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INFLUENCE OF BIO- FERTILIZER AND SPRAYING WITHPALM POLLENGRAINS EXTRACTS ON SOME GROWTH INDICATORS AND LEAVES CONTENT OF NUTRIENT IN POMEGRANATE cv. SALIMI

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Abstract

The study was Conducted in the lath house of the General Station of Hort. and Forestry Ministry of Agriculture / Al-Mahaweel / Babylon, during the growing season of 2017 to identify the influence of Bio- fertilizer mycorrhizae and Spraying with palm pollen grains extracts on the vegetative growth and leaves content of nutrient on Pomegranate seedlings cv. Salimi. This experiment conducted in a completely randomized block design (RCBD) as factorial experiment with three replicates, The first factor is Bio- fertilizer mycorrhizae with three levels (0, 10, 20) gm/seedling, while the two factor is Spraying with palm pollen grains extracts with four levels (0, 5, 10, 15) gm.L^{-1}. The results showed that the Bio- fertilizer mycorrhizae showed a significant influence on all characters studied) seedling length, stem diameter, leaves number, leaf area, chlorophyll content in leaves, dry weight of shoot and root system and nutrient concentration (NPK) in leaves .The treatments 20 gm/seedling gave a significant increased in all studied characters as compared to the control. However, the palm pollen grains extract showed a significant influence on all characters studied, and the treatment 15gm.L^{-1} gave the highest rate of those characteristics .The interaction treatment (20 gm/seedling+15 gm.L^{-1}) was effective on all characters studied and gave the highest rate of those characteristics compared to the control treatment.

Key words : Pomegranate, Salimi, Bio- fertilizer mycorrhizae, palm pollen grains.

Introduction

Pomegranate His scientific name He belongs to the Punicaceae It is a fruit tree falling leaves Which are cultivated in temperate areas (Mir *et al.,* 2012). The number of pomegranate trees in Iraq 11696 Million trees, The average production per tree is 26.0 kg. The total production of fruits is 304,096 tons (Central system of Statistics, 2015). Pomegranate is of great nutritional importance because it contains vitamins, especially vitamin C and many fats, dyes, acids, sugars, protein, fiber and nutrients (Opara *et al.,* 2009). It also has many therapeutic and medical benefits. It strengthens the heart, grips, repels worms, treats stomach diseases and indigestion to contain the juice on compounds with therapeutic properties, such as anthocyanins, phenols, tannins and some vitamins (B12), which proved to be effective against cancer and many pathogens, Pomegranate juice is one of the most important antioxidants (Glozer and Ferguson, (2008). Environmental protection organizations have devoted much importance to their active contribution to sustainable development through the exchange of organic, bio-chemical and organic fertilizers and their impact on the quantity, quality and productivity of various crops (Chaudhary and Iqubal, 2006). Mycorrhiza fungi are the most common fungi, affecting about 95% of plants (Smith and Read, 2008). They thus positively affect in the process of photosynthesis through the processing of plant nutrients and water from the soil, which contributes to the increased content of leaves of chlorophyll and nutrients (Wu and Zou, 2010). And enjoy many of the characteristics that made it the focus of researchers and these characteristics ability to form a relationship with the plant takes from the host source of energy (carbon) and provide the plant needs of nutrients, especially phosphorus as well as plant processing with nitrogen, zinc and manganese, Mycorrhiza fungi includes several species, the most important of which is Glomus, which is the most widespread and comes back with a variety of species: *G. mosseae* and *G. leptoticum* (Orcutt and Nilsen, 2000). Research has shown that bio-

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fertilizers improve the ecosystem of plants and soil by increasing the processing of nutrients and thus increasing the quality of crops (Rosen and Biermann, 2007). Nerwad (2014) found that the high levels of mycorrhiza fungi effect significantly affected the growth and yield of local orange trees (*C. sinensis*), which exceeded the length of the branch, the number of leaves, the leaf area and the leaf content of chlorophyll, as well as the leaves content of nutrients N, P, K, Mg, Ca, Fe, Zn, Mn EL_Shamy *et al.,* (1990) found that the mycorrhiza fungi caused a significant increase in branch length, number of leaves, leaf content of chlorophyll and plant content of nutrients N, P, K for citrus seedlings. Wu & Zou (2011) found that Inoculation of *Trifoliate Orange* seedlings of the mycorrhiza fungi significantly increased vegetative traits studied (branch length, leaf area, number of leaves and leaf content of chlorophyll) relative to comparison treatment. Recent studies have focused on the use of plant extracts to improve vegetative growth and increase the production of many plants because these extracts contain important nutrients and growth regulators, vitamins and organic acids, which vary in quantity and quality in different parts of the plant as well as easy to be absorbed by the plant and cheaply priced (Abed Al-Hussain and Ibriham, 2009). Among these extracts is the palm pollen extract, which is characterized by its chemical composition containing many mineral nutrients in addition to essential and non-essential amino acids, fatty acids, proteins and carbohydrates Hazem, (2011) table (A). Abo AL-Mikh (2017) found that the spray of the palm pollen extract improved vegetative qualities of plant height, leaf area, number of leaves and dry weight of vegetative and root group, as well as the proportion of NPK for pomegranate seedlings cv. Wonderful. Although the use of bio-fertilizers and plant extracts has increased dramatically in the last two decades of the twentieth century in various parts of the world, including the Arab region, but its use in Iraq is almost limited to some individual attempts, due to the lack of studies on this subject, especially on fruit, this study was conducted to investigate the effect of bio-fertilizers and plant extracts and their interaction in improving the growth of pomegranate seedlings.

Materials and Methods

This study was carried out in the vegetable canopy of the Hahaweel/Babel plantation of the General Company for Horticulture and Forestry for the period from the beginning of February until mid October 2017 on the pomegranate seedlings cv. Selimi, a total of 180 seedlings were selected with five seedlings of the experimental unit, one year old and homogeneous as possible in size, vegetative growth and growing in sandy mixed soil, planted with black plastic bags made of polyethylene with a capacity of 1.25 kg, it was converted on 15/2/2017 to bags measuring 30 × 25 cm and a capacity of 5 kg and was filled with sandy soil mixed table B.

The research was carried out as factorial experiment according to a completely randomized block design (RCBD) with two factors (3 ×4) and three replicates. The first factor was the bio-enriched mycorrhiza, is a fungal vaccine made up of fungus Glomus mosseae, it was added to the soil one month after it was transferred to the large bags (March 15) and at three levels (0, 10, 20) g/seedlings by making a hole around the root area. The Enriched with mycorrhiza was considered to be in contact with the roots of the seedlings (Matysiak and Falkowski, 2010). The second factor was spraying with the palm pollen extract at four levels (15, 10, 5, 0) gl^{-1} by taking the mature palm pollen seeds at the beginning of the opening of the covers of the male species and collecting pollen powder in March during the agricultural season, one weight was taken according to previously mentioned levels and was soaked with distilled water for 24 hours and mixed with electric mixer and filter the extract with filter paper. The seedlings were sprayed on

Table (A) : Components of palm pollen taken from Graystock *et al.,* (2013).

Subject	Ingredients
Water	11% for fresh pollen, 5% for pollen
Ash	6%
Vitamins	Vitamin A, H, E, D, K and group vitamins B (B1, B2, B6, B12, Niyasine, Butine, Anysitole, Rothine)
Hormones	Astron hormone
Food Ingredients	Carbohydrates 34%, Protein 35%, Fat 5%
Mineral salts	Ti, Mo, B, Si, Zn, I, Cu, Mn, Mg, Fe, Cl, Na, S, P, K, Ca
Enzymes	Phosphatase, Bactinase, Diaminase, Amylase, Lypase, Calase
Co-Enzyme	Cytochrome, Isomyrase, Lactic, Dihydrogenase
Pigments	Carotine, Xanthophyle
Other compounds	phenolic acids, amino acids, clycerides, mono acids, bilateral acids and triple acids

Table (B): Some physical and chemical properties of the soil study.

Type Analysis	Result Analysis
Electrical conductivity EC (extract 1-1)	1.23 DSM.m^{-1}
Soil reaction pH (Extract 1-1)	6.8
Sand	871 g. Kg^{-1}
Silt	41 g. Kg^{-1}
Clay	88 g. Kg^{-1}
Soil texture	Sand mixture
Nitrogen	25.70 mg. Kg^{-1} Soil
Phosphorus	0.48 mg. Kg^{-1} Soil
Potassium	30.00 mg. Kg^{-1} Soil

5/4, 25/4, 15/5 and 5/6 of 2017 using a 2 liter hand spray and add 1 cm^3. liter^{-1} of tween 20 with each concentration to reduce the surface tension of water molecules, the extract was sprayed with concentrations under study in the early morning and even achieve complete wetness of the vegetative parts, comparative treatment was sprayed with distilled water only after the seedlings were irrigated one day before spraying to increase plant efficiency to absorb the extract (El-Sheikh *et al.,* 2007). Agricultural service operations were conducted on a regular basis for all seedlings until the end of the experiment on 15/10/2017. Three seedlings were randomly selected from each treatment and the following measurements were taken:

First: vegetative qualities include

1. **Seedling height cm:** It was taken at the end of the experiment, use the metric tape from the soil of the bag to the top of the seedling and then take the rate for each treatment.
2. **Stem diameter mm:** Calculated at a distance of 5 cm from the surface of the soil by the vernier and then take the rate for each treatment.
3. **Leave area cm^2:** Calculated by taking 5 full-width sheets and different directions from each experimental unit using a Digital Plan meter device with a unit of cm^2, and then according to the average paper area.
4. **Leaves Content of chlorophyll SPAD unit:** Three pairs of leaves were collected from the bottom, center of the plant and above, and were estimated using Chlorophyll content meter (SPAD-502) and all plants in the treatment according to the method in Felixloh and Bassuk (2000).
5. **Total leaves number/seedlings:** The number of leaves per seedlings was calculated according to the average for each experimental unit.
6. **Dry weight of vegetative and root groups gm:** The seedlings were extracted at the end of the experiment carefully after irrigation seedlings well before the day to maintain the largest possible total root and then root was removed from the soil and washed with water and separated the vegetative from the roots and then transferred to the laboratory in paper bags written on each transaction number and left for a week in the laboratory to reducing its weight and size, it was then inserted into the oven at 70°C until fully dry (Al-Sahaf, 1989). It was weighed and calculated the dry weight of the vegetative and root groups for each treatment.

Second: the percentage of some nutrients in the leaves

The leaves were taken from the randomly selected seedlings in the experimental unit and each was then washed with distilled water to remove dirt and dust and dried in an electric oven at 70°C until the weight was stable and then grinded and placed in sealed plastic bags and kept in a dry place. The digestion process was then carried out by taking 0.2 g of the plant sample and digested with sulfuric and perchloric acids and by 4: 1 and according to the method suggested by Gresser and Parson (1979), and heated until the color of the solution was clear, was diluted with distilled water to 100 cm^3, and after the completion of the digestion was estimated the following elements:

1. **Nitrogen% (N):** Determination of nitrogen in plants using the microkijeldahl device as reported in Haynes (1980).
2. **Phosphorus% (P):** Determined using ammonium molybdate and ascorbic acid and reading absorptivity with the spectrophotometer at a wavelength of 882 nm as reported in Page *et al.,* (1982).
3. **Potassium% (K):** measured using the Flame photometer (Erwin and Houba, 2004).

Data were analyzed using the Anova Table according to the Genestate program. The statistical differences between the coefficients were tested using the least significant difference (L.S.D) at the probability level of 0.05 (Al-Rawi and Khalaf Allah, 2000).

Results and Discussion

First: Vegetative attributes

The results of the tables (1, 2, 3, 4, 5, 6 and 7) show that the addition of the bio-enriched mycorrhiza caused a significant increase in all vegetative traits under study, the concentration 20 g/s. gave the highest results in seedling height, stem diameter, leaf area, leaves number, leaf content of chlorophyll and dry weight of the

vegetative and root group at 130.99 cm, 7.96 mm, 5.92 cm 2, 345.28 leaves, 37.59 SPAD, 34.38 g, 16.62 g), respectively. This may be due to the ability of the mycorrhiza fungi to secrete many compounds that improve physiological processes and plant susceptibility to water absorption and major and minor elements which is reflected on vegetative growth and increases plant height (Kaschuk *et al.,* 2010; Martinez-Medina *et al.,* 2011), table 1. The mycorrhiza fungi have a positive effect on nitrogen biosynthesis, which contributes to the production of plant hormones such as oxins and cytokineins, which stimulate and expand cell division, which increases the stems diameter (Table 2). As well as the role of mycorrhiza fungi may increase the surface area of the roots through the secretion of IAA and the increase in the number of side roots, which increased the absorption of nutrients and positively reflected on the vegetable growth (Richardson *et al.,* 2009). As well as the plant height, which leads to the increase of the Sagittal nodes and thus increase the total paper area and the leaves number (Taiz and Zeiger, 2006), Table (3 and 4). That the secretion of the mycorrhiza of cytokinein, which is a receptor to receive the elements of potassium, iron and magnesium necessary in the composition of chlorophyll molecule, which is important in the process of carbon construction in addition to the role of mycorrhiza in increasing the processing of phosphorus important in the process of representation of carbohydrates and other materials resulting from the process of photosynthesis and its role in the formation of amino acids and proteins which is task in building plastids is thus increasing the chlorophyll (table 5). They also contribute to dissolving insoluble forms of phosphorus and some micronutrients such as iron, manganese and zinc, and improving the plant root and increasing its efficiency in nutrient uptake, which is reflected in increased plant growth indicators and increased vegetative and root weight Mahgoub *et al.,* (2006) (Table 6 and 7). These results were agreed with Wu and Zou (2009) on the citrus seedlings and Wu and Zou (2012) on *Trifoliate Orange* (L.) seedlings and Nerwad (2014) on local orange trees. In addition, the palm pollen extract had a significant effect on the increase in the growth indicators under study and the concentration 15 $g.L^{-1}$ gave the highest results in the above characteristics were (120.48 cm, 6.83 mm, 5.52 cm^2, 347.66 sheets, 36.41 SPAD, 32.79 g, 16.10 g) Respectively, this may be due to the fact that it contains many mineral elements, including K, Ca, Mg, P, in addition to proteins, vitamins and organic acids, which increase the process of photosynthesis, respiration and metabolism thus encouraging cell division and elongation in addition to the micro elements Fe, Mn, Zn, the Zn component plays an important role in activating many of the enzymes necessary to formation the oxins that cause cell division and elongation, thus increasing plant height, stem diameter, leaves number and area, in addition to the important role of Mn and Fe in the formation of chlorophyll and photosynthetic process, as well as several chemical compounds as shown in table A and then the accumulation of nutrients in the plant and its transfer to the roots and therefore positively reflected on the dry weight of the root total. These results are consistent with Abo AL-Mikh (2017) on pomegranate seedlings cv. Wonderful and with both Hazem (2011) and Bukhaev and others (1983). Interaction coefficients had a significant effect on the above characteristics and the interaction 20 g/s + 15 $g.L^{-1}$ gave the highest values for the same characteristics were 141.65 cm, 9.27 mm, 7.47 cm^2, 382.12 sheets, 39.25 SPAD, 37.75 g, 19.65 g respectively,

Table 1: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in plant height cm.

Mycorrhizae	Palm pollen extract $gm.L^{-1}$				
gm/s	0	5	10	15	mean
0	81.12	86.22	95.30	101.55	91.05
10	90.56	98.32	110.11	118.23	104.31
20	120.22	127.65	134.44	141.65	130.99
mean	97.30	104.06	113.28	120.48	
L.S.D 5%	Mycorrhizae 3.124.018.10	extrac tinteraction			

Table 2: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in stem diameter mm.

Mycorrhizae	Palm pollen extract $gm.L^{-1}$				
gm/s	0	5	10	15	mean
0	2.33	3.44	4.65	5.20	91.05
10	4.12	4.98	5.45	6.03	104.31
20	6.31	7.55	8.71	9.27	130.99
mean	4.25	5.32	6.27	6.83	
L.S.D 5%	Mycorrhizae 0.541.151.95	extrac tinteraction			

Table 3: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in leaf area cm^2.

Mycorrhizae	Palm pollen extract $gm.L^{-1}$				
gm/s	0	5	10	15	mean
0	2.53	3.10	3.55	3.97	3.29
10	3.62	4.38	4.55	5.13	4.42
20	4.33	5.15	6.71	7.47	5.92
mean	3.49	4.21	4.94	5.52	
L.S.D 5%	Mycorrhizae 0.220.340.71	extrac tinteraction			

Table 4: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in leaves number.

Mycorrhizae	Palm pollen extract gm.L^{-1}				
gm/s	0	5	10	15	mean
0	277.33	311.65	312.22	315.32	304.13
10	291.63	293.00	322.63	345.55	313.20
20	313.00	339.44	346.56	382.12	345.28
mean	293.99	314.70	327.14	347.66	
L.S.D 5%	Mycorrhizae 8.4910.3118.86	extrac tinteraction			

Table 5: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in leaf content of chlorophyll SPAD.

Mycorrhizae	Palm pollen extract gm.L^{-1}				
gm/s	0	5	10	15	mean
0	28.12	30.45	31.88	33.18	30.91
10	33.53	34.67	35.77	36.80	35.19
20	35.16	37.32	38.61	39.25	37.59
mean	32.27	34.15	35.42	36.41	
L.S.D 5%	Mycorrhizae 0.500.731.98	extrac tinteraction			

Table 6: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in dry weight of the vegetative group gm.

Mycorrhizae	Palm pollen extract gm.L^{-1}				
gm/s	0	5	10	15	mean
0	21.22	23.61	25.63	29.14	24.90
10	26.13	28.45	29.52	31.47	28.89
20	30.42	33.69	35.64	37.75	34.38
mean	25.92	28.58	30.26	32.79	
L.S.D 5%	Mycorrhizae 0.590.751.36	extrac tinteraction			

Table 7: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in dry weight of the vegetative group gm.

Mycorrhizae	Palm pollen extract gm.L^{-1}				
gm/s	0	5	10	15	mean
0	10.42	11.62	12.53	13.24	11.95
10	11.33	12.49	14.62	15.42	13.47
20	13.46	15.59	17.79	19.65	16.62
mean	11.74	13.23	14.98	16.10	
L.S.D 5%	Mycorrhizae 0.490.771.20	extrac tinteraction			

the increase may be due to the impact of single or combined factors as previously described.

Second: The percentage of nutrients (NPK%) in leaves

The results of the tables 1, 2 and 3 showed that the bio-enriched mycorrhiza had a significant effect on the percentage of NPK in the leaves, the concentration 20 g /s gave the highest results (2.14%, 0.54%, 1.45%) respectively, this is due to the possibility of the mycorrhiza fungi in increasing the processing of plants with nutrients as a result of the extension of its widespread fungal filaments (Haifa) in the soil, allowing them to explore more soil size, causing an increase in the widespread area of the plant roots, enabling it to absorb the nutrients available in the soil solution (Siddiqui *et al.,* 2008), as well as playing a major role in biological processes leading to the NO_3 release from the organic sources of nitrogen in soil and protein building (Abdel – Latif and Chaoxing, 2011), and that about 21% of the nitrogen absorbed by the roots comes from external mysilim as the form of amino acid Arginine, which decomposes an enzyme to end with the release of ammonia to the internal Mysilim (Toussaint *et al.,* 2004), and then increase the nitrogen concentration in the vegetable tissue table 1. The nitrogen element enters many biochemical processes in the plant and it enters into the construction of the chlorophyll molecule, which increases the paper area and the leaves number (Table 3 and 4). Ruiz *et al*., (2000) reported that there is a close relationship between the amount of chlorophyll in the leaves and the accumulation of nitrogen in the dry matter, which is an indicator of nitrogen uptake from the soil. The symbiotic relationship between host plant roots and fungi allows the plant to access the phosphorus sources found in the soil by extending the fungal filaments to the surrounding areas of the rhizosphere and withdrawing the phosphorus and thus increasing its absorption by the root, the processing of plants with phosphorus element is one of the most important roles of the fungi that are carried out because it is immobile in the soil solution and the largest proportion of it is not ready for absorption. The mycorrhiza takes its complex dietary needs, such as amino acids, vitamins and carbohydrates from the host plant, while absorbing nutrients such as nitrogen, which promotes absorption of potassium and increases its concentration in leaves table 3, which has the effect of stimulating the process of carbon construction and then the transmission of its products to other parts of the plant and thus improve growth rates (Kaschuk *et al.,* 2010). The results were agreed with Aubied (2014) on date palms and with Khalaf and Abdul Latif (2013), when pollinating the carnation with mycorrhiza fungi, the plant content increased from N.P.K, Allawi (2013) found the same result on peppers, the results also agree with EL-Shamy and others (1990) on the citrus seedlings. In addition, the palm pollen extract had a significant effect on the percentage of nutrients under study and achieved a concentration of 15 g.L^{-1} the highest results were (2.31%, 0.53%, 1.42%) respectively, the

Table 1: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in percentage of nitrogen element in leaves (N%).

Mycorrhizae	Palm pollen extract gm.L^{-1}				
gm/s	0	5	10	15	mean
0	1.32	1.63	1.71	1.84	1.63
10	1.43	1.67	1.75	2.18	1.76
20	1.81	1.89	1.94	2.90	2.14
mean	1.52	1.73	1.80	2.31	
L.S.D 5%	Mycorrhizae 0.090.160.26	extrac tinteraction			

Table 2: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in percentage of phosphorus element in leaves (P%).

Mycorrhizae	Palm pollen extract gm.L^{-1}				
gm/s	0	5	10	15	mean
0	0.30	0.32	0.36	0.38	0.34
10	0.37	0.43	0.49	0.56	0.46
20	0.45	0.50	0.56	0.64	0.54
mean	0.37	0.42	0.47	0.53	
L.S.D 5%	Mycorrhizae 0.010.020.04	extrac tinteraction			

Table 3: Effect of Biofertilizer Mycorrhizae and Palm Pollen Extract in percentage of potassium element in leaves (K%).

Mycorrhizae	Palm pollen extract gm.L^{-1}				
gm/s	0	5	10	15	mean
0	0.59	0.75	0.86	0.95	0.79
10	0.65	0.81	0.94	1.36	0.94
20	0.93	1.30	1.61	1.94	1.45
mean	0.72	0.95	1.14	1.42	
L.S.D 5%	Mycorrhizae 0.060.070.14	extrac tinteraction			

effect of the pollen extract may be due to increased vegetative growth rates, such as plant height, stem diameter, number and area of leaves, chlorophyll content and dry weight of vegetative and root group, which increased absorption of N, P, K elements to meet the plant's need for these elements, The results were agreed with Abo AL-Mikh (2017) when spraying the pollen extract on pomegranate seedlings caused a significant increase in the percentage of elements N, P, K in leaves, The results are also consistent with Hazem (2011) and Bukhaev and others (1983). Interaction coefficients had a significant effect on the above characteristics, giving the interaction 20 g/s + 15 g.L^{-1} the highest values were 2.90%, 0.64% and 1.94% respectively, This may be due to the positive effect of mycorrhiza and the palm pollen extract in increasing the vegetative growth indicators shown above, which was reflected in the increase in leaf content of N, P, K, this may be due to the importance of the study factors in improving the soil ecosystem, which encouraged vegetative growth and thus increase the susceptibility of seedlings to absorb a large amount of nutrients to meet their requirements of these elements and increase their accumulation in the plant.

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