Organic fertilizer (ml.l⁻¹)	Spray treatments (mg.l⁻¹)					Average effect of organic fertilizer
	0	Ascorbic 50	Ascorbic 100	Salicylic 150	Salicylic 300	
0	5.79	6.24	6.52	10.58	9.77	7.78
3	15.10	12.17	9.66	11.94	14.29	12.63
6	13.59	13.55	12.43	20.57	24.95	17.02
Average effect of spray treatments	11.50	10.65	9.54	14.36	16.34	

RLSD 0.05		
Organic fertilizer	Spray treatments	Organic fertilizer x Spray treatments
3.119	4.026	6.973

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2. **Vitamin C (mg 100 g^{-1} fresh weight)**

Table (14) shows the effect of soil application of organic fertilizer and spraying with ascorbic and salicylic acids on the concentration of vitamin C in the leaves. The highest concentration of vitamin C was observed in the treatment with 6 ml L^{-1} organic fertilizer, reaching 31.99 mg100 g^{-1} fresh weight, with a significant difference compared to the other treatments. The treatment with 3 ml L^{-1} organic fertilizer also significantly outperformed the control treatment, which recorded the lowest average concentration of 17.20 mg 100 g^{-1} fresh weight. The table also shows the significant effect of the spray treatments on the concentration of vitamin C. The treatment of spraying with salicylic acid at a concentration of 300 mg L^{-1} recorded the highest concentration of vitamin C, which reached 27.26 mg100g^{-1} fresh weight, with a significant differences from the rest of the treatments, followed by the treatment of spraying with salicylic acid at a concentration of 150 mg L^{-1}, which recorded 26.35 mg 100 g^{-1} fresh weight. The lowest concentration of vitamin C was observed in the control treatment, which yielded 20.86 mg100 g^{-1} (fresh weight). The ascorbic acid treatment spray at concentrations of 100 mg L^{-1} and 50 mg L^{-1} significantly outperformed the control, recordering 24.56 mg100 g^{-1} (fresh weight) and 22.95 mg mg100 g^{-1} (fresh weight), respectively.

The interaction between organic fertilizer and spray treatments was significant, with the highest concentration of vitamin C in the treatment of soil application of organic fertilizer at a concentration of 6 ml L^{-1} and spraying with salicylic acid at a concentration of 300 mg L^{-1}, reaching 35.89 mg 100 g^{-1} fresh weight, while the lowest concentration of vitamin C was in the treatment of organic fertilizer at a concentration of 0 ml L^{-1} and spraying with salicylic acid at a concentration of 0 mg L^{-1}, reaching 13.84 mg 100 g^{-1} fresh weight.

Table 2The effect of adding organic fertilizer and spraying treatments and the interaction between them on the leaf content of vitamin C (mg 100 g^{-1} fresh weight)

Organic fertilizer (ml.l^{-1})	Spray treatments (mg.l^{-1})					Average effect of organic fertilizer
	0	Ascorbic 50	Ascorbic 100	Salicylic 150	Salicylic 300	
0	13.84	15.70	17.57	19.35	19.53	17.20
3	20.82	22.93	24.25	25.63	26.37	24.00
6	27.92	30.21	31.87	34.09	35.89	31.99
Average effect of spray treatments	20.86	22.95	24.56	26.35	27.26	

RLSD 0.05		
Organic fertilizer	Spray treatments	Organic fertilizer x Spray treatments
0.531	0.685	1.187

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3 . **Proline (mmol g⁻¹)**

Table 15 shows the effect of soil application of organic fertilizer and spraying with ascorbic and salicylic acids on the proline content of Khastawi olive leaves. The highest proline concentration was in 6 ml L⁻¹ organic fertilizer treatment, reaching 9.326 mmol g⁻¹, significantly higher than the other treatments. The treatment with 3 ml L⁻¹ organic fertilizer also significantly outperformed the control treatment, which recorded the lowest average concentration of 5.467 mmol g⁻¹. The table also shows the significant effect of spray treatments on proline concentration. The treatment with salicylic acid at a concentration of 300 mg L⁻¹ recorded the highest value at 8.259 mmol g⁻¹, significantly higher than the other treatments, followed by the treatment with salicylic acid at a concentration of 150 mg L⁻¹, which recorded 7.991 mmol g⁻¹. The lowest proline concentration was in the control treatment, which yielded 6.972 mmol g⁻¹. The treatment with ascorbic acid at concentrations of 100 mg L⁻¹ and 50 mg L⁻¹ also showed a significant increases over the control treatment, recording 7.571 mmol g⁻¹ and 7.432 mmol g⁻¹, respectively.

The interaction between organic fertilizer and spray treatments was significant, with the highest concentration of vitamin C in the treatment of soil application of organic fertilizer at a concentration of 6 ml L⁻¹ and spraying with salicylic acid at a concentration of 300 mg L⁻¹, which reached 9.647 mmol g⁻¹ , while the lowest concentration of vitamin C was in the treatment of organic fertilizer at a concentration of 0 ml L⁻¹ and spraying with salicylic acid at a concentration of 0 mg L⁻¹, which reached 4.550 mmol g⁻¹ .

Table 3 The effect of adding organic fertilizer and spraying treatments and the interaction between them on the leaf content of proline (mmol g⁻¹)

Organic fertilizer (ml.l⁻¹)	Spray treatments (mg.l⁻¹)					Average effect of organic fertilizer
	0	Ascorbic 50	Ascorbic 100	Salicylic 150	Salicylic 300	
0	**4.550**	**5.107**	**5.223**	**5.883**	**6.570**	**5.467**
3	**7.427**	**7.920**	**8.267**	**8.450**	**8.560**	**8.143**
6	**8.940**	**9.270**	**9.223**	**9.550**	**9.647**	**9.326**
Average effect of spray treatments	**6.972**	**7.432**	**7.571**	**7.991**	**8.259**	
RLSD 0.05						
Organic fertilizer	**Spray treatments**	Organic fertilizer x **Spray treatments**				
0.1387	**0.1791**	**0.3102**				

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4 . **Total Phenols (%)**

Table (16) shows the effect of soil application of organic fertilizer and spraying with ascorbic and salicylic acids on the total phenols of olive leaves. The treatment with 6 ml L⁻¹ organic fertilizer was superior to other treatments by recording the highest concentration of total phenols reaching 136.0%, with no differences with 3 ml L⁻¹ organic fertilizer, which recorded 135.2%, and significantly higher than the control treatment, which recorded the lowest average at 93.7%. The table also shows the significant effect of the spray treatments on the concentration of total phenols. The treatment with 300 mg L⁻¹ salicylic acid recorded the highest value at 134.8%, with no significant differences to the treatment with 150 mg L⁻¹ salicylic acid, the treatment with 100 mg L⁻¹ ascorbic acid, and the treatment with 50 mg L⁻¹ ascorbic acid, and a significant difference from the control treatment, which gave 110.4%.

The interaction between the organic fertilizer and the spray treatments was also significant. The highest concentration of total phenols was observed in the treatment of 6 ml L⁻¹ soil application and 300 mg L⁻¹ salicylic acid spray, reaching 155.4%. Conversely, the lowest concentration was in the treatment with organic fertilizer at a concentration of 0 ml L⁻¹ and salicylic acid spray treatment at a concentration of 0 mg L⁻¹, reached 84.4%.

Table 4 the effect of adding organic fertilizer and spraying treatments and the interaction between them on the leaf content of total Phenols (%)

Organic fertilizer (ml.l⁻¹)	Spray treatments (mg.l⁻¹)					Average effect of organic fertilizer
	0	Ascorbic 50	Ascorbic 100	Salicylic 150	Salicylic 300	
0	84.4	86.5	89.2	95.2	113.2	93.7
3	116.4	138.5	140.4	144.7	135.8	135.2
6	130.5	142.9	145.4	145.9	155.4	136.0
Average effect of spray treatments	110.4	122.6	125.2	128.6	134.8	

RLSD 0.05		
Organic fertilizer	Spray treatments	Organic fertilizer x Spray treatments
11.89	15.35	26.59

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It is evident that the factors studied and their interactions significantly affected the studied parameters and contributed to increasing the chemical components of the leaves of the Khastawi olive cultivar. The positive effect of organic fertilizer treatment on these components is attributed to the increased release of nitrogen into the soil, which leads to its accumulation within the plant and consequently an increase in leaf chlorophyll content. Nitrogen is a component of chlorophyll, specifically in the porphyrin ring, a fundamental element in chlorophyll synthesis. A significant amount of nitrogen in the leaf is used in the formation of this pigment, as chloroplasts contain the majority of the nitrogen in plant leaves. Furthermore, the increased leaf chlorophyll content resulting from organic fertilizer treatment may be due to its role in providing substantial amounts of nitrogen for chlorophyll formation. This nitrogen, in turn, plays a role in the formation of amino acids that are essential for chloroplast synthesis, thus increasing leaf chlorophyll content. Additionally, organic fertilizers enhance carbon fixation, thereby increasing the plant's ability to produce the carbon structures required for the biosynthesis of the chlorophyll molecule. Organic matter also increases the availability of nitrogen and magnesium, which have a significant impact through their presence in the chlorophyll molecule (Rees et al., 2002).

Bojovic and Markovic (2009) showed that chlorophyll is directly linked to the nitrogen content of leaves, and nitrogen concentration in leaves can be determined by their chlorophyll concentration. Phosphorus improves root growth and thus increases their absorption efficiency. Potassium contributes to carbohydrate synthesis. Iron, manganese, and magnesium help in the synthesis of the chlorophyll molecule. Boron maintains chlorophyll pigmentation by increasing the activity and effectiveness of certain growth hormones, especially cytokinins, which increases the greenness of plants (Mengel and Kirkby, 2001), hence increases photosynthesis in the leaves and, consequently, the synthesis and transport of nutrients to different parts of the plant.

Salicylic acid increases ion absorption and regulates water relations during stomatal opening and closing. It also accelerates the formation of chlorophyll and carotene pigments and contributes to photosynthesis. Furthermore, it regulates enzyme and cell membrane functions, fixes carbon dioxide, and regulates the level of secondary metabolites by accumulating phenolic compounds at a level consistent with the plant's physiological and nutritional status (Mateo et al., 2006) .

The role of salicylic acid in increasing vitamin C content may be attributed to its antioxidant properties and its ability to enhance the activity of the enzyme ascorbate oxidase, which contributes to vitamin C synthesis (Gil et al., 1999 ; Buescher et al., 1999) . Furthermore, salicylic acid promotes vegetative growth and increases the absorption of minerals involved in vitamin synthesis, including vitamin C. This aligns with the findings of Masoud and El- Sahrawy (2012), who observed an increase in vitamin C content in orange fruits when trees were sprayed with salicylic acid in conjunction with a group of vitamins. The results also in the same line with those of Kazemi (2013) , who observed an increase in vitamin C content in strawberry plants from 20.12 mg100 g^{-1} fresh weight to 30.12 mg100 g^{-1} fresh weight, along with a doubling of total phenols compared to the control treatment.

Ascorbic acid regulates nutrient absorption, activates photosynthesis, protects cells, and increases carbohydrates. These functions contribute to increased lateral stem growth. Ascorbic acid also improves hormonal balance within the plant, protects against environmental stresses, and promotes carbohydrate formation. Ascorbic acid has many physiological roles in plants, including stimulating the formation of nucleic acids and proteins, acting as a strong electron donor (Swaraj and Garg, 1970) , and acting as a coenzyme in the enzymatic reactions of carbohydrate and protein metabolism. It is involved in respiration and photosynthesis (Robinson, 1973) , and acts as a cofactor in the synthesis of ethylene,

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gibberellins, and anthocyanins. It also controls cell growth, as it plays a role in cell elongation and division (Smirnoff and Wheeler, 2000) .

Foliar spraying with ascorbic acid is an effective and sustainable method for improving plant growth and productivity. Numerous studies have shown that ascorbic acid is not just an antioxidant, but plays a pivotal role in enhancing plant physiological processes, including photosynthesis, nutrient absorption, and regulating the plant's response to various environmental stresses (Smirnoff, 2018) .

V. Conclusion

The results of the current study indicate a significant effect of adding organic fertilizer to the soil, especially at a concentration of 6 $ml.l^{-1}$, on total soluble carbohydrates, vitamin C, proline and total phenols, thus improving chemical components of the olive plant cv. Khestawi. Spraying with ascorbic acid at a concentration of 100 $mg.l^{-1}$ and salicylic acid at a concentration of 300 $mg.l^{-1}$ also improved the above characteristics. Therefore, to improve the chemical indicators of olive leaves cv. Khestawi, could be recommended adding organic fertilizer to the soil, especially at a concentration of 6 ml L^{-1}, and spraying with salicylic acid, especially at a concentration of 300 mg L^{-1}, and ascorbic acid, especially at a concentration of 100 mg L^{-1}.

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