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Response of Young Olive (*Olea europaea* L.) Trees cv. "K18" to Foliar Application with Promalin and Salicylic Acid Under Field Conditions

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Abstract. This study was conducted in the olive orchard of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Iraq for the period from April 2019 to December 2019 to investigate the influence of foliar application with Promalin and Salicylic acid in some vegetative growth traits and chemical content of young olive (*Olea europaea* L.) trees cv."K18", Where 72 young trees were selected at the age of five years, homogeneous in their growth as much as possible. The experiment included a foliar spraying with growth regulator of promalin at three concentrations: (0, 25 and 50) mL⁻¹ which is symbolized by (P0, P1 and P2) respectively and foliar spraying with salicylic acid at four levels: (0, 50, 100 and 200) mgL⁻¹ which is symbolized by (S0, S1, S2 and S3) respectively. A factorial experiment was conducted with Randomized Complete Block Design (RCBD) with three replicates for each treatment. The two factors of the study showed a significant effect in all of the studied growth traits especially the foliar application treatment of promalin with P2 concentration and the spraying with salicylic acid at a concentration of S3 where achieved a significant superiority in average number and length of new branches, average increase in the number of leaves, leaf area, the percentage of dry matter in leaves, percentage of carbohydrates in branches, leaves content of nitrogen as well as total chlorophyll compared to the control treatment which achieved the lowest values.

Keywords: Olive, Vegetative growth, Foliar application, Promalin, Salicylic Acid.

1. Introduction

Olive tree (*Olea europaea* L.) is one of the oldest agricultural crops of remarkable economic and cultural importance in the Mediterranean Basin that known in the history of the world about 8000 years ago. It also represents a widely distributed fruit tree in the world [1, 2]. The olive is an evergreen tree, belongs to the family of Oleaceae which includes many cultivars which are used for pickling and oil extraction [3]. In addition, olive is well adapted to a wide range of growing conditions and soils because of its special leaf structure and ramified root system [4].

K18 is one of the Palestinian dual-purpose olive cultivars that were recently introduced to Iraq. Its trees are medium to strong growth with a standing and vertical growth nature, which characterizes the shape of this cultivar as it is suitable for intensive cultivation and mechanical harvest. Furthermore, it is one of the cultivars with high productivity and constant, the proportion of oil in its fruits range between 20-22 % [5].

Despite the availability of all appropriate environmental conditions for large areas of olive cultivation in Iraq, it has not spread widely for several reasons, including the negligence of the existing orchards and the low productivity of the tree compared to neighboring countries. In addition to secure seedlings suitable for cultivation as well as the slow growth in the nursery after transportation to the sustainable place comes on top of the obstacles facing the expansion of olive cultivation. This calls for the use of means to improve and accelerate growth, including foliar application with plant growth regulators such as Promalin, which is a mixture of (GA₄₊₇ + BA) which was used in the early 1980s [6] for many types of fruit trees and ornamental plants where it stimulates cell division and elongation, growth of lateral buds, increases the number and growth of branches as well as the other multiple physiological effects of both



growth regulators [7][8]. Many researchers have indicated the importance of foliar spraying with Promalin in improving the vegetative growth of many saplings and fruit trees species such as [9] on apple trees, [10] on two cultivars of apple saplings, [11] on orange trees, [12] on pear trees, [13] and on two cultivars of apple saplings.

In addition, salicylic acid (SA) or ortho-hydroxy benzoic acid is a phenolic compound was recognized as an endogenous growth regulator in plants, which are safe to human and environment. Salicylic acid plays an important role in the regulation of many physiological processes in plants such as enhancing photosynthesis, uptake and transport of nutrients, cell division, biosynthesis of plant pigments, stomatal regulation, inhibition of ethylene biosynthesis, prevents the accumulation of reactive oxygen species (ROS) that resulted in protecting plant cells from death. It also plays an important role in enhancing plant resistance against different biotic and a biotic stress [14-18]. Furthermore, previous studies supported the beneficial effects of spraying salicylic acid in improving the growth of many fruit trees species, including [19-23] spraying olive trees.

1.1. Objectives

This study aims to highlight the effect of foliar application with promalin and salicylic acid on the vegetative growth and chemical content of young olive trees cv. "K18".

2. Materials and methods

This study was conducted in the olive orchard of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Iraq for the period from April 2019 to December 2019. The study aims to investigate the effect of spraying with promalin and salicylic acid in vegetative growth and chemical content of young olive (*Olea europaea* L.) trees cv. "K18", where 72 young trees were selected at the age of 5 years, homogeneous in their growth vigor as much as possible planted at 5 x 5 m apart under drip irrigation. All service operations were conducted in an identically for all experimental units, including fertilization, weeding, diseases and insects control as needed. Four main shoots were chosen and were distributed on the perimeter of each tree, then each shoot was marked for the purpose of making measurements of the traits that were studied. Soil samples were taken to conduct some chemical and physical analyzes prior to implementing the experiment (Table 1).

Table 1. Physical and chemical properties of the experimental soil

Particle size distribution (g kg ⁻¹ soil)						Available nutrients (mg kg ⁻¹ soil)			
Sand	Loam	Clay	Texture	pH	EC (1:1) ds.m ⁻¹	N	P	K	
703.6	193.3	103.1	Sandy loam	7.32	4.52	67.8	13.9	145.2	

The experiment included two factors: the first factor included foliar application with Promalin at three concentrations (0, 25 and 50) mL⁻¹, which is symbolized by (P0, P1 and P2) respectively. The growth regulator of Promalin produced by Valent Bio Sciences Corporation / USA contains Gibberellins A₄ & A₇ (1.8%) and (BA) 6-benzyl amino purine (1.8%). As for the recommended concentrations according to the manufacturer, they are in the range of 12.5-50 mL⁻¹. While the second factor included spraying with salicylic acid (SA) at four concentrations (0, 50, 100 and 200) mgL⁻¹ which are symbolized by (S0, S1, S2 and S3) respectively. A salicylic acid (SA) was produced by HI-SKY Chemicals Co. Ltd. / China, the active ingredient (I.A.%) 99.8%. The foliar application with Promalin was conducted according to the following dates (5/4, 5/5, 5/6 and 5/9). As for spraying with salicylic acid, it was done a day after spraying with Promalin and for each appointment by using a backpack sprayer, with the capacity of 16 L until full coverage with the addition of a wetting agent (Triton B) to spraying solution at a concentration of 0.1%.

2.1. Experimental design

A factorial experiment (3×4) was conducted within the Randomized Complete Block Design (RCBD), with three replicates and each replicate was represented by two young trees per treatment. The data were analyzed according to the Genestat statistical program, and the arithmetic averages were compared using the least significant difference at a probability level of 0.05 [24].

2.2. Studied traits

2.2.1 Vegetative growth traits.

- The Number and length of the new branches: The number of new branches (branch. main shoot⁻¹) was calculated and the lengths of new branches (cm) were measured on the main marked shoots of the young olive tree at the end of the experiment in December of 2019 and the average was extracted for each replicate and for each treatment.
- The average increase in the number of leaves (leaf. main shoot⁻¹): The number of leaves for the main shoots marked at the beginning of the implementation of the experiment in April of 2019 (before conducting the treatments) was calculated. Then, it was calculated at the end of the experiment. The average increase was calculated by the difference between the two readings.
- Leaf area (cm²): 10 fully expanded leaves were taken from the fourth leaf to the sixth from the tip of the new branches (40 leaves/ experiment unit) in December of 2019 the leaf area was calculated by using the following equation:

$$\text{Leaf area (cm}^2\text{)} = 0.53 (\text{length} \times \text{width}) + 1.66 \text{ [25].}$$

- The percentage of dry matter in the leaves: The 40 leaves were taken from the fourth leaf to the sixth leaf from the tip of new branches in December of 2019 and washed with distilled water several times to remove the stuck from the soil and left to dry air then, weighed using a sensitive electrical balance and dried in an electric oven containing a vacuum (Oven) at a temperature of 70°C until the weight stabilized and the percentage of dry matter was calculated according to the following equation:

$$\text{The dry matter percentage in leaves} = \text{dry sample weight (g)} / \text{fresh sample weight (g)} \times 100.$$

2.2.2 Chemical traits

- The percentage of total carbohydrates in the branches:* Was estimated at the end of the experiment and according to what he mentioned by [26].
- The leaves content of nitrogen (%):* Mature leaf samples were taken from the middle of the branches at the end of the experiment, the nitrogen content in leaves was estimated using the Microkjeldahl device according to the method mentioned by [27].
- The leaves content of total chlorophyll (mg.g⁻¹ fresh weight):* The fully expanded leaves were taken from the fourth leaf to sixth leaf from the tips of the branches in December of 2019. Chlorophyll A and B were extracted by taking 0.5g of the fresh weight of the leaves in tablet form and placed in dark-colored tubes and 20 ml of acetone (98%) was added to it and left in the dark for 72 hours. This process was repeated until complete extraction of chlorophyll, where the final volume of the extraction solution was 50 ml, light absorption of the solution was determined at wavelengths (663 and 645 nm) using a spectrophotometer. The leaves content of the total chlorophyll was calculated according to the method mentioned by [28].

3. Results and discussion

3.1. The number of new branches (branch. main shoot⁻¹) and their length (cm)

Table (2) shows the significant effect of foliar spraying with Promalin in increasing the number and length of new branches, especially the P2 concentration, which achieved the highest average of 23.28 branch, main shoot⁻¹ and 16.33 cm. While the lowest average was at P0 concentration, which amounted to 11.56 branch, main shoot⁻¹ and 13.27 cm for the number and length of new branches respectively. The spraying with salicylic acid also led to a significant increase in these two traits, where the S3 spraying

treatment achieved the highest average of 22.00 branch, main shoot¹ and 19.41 cm, which a significantly excelled on the rest of the treatments. While the lowest average was at S0 treatment, which reached 12.17 branch, main shoot¹ and 11.46 cm sequentially. The results shown in the same table reflect the significant effect of the interaction of spraying between both growth regulators in increasing the number of new branches, where the treatment P2 × S3 achieved the highest value of 27.75 branch, main shoot¹ and without significant difference from the treatment P2 × S2, P2 × S1 and P1 × S3. While the lowest number of new branches, which amounted to 6.75 branch, main shoot¹ at the treatment P0 × S0. Thus, the interaction between the two study factors had no significant effect in increasing the length of new branches.

Table 2. Effect of foliar spraying with Promalin and salicylic acid and their interaction in the number and length of new branches

Promlin (P) ml L ⁻¹	Number of new branches (branch . main shoot ¹)					Length of new branches (cm)				
	S0	S1	S2	S3	Means (P)	S0	S1	S2	S3	Means (P)
	Salicylic acid mg L ⁻¹					Salicylic acid mg L ⁻¹				
P0	6.75	10.25	12.50	16.75	11.56	9.32	12.45	14.87	16.43	13.27
P1	11.50	15.75	16.25	21.50	16.25	12.27	14.93	16.56	20.64	16.10
P2	18.25	23.50	24.00	27.75	23.38	12.78	15.13	16.26	21.15	16.33
Means (S)	12.17	16.50	17.58	22.00	Means (S)	11.46	14.17	15.90	19.41	
L.S.D.	P	S	P × S			P	S	P × S		
0.05	3.43	3.96	6.87			0.83	0.96	N.S.		

The reason for the excel of treatment with Promalin in increasing the number and length of new branches may be due to the regulators (GA_{4 + 7} and benzyl adenine) that play an important role in a wide range of aspects of regulating plant growth and development as gibberellins work to stimulate cell division indirectly as well as their role in stimulating cell elongation and expansion [7] As for the benzyl adenine (BA), it may be due to its role in breaking the apical dominance induced by auxin and stimulating the growth of lateral buds as well as increasing cell division in the apical meristems and adding new cells to the plant [29, 30, 31]. The reason may also be due to the role of both two growth regulators in stimulating the activity of the enzymes responsible for biosynthesis nucleic acids, especially RNA and proteins that contribute to the bio processes activity in plant such as photosynthesis and carbohydrates metabolism [32], which is positively reflected in the increase in the number and length of branches. These results are in agreement with Kiang [35] who found that the foliar spraying with Promalin at a concentration of 25 mlL⁻¹ on trifoliolate orange saplings significantly increased the number and length of branches. It also agrees with the results of Radivojevic et al. [13] who indicated that there was a significant increase in the number and length of branches for two cultivars of apple saplings when spraying with Promalin at a concentration of 2000 mgL⁻¹. As for the increase in the number of branches and their lengths as a result of foliar spraying with salicylic acid, the reason may be due to its role in stimulating cell division as well as its role in providing the energy needed for plant growth and development by stimulating some of the biological pathways responsible for biosynthesis amino acids, nucleic acids and proteins as well as its role in accelerating the formation of the chlorophyll and increasing the efficiency of the photosynthesis [14][15][18]. This leads to the activity of vegetative growth, including the number and length of branches. These results are consistent with the results of Abd-El-Rhman and Attia [19] who showed that spraying with salicylic acid for olive trees at a concentration of 1000 mgL⁻¹ had a significant effect in increasing the number and length of branches. It agreed with Hussein and Maha [21], where their results showed a significant increase in the length of the main shoot when olive trees were sprayed with salicylic acid at a concentration of 200 mgL⁻¹. Hagagg et al. [23] also

found that the number of branches increased significantly in olive saplings as a result of spraying with salicylic acid at a concentration of 400 mgL⁻¹.

3.2. The average increase in the number of leaves (leaf. main shoot⁻¹) and leaf area (cm²)

It is noted from the results in Table (3) that the number of leaves and leaf area have increased significantly by increasing the levels of Promalin spraying, as the P2 concentration achieved the highest values of 263.06 leaf, main shoot⁻¹ and 3.62 cm². While the lowest values were 132.44 leaf, main shoot⁻¹ and 3.15 cm² at P0 concentration. The spraying with salicylic acid also showed an increase in these two traits, especially treatment S2, which achieved the highest values reaching 241.92 leaf, main shoot⁻¹ and 3.63 cm². with a significant excel on the rest of the treatments. However, the treatment S0 recorded the lowest values for these two traits, which amounted to 140.25 leaf. main shoot⁻¹ and 3.18 cm². The interaction between the two growth regulators had a significant effect in increasing the number of leaves and leaf area, where the interaction treatment P2 × S3 achieved the highest value which amounted 312.25 leaf, main shoot⁻¹ and 3.87 cm² while the treatment P0 × S0 recorded the lowest value, which amounted to 71.25 leaf. main shoot⁻¹ and 2.94 cm² respectively for both traits.

Table 3. Effect of foliar spraying with Promalin and salicylic acid and their interaction in the average increase in number of leaves and leaf area

Promlin (P) ml L ⁻¹	Average increase in leaves number (leaf. main shoot ⁻¹)					Leaf area (cm ²)				
	S0	S1	S2	S3	Means (P)	S0	S1	S2	S3	Means (P)
	Salicylic acid mg L ⁻¹					Salicylic acid mg L ⁻¹				
P0	71.52	125.75	143.00	189.75	132.44	2.94	3.09	3.17	3.41	3.15
P1	132.00	182.50	195.00	223.75	183.31	3.15	3.31	3.34	3.62	3.35
P2	217.50	255.00	267.50	312.25	263.06	3.47	3.60	3.55	3.87	3.62
Means (S)	140.25	187.75	201.83	241.92	Means (S)	3.18	3.33	3.35	3.63	
L.S.D.	P	S	P × S			P	S	P × S		
0.05	9.53	11.00	19.06			0.17	0.20	0.35		

The reason for the increase in the number of leaves as well as the leaf area resulting from the treatment with Promalin may be due to the role of gibberellins in increasing the accumulation of mineral elements in the places where they are concentrated [34] as well as their role in increasing the size and expansion of cells [7]. As for benzyl adenine. It is represented through its role in promoting the growth of leaves primordia through cell division and differentiation [35], BA also influence the allocation of nutrients and assimilates in the plant towards treated tissues with it [31, 36] and its use in building the vegetative system, including increasing the number of leaves and leaf area. It may also be attributed to the fact that foliar spraying with Promalin has improved the vegetative growth traits, including the number and length of branches as shown in Table (2) which leading to an increase in the number of leaves and leaf area. These results are consistent with what was found by Youn et al. [9] where the leaf area of apple trees increased significantly when spraying with Promalin at a concentration of 18 mgL⁻¹. The results were also consistent with Kundu et al. [12], where their results showed a significant increase in the number of leaves as well as the leaf area of pear trees when spraying with Promalin at a concentration of 250 mgL⁻¹. The reason for the increase in the number of leaves and leaf area as a result of foliar spraying with salicylic acid may be due to its positive effect and its physiological role in improving vegetative growth indicators, including the number and length of branches as well as improving the nutritional status and accumulation of carbohydrates and its relationship to the increase in the number and area of leaves. These results are consistent with Abd-Alhamid et al. [22] who found when olive trees were sprayed with salicylic acid at a concentration of 300 mgL⁻¹ which significantly increased the number of leaves as well as leaf area.

3.3. The percentage of dry matter in the leaves and the branches contents of total carbohydrates (%)

Table (4) shows significant differences as a result of the treatment with Promalin, where the percentage of dry matter in the leaves and the carbohydrate content of the branches increased with the increase in spraying levels, as treatment P2 achieved the highest rates of 54.68% and 9.06%. While treatment P0 recorded the lowest averages, which amounted to 53.25% and 8.66%, respectively, for both traits. In addition, spraying salicylic acid also led to an increase in these two traits, especially the concentration of S3, which was significantly excelled to the rest of the treatments, as it achieved the highest averages of 54.54% and 9.04%. While the lowest averages of 53.39% and 8.66% when treatment S0, the effect of the interaction between the two factors was significant in the percentage of dry matter in the leaves and the carbohydrate content of the branches, where the treatment P2 × S3 achieved the highest percentages which achieved 55.30% and 9.27%. while the treatment P0 × S0 recorded the lowest values, which amounted to 52.16% and 8.53% respectively for both traits.

Table 4. Effect of foliar spraying with Promalin and salicylic acid and their interaction in the percentage of dry matter in leaves and the branches' content of total carbohydrates

Promlin (P) ml L ⁻¹	Percentage of dry matter in leaves (%)					Branches contents of total carbohydrates (%)				
	S0	S1	S2	S3	Means (P)	S0	S1	S2	S3	Means (P)
	Salicylic acid mg L ⁻¹					Salicylic acid mg L ⁻¹				
P0	52.61	53.13	53.37	53.92	53.25	8.53	8.60	8.68	8.84	8.66
P1	53.32	53.74	53.85	54.41	53.83	8.61	8.73	8.81	9.03	8.79
P2	54.24	54.59	54.62	55.30	54.68	8.84	9.05	9.11	9.27	9.06
Means (S)	53.39	53.82	53.94	54.54	Means (S)	8.66	8.79	8.86	9.04	
L.S.D.	P	S	P × S			P	S	P × S		
0.05	0.42	0.49	0.85			0.12	0.14	0.25		

The reason for the increase in the percentage of dry matter in the leaves as well as the branches content of carbohydrates may be due to the vigor and activity of the vegetative growth of the olive trees resulting from the foliar spraying with Promalin and salicylic acid represented by the increase in the number and area of the leaves (Table 3). In addition to its chlorophyll content, and its reflection in increasing the efficiency of photosynthesis process and increase of manufactured carbohydrates [37], Hamman et al. [38] indicated that high growth efficiency has a significant correlation with the level of stored carbohydrates. These results agree with Kiang [33] finding that spraying trifoliolate orange saplings with Promalin significantly increased the dry weight of the branches. It also agreed with the results of Youn et al. [9] where the dry weight of apple trees leaf increased significantly when sprayed with the Promalin, and it also agreed with the findings of Jumaa and Zain aldeen [39] when spraying *Ziziphus spina* saplings with salicylic acid at a concentration of 200 mg L⁻¹ which it led to a significant increase in the percentage of dry matter in the leaves as well as the branches content of the carbohydrates.

3.4. The percentage of nitrogen in leaves and their content of total chlorophyll (mg gm⁻¹ fresh weight)

Table (4) indicates significant differences in the percentage of nitrogen in leaves as well as their content of chlorophyll as a result of spraying with Promalin, where the concentration of P2 was significantly excelled on the rest of the concentrations by giving it the highest values of 1.67% and 1.37 mg gm⁻¹ fresh weight. While the concentration P0 showed the lowest values, which were 1.52% and 1.12 mg gm⁻¹ fresh weight, the foliar spraying with salicylic acid led to a significant increase in these two traits, especially the S3 treatment which achieved the highest values 1.71% and 1.36 mg gm⁻¹ fresh weight. While the lowest values were 1.52% and 1.15 mg gm⁻¹ fresh weight at S0 treatment, the interaction between the two factors was significantly affect in the both traits, the treatment P2 × S3 achieved the highest values

amounted to 1.81% and 1.47 mg gm⁻¹ fresh weight compared to the treatment P0 × S0 which recorded the lowest values of 1.47% and 1.02 mg gm⁻¹ fresh weight respectively for both traits.

Table 5. Effect of foliar spraying with Promalin and salicylic acid and their interaction in the percentage of nitrogen in the leaves and their total chlorophyll content

Promlin (P) ml L ⁻¹	Leaves content of nitrogen (%)					Leaves content of total chlorophyll (mg.g ⁻¹ fresh weight)				
	S0	S1	S2	S3	Means (P)	S0	S1	S2	S3	Means (P)
	Salicylic acid mg L ⁻¹					Salicylic acid mg L ⁻¹				
P0	1.47	1.51	1.53	1.58	1.52	1.02	1.09	1.14	1.26	1.12
P1	1.52	1.57	1.60	1.74	1.60	1.13	1.18	1.24	1.35	1.22
P2	1.59	1.63	1.65	1.81	1.67	1.31	1.33	1.40	1.47	1.37
Means (S)	1.52	1.57	1.59	1.71	Means (S)	1.15	1.20	1.26	1.36	
L.S.D.	P	S	P × S			P	S	P × S		
0.05	0.04	0.05	0.08			0.06	0.07	0.13		

The reason of the increase in the percentage of nitrogen in the leaves was a result of treatment with promalin as well as salicylic acid which may be due to their synergism action in stimulating the uptake and transport of nutrients in the plant towards the treated tissues [7, 18, 36]. As for the reason for the increase in the leaves content of chlorophyll resulting from foliar spraying with Promalin, it may be due to the role of gibberellins in inhibiting the activity of the enzyme Chlorophyllase responsible for the degradation and loss of the chlorophyll pigment [34] as well as the effect of cytokinins that stimulate the biosynthesis of chlorophyll by activating the enzyme NADH-Protochlorophyll. This is in addition to its role in stimulating chloroplast development [7, 40, 41]. The reason for the increase in leaves content of chlorophyll as a result of the treatment with salicylic acid may be due to its role in biosynthesis that pigment in addition to its role in delaying the senescence of leaves by regulating the signal transduction during the gene expression process by activating of RNA Polymerase, which increases the formation of the nucleic acid (RNA) during chloroplast development [42][43]. These results confirm the results of Swiercznski et al. [10] when spraying saplings of two apple cultivars with Promalin at a concentration of 25 ml.L⁻¹, which resulted in a significant increase in nitrogen concentration in the leaves of the cultivar "Idared". It also agrees with Abo El-Ez et al. [20] who obtained a significant increase in the percentage of nitrogen in the leaves of two cultivars of olive trees when foliar spraying with salicylic acid at a concentration of 200 mgL⁻¹. These results agree with Abd-Alhamid et al. [22] who obtained a significant increase chlorophyll content of leaves when spraying with salicylic acid at a concentration of 300 mgL⁻¹ for olive trees.

4. Conclusion

The results showed the significant effect of both study factors in all the growth traits of young olive trees, which reflected positively in building a strong structure for the trees able to grow balanced in a sustainable place.

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