



## ROLE OF GROWTH REGULATORS BRS AND CPPU IN GROWTH AND LEAVES MINERAL AND HORMONAL CONTENT OF OLIVE TRANSPLANTS

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### Abstract

This study was conducted in the olive orchard, in Habbaniyah city, Anbar province 84 km west of Baghdad during 2018 growing seasons to investigate the influence of brassinolide (BRs) and CPPU spray on 2 year's old trees of "Nebali" olive cultivar. This study included two treatments: three levels of spraying of BRs, 0 (BR0), 1mg L<sup>-1</sup> (BR1) and 2mg L<sup>-1</sup> (BR2) and four levels of spraying of cytokinins (CPPU), 0 (C0), 2.5mg L<sup>-1</sup> (C2.5), 5mg L<sup>-1</sup> (C5) and 10mg L<sup>-1</sup> (C10) and their interaction. Treatments were replicated three times with two transplants in experimental unit at factorial experiment in a RCBD. The number of trees used was 72 trees. The experimental results showed that BRs spray at 2 mg L<sup>-1</sup> gave the highest leaves chlorophyll content of 62.69 SPAD units, leaves dry weight of 29.49 %, highest leaf area of 6.80 cm<sup>2</sup>, leaf nitrogen content of 1.169 %, highest leaf IAA content of 44.49 µg g<sup>-1</sup> FW and highest leaf GA content of 150.24 µg g<sup>-1</sup> FW. Results also showed that CPPU spray at levels 10 mg L<sup>-1</sup> superiority of the control treatment and gave the highest leaves chlorophyll content of 62.89 SPAD units, leaves dry weight of 28.92 %, leaf IAA content of 41.82 µg g<sup>-1</sup> FW and highest leaf GA content of 134.00 µg g<sup>-1</sup> FW. The lowest value of these parameters was found in the control (BR0C0) treatment.

**Keywords:** Growth, Regulators, BRs, CPPU, Olive.

## دور منظمي النمو BRs و CPPU في النمو و المحتوى المعدني و الهرموني للأوراق

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### المستخلص

اجريت هذه التجربة في بستان الزيتون الواقع في مدينة الحبانية / محافظة الأنبار 84 كم غرب بغداد خلال الموسم 2018 لمعرفة تأثير رش البراسينولايد (BRs) و CPPU على شتلات زيتون بعمر سنتين صنف نبالي. تضمنت التجربة عاملين، الأول هو رش ثلاثة مستويات من BRs هي صفر (BR0) و 1 ملغم لتر<sup>-1</sup> (BR1) و 2 ملغم لتر<sup>-1</sup> (BR2) ورش اربعة مستويات من الساييتوكانينات (CPPU) هي صفر (C0)، 2.5 ملغم لتر<sup>-1</sup> (C2.5)، 5 ملغم لتر<sup>-1</sup> (C5) و 10 ملغم لتر<sup>-1</sup> (C10) والتداخل فيما بينهما. صممت التجربة بتصميم التجارب العاملية ضمن تصميم RCBD وبثلاثة مكررات وبشتلتين للوحدة التجريبية الواحدة، و بذلك يكون عدد الشتلات الداخلة في التجربة 72 شتلة. اظهرت نتائج التجربة ان رش BRs بالمستوى 2 ملغم لتر<sup>-1</sup> قد اعطى اعلى محتوى للأوراق من الكلوروفيل والبالغ 62.69 وحدة Spad واعلى وزن جاف للأوراق و البالغ 29.49 % واعلى مساحة ورقة و البالغة 6.80 سم<sup>2</sup>، اعلى محتوى للأوراق من النتروجين والبالغ 1.169 % ومن IAA والبالغة 44.49 مايكوغرام غرام<sup>-1</sup> وزن طري ومن GA والبالغة 150.24 مايكوغرام غرام<sup>-1</sup> وزن طري. كما اظهرت النتائج ايضاً تفوق رش CPPU بالمستوى 10 ملغم لتر<sup>-1</sup> على معاملة المقارنة واعطى اعلى محتوى للأوراق من الكلوروفيل و البالغ 62.89 وحدة Spad واعلى وزن جاف للأوراق والبالغ 28.92 % واعلى محتوى للأوراق من IAA والبالغة 41.82 مايكوغرام غرام<sup>-1</sup> وزن طري ومن GA والبالغة 134.00 مايكوغرام غرام<sup>-1</sup> وزن طري. اقل القيم لهذه الصفات وجدت في معاملة المقارنة (BR0C0).

كلمات مفتاحية: نمو، منظمات، BRs، CPPU، زيتون.

### Introduction

Olive is the fruit tree, which is economically important in the Oleaceae family and follows the Olea Genus. It was and still with economic importance especially in the life of peoples. Most scientists agree that the olive tree originated in the eastern Mediterranean. Its fruits are used as food and its leaves are extracted for medical preparations and its oil are used in cooking, making soap and cosmetics. Olive oil is one of the best vegetable oils because it protects against atherosclerosis, heart disease and gall bladder activity. It contains high levels of Oleic acid, Linoleic acid and vitamin K (18 and 24). In 2016, the acreage of olive in the world reached about 10650068 hectare, with production of 19267493 tons (15). The main producing countries are Spain then Greece, Italy, Turkey and Morocco (15). In 2017, the estimated number of olive fruit trees in Iraq, including nearly 487458 tree produces up to 10203 tons, and the average production per tree about 20.93 kg (23).

Brassinosteroids (BRs) are one of the plant hormones; the first to be discovered is the world of Mitchell when they studied the effect of rape (*Brassica napus* L.) extract on cell division and elongation (17). Montoya *et al.* (22) noted that brassinosteroids are a basic hormone in the most physiological functions of plants, yet little is known about where and when to make them in plants. This hormone plays a major role in stimulating growth through its relationship to increasing the DNA and RNA content,

increasing the efficiency of polymerase and protein synthesis, using this hormone in very small amounts (17).

Several studies have been conducted to determine the role of brassinosteroids in growth, leaf mineral and chlorophyll content, (22) Mentioned that the foliar spray with 0.00, 0.010 and 0.020 mg BRs L<sup>-1</sup> caused significant increase in leaves area and leaves chlorophyll content, especially at 0.020 mg L<sup>-1</sup> in his study on orange trees (5) reported that there was a positive correlation between the vegetative growth, leaf mineral content, leaf chlorophyll content and brassinolide concentrations of apricot trees. (10) They have studied the effect of spray three levels of Brassinolide (0, 0.003 and 0.005) mg L<sup>-1</sup>, and found the concentration of brassinolide at 0.005 mg L<sup>-1</sup> caused significant increase in leaves hormonal content and leaves carbohydrates content in Orange transplants. Al-Khattab (8) Recorded that, highest content of leaves N, K, Fe and Zn and leaf chlorophyll content it was in the 3 year's old trees of "Nebali" olive cultivar treated with brassinolide as foliar spray.

CPPU is industrial cytokinins that has significant physiological efficacy in many plants, leading to a clear increase in plant growth. The compound was prepared in the mid-1980 and then tested and recorded for use on many fruit trees. CPPU is absorbed by stems, leaves and seeds. It stimulates cell division, differentiation and development (9). It regulates capillary sovereignty, breaks the buds dormancy and improves germination. It inhibits aging and preserves the retention of chlorophyll in isolated leaves, regulates the movement of nutrients and improves fruit formation (21). Shalash and AL\_Esawi (26) Mentioned that the foliar spray with 200 mg CPPU.L<sup>-1</sup> caused significant increase in shoot number, shoot length, leaves area, leaves chlorophyll content, leaves carbohydrates content and leaves nitrogen content compared with the control treatment in his study on orange rootstocks. Abd-alwahab and Al-Mashari (2) Found that foliar spray with CPPU in two levels 4, 8 mg.L<sup>-1</sup> caused significant increase in leaves dry weight, leaves chlorophyll content, leaves carbohydrates content and leaves nitrogen content in orange and lemon trees. Al-janabi and Al-shabani (7) recorded that, highest content of leaves dry weight, chlorophyll, carbohydrates and nitrogen it was in sour orange trees treated with CPPU as foliar spray. Therefore, due to few of similar studies in Iraq, this study aims to determine the effect of the growth regulators of BRs and CPPU on vegetative growth and mineral and hormonal content of olive transplants.

### Materials and Methods

This study was conducted in the olive orchard, in Habbaniyah city, Anbar province 84 km west of Baghdad during 2018 growing seasons to investigate the influence of brassinolide (BRs) and CPPU spray on 2 year's old trees of "Nebali" olive cultivar. This study included two treatments: three levels of spraying of BRs, 0 (BR<sub>0</sub>), 1mg L<sup>-1</sup> (BR<sub>1</sub>) and 2mg L<sup>-1</sup> (BR<sub>2</sub>) and four levels of spraying of cytokinins (CPPU), 0 (C<sub>0</sub>), 2.5mg.L<sup>-1</sup> (C<sub>2.5</sub>), 5mg L<sup>-1</sup> (C<sub>5</sub>) and 10mg L<sup>-1</sup> (C<sub>10</sub>) and their interaction. Treatments were replicated three times at factorial experiment in a RCBD. The number of trees

used was 72 trees (Two trees for experimental unit). The following parameters were determined in the two successive seasons:

Leaf chlorophyll contents (SPAD unit).

Leaf dry weight (%): Various leaves were taken from the trees was weighing then drained and calculated the percentage of dry matter by dividing weight after drying on weight before drying  $\times 100$ .

A leaves number and leaf area  $\text{cm}^2$  according to (3) using the following equilibration: Leaf area =  $0.53 (\text{length} \times \text{width}) + 1.66$ .

Leaves mineral content: Leaves samples were collected for chemical analysis at the 2<sup>nd</sup> week of June. Each sample consisted of 10 leaves.  $\text{Tree}^{-1}$ . Leaves were washed with tap water, rinsed with distilled water, and then dried at  $70\text{ }^{\circ}\text{C}$  until a constant weight, ground and digested according (11). Nitrogen was estimated by micro-kjeldahl method of (1). Phosphorus was estimate the chromatic by using spectrophotometer by (14).

Leaves hormonal content (IAA, and gibberellins) were assayed according to (27).

The obtained results were subjected to analysis of variance according to (13) using L.S.D 0.05 for comparing differences between various treatment means.

### Results and Discussions

Effects of BRs and CPPU spray on chlorophyll content, dry weight, leaves number and leaf area:

Data concerning the effect of treatments on leaves chlorophyll content, leaves dry weight, leaves number and leaf area are listed in Table 1. The data cleared that, BRs spray at  $2\text{ mg L}^{-1}$  significantly increased leaves chlorophyll content of 62.69 SPAD units, leaves dry weight of 29.49 % and the highest leaves number of 660.33 leaf  $\text{plant}^{-1}$  and highest leaf area of  $6.80\text{ cm}^2$ , while lower values of these traits was in control treatment. Table 1 also shows that the sprayed CPPU at levels  $10\text{ mg L}^{-1}$  superiority of the control treatment and gave the highest leaves chlorophyll content of 62.89 SPAD units, leaves dry weight of 28.92 % and leaves number of 630.89 leaf  $\text{plant}^{-1}$ . The interactions between BRs and CPPU significantly affected in all studied traits.

The positive effects of BR on vegetative growth may be due to the fact that BR helps to increase the absorption of mineral elements from the soil and to benefit from plant growth, as well as to stimulate processes responsible for elongation and division of the cell (12). Generally, these results are in harmony with those reported by (5) on apricot trees, (8) when they worked on olive trees. These results are attributed to the effect of cytokinin in stimulate the formation of buds and increasing the number of leaves, by encouraging lateral branching and increased lateral buds, as well as the vital role of cytokinin in reducing the inhibitory effect of auxins in lateral buds (16). These results are in agreement with those obtained by, (20) on olive transplants, they found that the leaves chlorophyll content and leaves dry weight and leaves carbohydrates content positively correlated with cytokinin spray in those transplants.

**Table 1 Effects of BRs and CPPU spray and their interaction on chlorophyll content, leaves dry weight, leaves number and leaf area of Nebali olive trees.**

CPPU	Leaf chlorophyll content (SPAD unit)				Leaf dry weight (%)			
	BRs			Mean	BRs			mean
	0	1	2		0	1	2	
0	61.13	61.89	62.00	61.67	26.43	29.00	29.25	28.23
2.5	61.37	62.19	62.28	61.95	27.19	28.18	29.40	28.26
5	61.94	63.03	63.12	62.70	27.34	28.88	29.58	28.60
10	62.11	63.18	63.37	62.89	27.90	29.11	29.74	28.92
mean	61.64	62.57	62.69		27.22	28.79	29.49	
L.S.D 5%	CPPU	BRs	Int.		CPPU	BRs	Int.	
	0.56	0.48	0.96		0.65	0.56	1.12	
	Leaves number				Leaf area(cm <sup>2</sup> )			
0	470.33	510.67	590.00	520.67	5.33	5.64	6.07	5.68
2.5	480.67	550.33	630.67	550.89	5.39	5.74	6.43	5.85
5	500.00	600.67	700.33	600.33	5.63	5.93	7.17	6.24
10	530.67	650.67	720.33	630.89	5.66	6.17	7.52	6.45
mean	490.92	580.33	660.33		5.50	5.87	6.80	
L.S.D 5%	CPPU	BRs	Int.		CPPU	BRs	Int.	
	34.74	30.09	60.20		0.26	0.22	0.44	

Effects of BRs and CPPU spray on leaf N, P, IAA and GA<sub>3</sub> content:

Data concerning the effect of treatments on leaves N, P, IAA and GA content are listed in Table 2. The data cleared that BRs spray at 2 mg L<sup>-1</sup> significantly increased and gave the highest leaf nitrogen content of 1.169 %, highest leaf IAA content of 44.49 µg g<sup>-1</sup> FW and highest leaf GA content of 150.24 µg g<sup>-1</sup> FW, while BRs did not affect on leaf phosphor content. Table 2 also shows that sprayed CPPU at levels 10mg L<sup>-1</sup> significantly superiority of the control treatment and gave the highest leaf nitrogen content of 1.151 %, leaf IAA content of 41.82 µg g<sup>-1</sup> FW and highest leaf GA content of 134.00 µg g<sup>-1</sup> FW. The interaction between BRs and CPPU significantly affected all studied parameters. The reason for these results may be due to increase growth as a result of foliar spray for brassinolide and its role in the division and cell elongation (9), which leads to increased uptake of soil elements, thus increasing their content in the leaves. Generally, these results are in harmony with those reported by (5) when they worked on apricot trees. As for the effect of the CPPU had a significant effect on N, IAA and GA. Or may be due to CPPU role in stimulate new leaves table 1 this may have increased the production of IAA, especially since it is mainly built in the tops of shoots and leaves (19). These results are in agreement with those obtained by, (4 and 6) on fig trees; they found that the leaves mineral and hormonal content positively correlated with cytokinins spray in those trees.

**Table 2 Effects of BRs and CPPU spray and their interaction on leaf N, P, IAA and GA<sub>3</sub> content of Nebali olive trees.**

CPPU	N (%)				P (%)			
	BRs				BRs			
	0	1	2	mean	0	1	2	mean
0	1.120	1.128	1.153	1.134	0.142	0.151	0.156	0.150
2.5	1.129	1.147	1.166	1.147	0.147	0.150	0.152	0.150
5	1.133	1.139	1.152	1.141	0.147	0.146	0.143	0.145
10	1.136	1.152	1.204	1.164	0.149	0.144	0.160	0.151
mean	1.130	1.142	1.169		0.146	0.148	0.153	
L.S.D 5%	CPPU	BRs	Int.		CPPU	BRs	Int.	
	0.018	0.016	0.032		N.S	N.S	N.S	
	IAA ( $\mu\text{g g}^{-1}$ FW)				GA ( $\mu\text{g g}^{-1}$ FW)			
	0	1	2	mean	0	1	2	mean
0	30.31	32.96	36.68	33.32	94.38	101.87	129.90	108.72
2.5	30.76	33.80	41.60	35.39	96.11	108.56	142.27	115.65
5	32.11	36.87	47.84	38.94	100.12	119.37	160.70	126.73
10	32.89	40.75	51.82	41.82	103.46	130.45	168.08	134.00
mean	31.52	36.10	44.49		98.52	115.06	150.24	
L.S.D 5%	CPPU	BRs	Int.		CPPU	BRs	Int.	
	5.01	4.34	8.68		6.74	5.84	11.68	

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