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Effect of Bio_fertilizers on Growth and Medicinal active compounds Content of Olive Transplants

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Abstract:

A study was carried out in central nursery in Kut affiliated to Wasit Agriculture Directorate on 18/09/2020 to study effect of adding *Azotobacter chroococcum* and *Bacillus megatherium* on growth, leaf mineral content and medicinal compounds of "Qaisi" olive transplants. This experiment was carried out on 81 olive transplants with homogeneous vegetative growth as much as possible. Two factors were used in experiment; first factor is addition *Azotobacter chroococcum* bacteria (A) with three levels: no addition (A_0), addition of 30 ml.pot^{-1} (A_{30}) and addition of 60 ml.pot^{-1} (A_{60}) and second factor is three levels of *Bacillus megatherium* (P) is without addition (P_0), adding 30 ml.pot^{-1} (P_{30}) and adding 60 ml.pot^{-1} (P_{60}).Treatments were replicated three times (three transplants in experimental unit) at factorial experiment in a RCBD. experimental results showed; adding *Azotobacter chroococcum* to soil at 60 ml.pot^{-1} (A_{60}) significantly increased in transplant height of 5.78 cm, highest leaf area of 8.99 cm^2, highest Branches number of 10.67 branches transplant^{-1}, highest Leaves Carbohydrates content of 4.54 %, highest leaf Caffeic acid content of 14.63 mg.g^{-1} and highest leaf Vanillic Acid content of 16.90 mg.g^{-1}, also results shows that adding *Bacillus megatherium* to soil at 60 ml.pot^{-1} (P_{60}) gave highest increased in transplant height of 5.64 cm, highest leaf area of 8.84 cm^2, highest Branches number of 9.78 branches transplant^{-1}, highest Leaves Carbohydrates content content of 4.62 %, highest leaf Caffeic acid content of 15.59 mg.g^{-1} and highest leaf Vanillic Acid content of 17.89 mg.g^{-1},

I. INTRODUCTION

Olive tree Olea europaea L. a fruitful and economically important tree belonging to Oleaceae family, which follows genus Olea. was and still is an economically important tree, especially in people's lives, so fruits are used as food and its leaves are extracted from medicinal preparations. Olive oil is one of the best vegetable oils because it protects against atherosclerosis, treats heart diseases, and increases bile gland activity because it contains high levels of oleic acid, linoleic and vitamin K (Preedy and Watson, 2010). In 2020, Estimated number of olive fruitful trees growing in Iraq, including nearly 1329191 tree produces up to 33912 tons, and average production per tree about 25.51 kg (PCBS, 2020). While acreage of olive in world reached about 10578246 hectare, with production of 19464495 tons (FAO,2021). A main producing countries are Spain then Italy, Morocco, Turkey and Greece (FAO,2021). And in order to grow well fruit transplants need availability of nutrients in soil As growth of plants is directly proportional to a fertility of soil and its suitability for each type of fruit and its sufficient containment of various mineral elements, which are greatly depleted in later stages of growth of different parts of plant. Therefore, this lack of nutrients must be remedied by carrying out composting process in order to compensate for soil fertility from these elements that may not exist or exist in insufficient

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quantities for needs of trees, so adding mineral, organic and biofertilizers compensates for what soil loses or what plant needs for ideal nutrition(Ali, 2012). A continuous addition of mineral (chemical) fertilizer leads to deterioration of soil properties and fertility and also leads to accumulation of heavy metals in plant tissues, which greatly affects nutritional value of fruits and their marketing (Mosa *et al*., 2014). For this reason, scientists searched for alternative methods of chemical fertilizers that are safe for human health and do not cause pollution in environment, and alternative was to use biotechnology to solve these problems, and biotechnologies include any technology in which a living organism (Mahdi et al., 2010). Environmental protection organizations have paid much attention to biofertilizers and are preparations that contain micro-organisms capable of supplying plants with nutrients they need from natural sources thus reducing dependence on various chemical fertilizersSeveral studies have been conducted to determine role of biofertilizers in growth and leaves mineral content of fruit trees, Al-Hadethi (2015) studied effect of biofertilizer on apricot trees and found biofertilizer (Phosphorene + Nitrobeine) caused significant increases in leaf area, leaf chlorophyll content, leaves nitrogen ,phosphor and potassium content compared with control treatment. Torshiz *et al*., 2017, studied effect of biofertilizer on pomegranate trees and found biofertilizer caused significant increases in leaf chlorophyll content and leaf NPK content compared with control treatment. Also Al-Hadethi, (2019) found added Azospirillum brasilense + Bacillus megatherium gave highest leaves number, shoots length and highest leaves NPK content when his studied on hawthorn transplants. Due to the lack of studies on role of biofertilizers in growth and leaves mineral content of fruit transplants present study aims to know effect of biofertilizers addition on growth and leaves mineral content in olive transplants.

II. MATERIALS AND METHODS

A study was carried out in central nursery in Kut affiliated to Wasit Agriculture Directorate on 18/09/2020 to study effect of adding *Azotobacter chroococcum* and *Bacillus megatherium* on growth, leaf mineral content and medicinal compounds of "Qaisi" olive transplants. This experiment was carried out on 81 olive transplants with homogeneous vegetative growth as much as possible. Two factors were used in experiment; first factor is addition *Azotobacter chroococcum* bacteria (A) with three levels: no addition (A_0), addition of 30 ml.pot^{-1} (A_{30}) and addition of 60 ml.pot^{-1} (A_{60}) and second factor is three levels of *Bacillus megatherium* (P) is without addition (P_0), adding 30 ml.pot^{-1} (P_{30}) and adding 60 ml.pot^{-1} (P_{60}).Treatments were replicated three times (three transplants in experimental unit) at factorial experiment in a RCBD. The results of this study were statistically analyzed and averages were compared according to (L.S.D) at 0.05 according to Elsahookie and Wuhaib (1990) and thus number of transplants used was 81 transplants. a following parameters were determined in experimental season:

1. **Transplant height (cm):** Transplant heights were measured by metric tape measure at beginning of experiment in 9-12-2020 and end of experiment in 10-04-2021, according to difference between them and that such an increase in transplant height.

2. **Leaf area (cm^2):** in first week of May, five leaves were taken from middle position of shoot randomly and measuring leaf area (cm^2). Using a Digimizer program Windows 7 operating system.

3. **Branches number (branches transplant^{-1}):** Branches number was calculated at beginning of experiment on 9/12/2020, end of experiment on 11/4/2021 and according difference between them, which represented increase in Branches number.

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4. **Leaves Carbohydrates content (%):** Total carbohydrate content was determined by method Hedge and Hofreiter (1962) so preparation (0.2 gm) of a sample to be measured was adding to it (25ml) of perchloric acid (1N) and placed in a test tube, then tubes were placed in a water bath at a temperature of (60) for (30 minutes) after which sample was filtered using filter papers. (1 ml) of filtrate is added to it (9 ml) of distilled water to complete the volume to (10 ml) in a volumetric vial. Take from last (1 ml) and add to it a concentration of phenol (5%) + (5 ml) concentrated sulfuric acid and leave it until it cools. It is measured at a wavelength (490 nm) with a spectrophotometer that prepares several concentrations of glucose (0.1,0.2,0.3,0.4,0.5,0.6,0.7) and absorbance of above readings is recorded (to make a calibration curve). Then absorbance of model is read and projected onto calibration curve and concentration is extracted from it.

$$X = \frac{(25) \times (10ml) \times Focus\ of\ calibration\ curve}{(0.2gm \times 1000)} \times 100\%$$

5. **Caffeic acid:** leaf samples were collected from middle of previous year's shoots; approximately 50 g of leaf samples was collected from each transplant. All of samples were immediately frozen and stored until used for analysis. After extraction caffeic in hydrolyzed extracts were determined using a Schimadzu 10 Series HPLC equipped with a UV detector, According to (Montedoro *et al*., 1992).

6. **Vanillic Acid:** Vanillic acid: leaf samples were collected from middle of previous year's shoots; approximately 50 g of leaf samples was collected from each transplant. All of samples were immediately frozen and stored until used for analysis. After extraction vanillic in hydrolyzed extracts were determined using a Schimadzu 10 Series HPLC equipped with a UV detector, According to (Montedoro *et al*., 1992).

III. RESULTS

Effects of adding *Azotobacter chroococcum* and *Bacillus megatherium* on Increase in transplant height, Leaf area, Branches number and Leaves Carbohydrates content: Data concerning effect of treatments on increase in transplant height, leaf area in Table (1). A data cleared that, adding *Azotobacter chroococcum* to soil at 60 ml.pot^{-1} (A60) significantly increased in transplant height of 5.78 cm, highest leaf area of 8.99 cm^2, Branches number of 10.67 branches transplant^{-1} and highest Leaves Carbohydrates content of 4.54 %, lower values of these traits was in control treatment (A0). Table (1) also shows that adding *Bacillus megatherium* to soil at 60 ml.pot^{-1} (P60) gave highest increased in transplant height of 5.64 cm, highest leaf area of 8.84 cm^2, Branches number of 9.78 branches transplant^{-1} and highest Leaves Carbohydrates content 4.62 %, Also, lower values of these traits were in control treatment (P0).

Table (1) Effects of adding *Azotobacter chroococcum* and *Bacillus megatherium* to soil on Increase in transplant height, Leaf area, Branches number, Leaves Carbohydrates content of "Qaisi" olive transplants.

Increase in transplant height (cm)					Leaf area (cm^2)			
A	P				P			
	0	30	60	Mean	0	30	60	Mean
A_0	2.25	3.50	4.58	3.44	8.11	8.25	8.43	8.26
A_{30}	4.92	5.42	5.92	5.42	8.27	8.58	8.78	8.54
A_{60}	5.42	5.50	6.42	5.78	8.64	9.02	9.30	8.99
Mean	4.20	4.81	5.64		8.34	8.62	8.84	

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L.S.D 5%	A	P	Int.		A	P	Int.	
	1.05	1.05	N.S		0.073	0.073	0.127	
	Branches number (branches transplant^{-1})				Leaves Carbohydrates content (mg.g-1 fresh weight)			
A_0	4.33	5.33	6.33	5.33	4.13	4.22	4.37	4.24
A_{30}	5.33	7.67	10.33	7.78	4.20	4.41	4.69	4.43
A_{60}	8.00	11.33	12.67	10.67	4.54	4.29	4.79	4.54
Mean	5.89	8.11	9.78		4.21	4.39	4.62	
L.S.D 5%	A	P	Int.		A	P	Int.	
	0.67	0.67	1.15		0.023	0.023	0.040	

A results show addition of soil microorganisms has had a positive effect on studied vegetative growth characteristics, and increase in vegetative characteristics may be attributed to effect of biofertilizers in improving soil biological and physical properties in addition to chemical properties, which resulted from release of larger quantities of nutrients available for absorption by roots and consequently effect on physiological processes such as increasing efficiency of leaves photosynthesis (Yu *et al*., 2014) and increasing its products represented by carbohydrates and thus increasing vegetative growth of transplants. A reason is also due to increased ability of microorganisms added to soil to produce plant growth regulators such as auxins, cytokinins and gibberellins (Sumbul *et al*., 2020). Auxin produced from these organisms increases vegetative growth of transplants, due to its role in increasing divisions and thus its reflection on vegetative growth, cytokinin resulting from these organisms added also encourages buds formation on vegetative growths on transplants and increases leaves number, as well as vital role of cytokinin in reducing inhibitory effect of auxins in lateral buds, and then encouraging these buds to grow, which works on improving vegetative characteristics , Thus, it affects increase in vegetative growth of olive transplants (Bhardwaj *et al*., 2014).These results are in harmony with those obtained by (Hassan, 2015) who worked on olive trees, (Al-Hadethi, 2019) who worked on hawthorn transplants.

Effects of adding *Azotobacter chroococcum* and *Bacillus megatherium* on Caffeic acid and Vanillic acid content: Data concerning effect of extracts spray on Caffeic acid and Vanillic acid content are listed in Table (2). A data cleared that adding *Azotobacter chroococcum* to soil at 60 ml.pot^{-1} (A_{60}) significantly increased and gave highest Caffeic acid content of 14.63 mg.g^{-1}, highest Vanillic acid content of 16.90 mg.g^{-1}. Table (2) also shows that adding *Bacillus megatherium* to soil at 60 ml.pot^{-1} (P_{60}) significantly superiority of control treatment and gave highest Caffeic acid content of 15.59 mg.g^{-1}, highest Vanillic acid content of 17.89 mg.g^{-1}. Interaction between treatments especially interaction treatment ($A_{60}P_{60}$) as it gave highest Caffeic acid content of 17.77 mg.g^{-1}, highest Vanillic acid content of 19.60 mg.g^{-1}.

Table (2) Effects of adding Azotobacter chroococcum and Bacillus megatherium on Caffeic acid and Vanillic acid content of "Qaisi" olive transplants.

A	Leaves Caffeic acid content (mg. g^{-1})				Vanillic acid content (mg. g^{-1})			
	P				P			
	0	30	60	Mean	0	30	60	Mean
A_0	6.47	8.57	12.37	9.13	8.93	11.60	15.47	12.00
A_{30}	7.33	13.77	16.63	12.58	10.23	16.83	18.60	15.22
A_{60}	10.23	15.90	17.77	14.63	13.50	17.60	19.60	16.90
Mean	8.01	12.74	15.59		10.89	15.34	17.89	

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L.S.D 5%	A	P	Int.		A	P	Int.	
	0.322	0.322	0.559		0.313	0.313	0.543	

Increase in some compounds may be due to organisms added to soil in increasing plant's content of hormones (Al-Hadethi, 2019). It is initiator of biosynthesis of other phenolic compounds in olive leaves such as Caffeic Acid (Otero *et al*., 2020). In addition, there is a second pathway for construction of phenolic compounds in olive leaves, which is plastidial 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway, as this pathway is affected by leaves' content of chlorophyll and phosphorous, which had a significant increase as a result of adding Reviving soil microscopic and thus increasing concentration of phenolic compounds in leaves (Alagna *et al*., 2012).

IV. CONCLUSIONS

An addition of *Azotobacter chroococcum* to soil had a significant effect for increasing all indicators of vegetative growth of studied in olive transplant, especially when adding a concentration of 60 ml of it, An addition of *Bacillus megatherium* to soil had a significant effect for increasing all indicators of vegetative growth and leaf content of Oleuropein, Caffeic acid and Vallinic acid in olive transplant when adding a concentration of 60 ml of it.

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