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INFLUENCE OF BIO AND MINERAL FERTILIZATION AND PALM POLEN EXTRACT IN GROWTH OF LIME SEEDLING

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Abstract

The research was conducted in the lath house belongs to the Al-Mussaib Technical College, AL-Furat Al-Awsat Technical University, Babylon Iraq during the period from March to November 2019, For the purpose of studying the effect of biofertilization treatment at four levels (without a biological vaccine, *Glomus mossa*, bacterial vaccine + mixture of fungal and bacterial vaccine), and the second factor is mineral fertilization at three levels (0, 7, 14) $g.L^{-1}$ and The third factor is palm pollen extract at three levels (0, 6, 12) $g.L^{-1}$. The factorial experiment conducted according to the Randomized Complete Block Design (R.C.B.D) and with three replicates, by three seedlings for the experimental unit, These results were analyzed using the variance analysis table (ANOVA table) according to the Genstat program. Statistical differences between the treatments were tested using the least significant difference (L.S.D)at probability level 0.05. The results showed that the bio fertilization factors (Mycorrhiza fungi and *Azospirillium snee brasil*) achieved significantly excelled in studied vegetative growth traits (leaf area and chlorophyll content) and chemical traits (NPK) in the leaves where they reached (1988 cm^2, 94.67 SAPD, 2.82%, 0.03%, 2.37% g), respectively. and The spraying with palm pollen extract at a concentration of 12 g. L^{-1} achieved a significant increase in most of the above mentioned vegetative growth and the chemical traits (nitrogen, phosphorus, and potassium) in the leaves. Bi-interaction treatments (B1C1, B1E2 and C1E2) had a significant effect on most of the studied traits.The triple interaction treatments (B1C1E1, B1C1E2, B1C2E1) significantly increased most studied vegetative and chemical traits (nitrogen, phosphorus, and potassium) in the leaves.

Key words: Bio- fertilization, Mycorrhiza, Bacteria, palm pollen grains extract, lime.

Introduction

Citrus belongs to the Rutaceae family, where it is characterized by the presence of oil glands in most parts of the plant, which gives the citrus a distinct aromatic scent. This family includes many genera, The most important genus (Citrus) of economic importance and given the abundance of species of the genus Citrus has been divided into five groups (orange group - mandarin - acid group - grapefruit group - hybrid group) (Al-Khafaji *et al.,* 1990). lime (*Citrus aurantifolia* L). is considered one of the evergreen trees and belongs to the Rutaceae family that grows in most countries such as Mexico, Italy, India, and America. These countries are the original country of lime (*Citrus aurantifolia* L) and then its cultivation began to spread in the rest of the countries (Murata, 1997). Biofertilizers are important matters in sustainable agriculture to regulate the production, protect the environment and produce crops free from vaccines as microbial vaccines supply the plant with its need of nutrients and facilitate its absorption by converting the elements form the that is not available to the form available for absorption by the plant, Biofertilizers also lead to the provision of some plant growth regulators and atmospheric nitrogen fixation through their symbiotic and non-symbiotic living, as well as plant protection from some pathogens, thereby reducing production costs and reducing environmental pollution (Al-Haddad, 2003, Al-Badawi, 2008). Chemical fertilization plays an important role in improving plant growth and increasing productivity, but the problem lies in the abundance of material burdens and the negative effects resulting from the use of chemical fertilizers. Therefore, we went to organic production (Al-Shaibani, 2005). Recent studies have tended to use plant organic extracts to improve vegetative growth and increase production for many plants, where these extracts contain nutrients, including growth regulators, organic acids and vitamins B1 and B2 (Abed Al-Hussain and Ibriham, 2009) Among these extracts is the pollen palm

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extract, and its chemical composition contains many nutrients in addition to amino acids, fatty acids, proteins and carbohydrates (Hazam, 2011).

Materials and Methods

The research was conducted in the lath house belongs to the Al-Mussaib Technical College, AL-Furat Al-Awsat Technical University, Babylon Iraq during the period from March to November 2019, For the purpose of studying the effect of biofertilization treatment at four levels (without a biological vaccine, *Glomus mossa*, bacterial vaccine + mixture of fungal and bacterial vaccine) and the second factor is mineral fertilization at three levels (0, 7, 14) $g.L^{-1}$ and The third factor is palm pollen extract at three levels (0, 6, 12) $g.L^{-1}$. on the growth of lime (*Citrus aurantifolia* L.) seedlings, The factorial experiment (4 * 3 * 3) conducted according to the Randomized Complete Block Design (R.C.B.D) and with three replicates by three seedlings for the experimental unit, the number of treatments reached 36 treatments distributed randomly to 324 seedlings with a one-year age and as homogeneous as possible cultivated in 5 kg bags It was converted into an 8 kg pot , filled with river soil +German Peat moss, in a ratio of 1: 1, The results were analyzed using the ANOVA table according to the Genstat program. Statistical differences between the treatments were tested using the least significant difference (LSD) test at probability level 0.05.

Studied traits

First : The vegetative traits

1-The leaf area (cm^2.seedlings^{-1}): measured according to (Dvornic *et al.,* 1965).

2- Chlorophyll (SPAD Unit) was measured with the SPAD-502 Chlorophyll meter.

Second - The percentage of some nutrients in the leaves:

Nitrogen: measured according to (Al-Sahaf, 1989)

Phosphorus: measured according to (Al-Sahaf, 1989).

Potassium: measured according to Horneck and Hanson (1998).

Results

First: the effect of bio and mineral fertilization and palm pollen extract on the vegetative traits of the lime (*Citrus aurantifolia* L).

The results in table 1 and 2 indicate that the use of bio fertilization caused a significant difference in the leaf area and the leaf content of chlorophyll, And the treatment of fungicide bio fertilization (B1) gave the highest values (1988 cm^2. Seedlings and 94.68 SPAD Unit) respectively. There was also a significant effect when adding mineral fertilizer, and all the treatments excelled on the control treatment, and the mineral fertilization treatment (concentration of 14 $g.L^{-1}$) gave the highest values (1982 cm^2, seedlings and 93.56 SPAD Unit), respectively. A significant effect was obtained when adding the palm pollen grain extract, so all the treatments excelled on the control treatment and gave the pollen extract treatments E2 (a concentration of 12 $g.L^{-1}$) and E1 (a concentration of 6 $g.L^{-1}$) with the highest values reached (1965 cm^2.Seedlings^{-1} and 93.73 SPAD Unit) respectively. The results of the tables also show the positive significant effect of bi interaction between bio and mineral fertilization. Both treatments significantly (B3C2 and B1C2) significantly excelled them by giving (2125 cm^2.Seedlings^{-1} and 101.37 SPAD Unit), respectively. , the bi-interaction between bio fertilization and palm pollen

Table 1: Effect of bio and mineral fertilization and palm pollen grain extract on the leaf area of seedlings (cm^2. Seedlings^{-1}).

C × B	Palm pollen extract			Mineral fertilization	Bio fertil -ization
	E 2	E 1	E 0		
1241	1402	1366	955	C 0	B 0
1652	1723	1756	1477	C 1	
1689	1740	1810	1517	C 2	
1746	1877	1883	1477	C 0	B 1
2121	2304	2209	1850	C 1	
2098	2192	2258	1844	C 2	
1743	1782	1893	1553	C 0	B 2
1987	2077	2078	1806	C 1	
2015	2165	2090	1789	C 2	
1716	1847	1768	1533	C 0	B 3
2040	2152	2110	1859	C 1	
2125	2323	2208	1843	C 2	
	1965	1952	1625	Average (E)	
C × B	E	E × C × B		L.S.D$_{(0.05)}$	
108.2	105.4	187.4			
Average (B)	Interaction bio fertilization × Palm pollen extract				
1527	1622	1644	1316	B 0	E × B
1988	2124	2117	1724	B 1	
1915	2008	2020	1716	B 2	
1960	2107	2029	1745	B 3	
62.5	108.2				L.S.D$_{(0.05)}$
Average (C)	Interaction Mineral fertilization × Palm pollen extract				
1611	1727	1727	1380	C 0	E × C
1950	2064	2038	1748	C 1	
1982	2105	2091	1748	C 2	
54.1	93.7			L.S.D$_{(0.05)}$	

extract showed significant excelled for the two treatments (B1E2 and B1E1) on the rest treatments by giving them (2124 $cm^2.Seedlings^{-1}$ and 104.86 SPAD Unit) respectively. Notice the significant effect of the interaction between mineral fertilization and palm pollen grain extract, the two treatments (C2E2) and (C2E2) excelled on the rest of the treatments by giving them (2105 $cm^2.Seedlings^{-1}$ and 102.10 SPAD Unit), respectively. B1C2E1) on the rest treatment by giving them (2323 $cm^2.Seedlings^{-1}$ and 113.07 SPAD Unit) respectively.

Second- The effect of bio and mineral fertilization and palm pollen extract on the percentage of nutrients (NPK) in the leaves.

The use of bio-fertilization caused a significant increase in the percentage of nutrients (NPK) in the leaves, and tables (3-5) show a significant difference of biofertilizers among them, despite all of them excelled on the control treatment (B0), where the fungal biofertilizer treatment (B1) (2.82 %) The mixture (B1) was 0.56% and the mixture (B2)2.37%, respectively . Also, there was a significant effect when adding mineral fertilizer in all the treatments. The control treatment was excelled and the mineral fertilization treatment (concentration of 14 $g.L^{-1}$) gave the highest values (2.84, 0.58 and 2.40)% respectively. A significant effect was obtained when adding the palm pollen extract. All the treatments excelled on the control treatment. The treatment for the palm pollen extract E1 (concentration of 6 $g.L^{-1}$) and E2 (at concentration 12 $g.L^{-1}$), the highest values were (2.81, 0.55 and 2.39)%, respectively. The results of the tables also show the positive significant effect of bi-interaction between bio and mineral fertilization. treatments(B1C2, B3C2 and B3C2) significantly differed on all treatments by giving them the values (3.27, 0.67 and 2.76)%, respectively. As for the bi-interaction between bio fertilization and palm pollen extract, there was significantly excelled of the two treatments (B3E2, B3E2 and B3E1)

Table 2: Effect of bio and mineral fertilization and palm pollen grain extract on the Chlorophyll content (SPAD Unit).

C × B	Palm pollen extract			Mineral fertilization	Bio fertil -ization
	E 2	E 1	E 0		
67.29	68.85	69.70	63.31	C 0	B 0
76.33	77.88	77.25	73.85	C 1	
80.90	88.31	82.59	71.80	C 2	
81.72	79.40	90.35	75.41	C 0	B 1
100.94	102.84	111.16	88.83	C 1	
101.37	112.47	113.07	78.59	C 2	
78.36	79.77	79.62	75.70	C 0	B 2
96.37	102.84	104.40	82.25	C 1	
95.77	101.57	104.20	81.53	C 2	
79.03	78.86	83.59	74.66	C 0	B 3
95.03	101.77	103.60	79.73	C 1	
96.21	106.07	105.20	77.37	C 2	
	91.68	93.73	76.92	Average (E)	
C × B	E	E × C × B		$L.S.D_{(0.05)}$	
5.924	2.962	10.261			
Average (B)	Interaction bio fertilization × Palm pollen extract				
74.84	78.34	76.52	69.65	B 0	E × B
94.68	98.23	104.86	80.94	B 1	
90.17	94.60	96.07	79.83	B 2	
90.09	95.56	97.46	77.25	B 3	
3.420	5.924				$L.S.D_{(0.05)}$
Average (C)	Interaction Mineral fertilization × Palm pollen extract				
76.60	76.72	80.81	72.27	C 0	E × C
92.17	96.23	99.10	81.17	C 1	
93.56	102.10	101.27	77.32	C 2	
2.962	5.131			$L.S.D_{(0.05)}$	

Table 3: Effect of bio and mineral fertilization and palm pollen grain extract on the percentage of Nitrogen(%) in leaves.

C × B	Palm pollen extract			Mineral fertilization	Bio fertil -ization
	E 2	E 1	E 0		
1.79	1.95	2.02	1.40	C 0	B 0
2.25	2.15	2.58	2.01	C 1	
2.15	2.27	2.23	1.94	C 2	
2.26	2.41	2.48	1.88	C 0	B 1
2.93	3.49	3.14	2.16	C 1	
3.27	3.14	3.54	3.13	C 2	
2.32	2.40	2.41	2.15	C 0	B 2
2.91	3.17	2.93	2.63	C 1	
2.95	3.10	3.49	2.27	C 2	
2.24	2.43	2.73	1.93	C 0	B 3
2.99	3.27	3.38	2.32	C 1	
3.00	3.51	3.10	3.38	C 2	
	2.77	2.81	2.18	Average (E)	
C × B	E	B × C × E		$L.S.D_{(0.05)}$	
0.166	0.083	0.288			
Average (B)	Interaction bio fertilization × Palm pollen extract				
2.06	2.13	2.28	1.78	B 0	E × B
2.82	3.01	3.05	2.39	B 1	
2.73	2.89	2.94	2.35	B 2	
2.74	3.07	2.95	2.21	B 3	
0.096	0.166				$L.S.D_{(0.05)}$
Average (C)	Interaction Mineral fertilization × Palm pollen extract				
2.15	2.30	2.32	1.84	C 0	E × C
2.77	3.02	3.01	2.28	C 1	
2.84	3.01	3.09	2.43	C 2	
0.083	0.144			$L.S.D_{(0.05)}$	

on the rest of the treatments by giving them the values (3.07, 0.62 and 2.81)% respectively. It is noted that the combined effect between mineral fertilization and palm pollen extract has a significant effect. All the treatment excelled on the control treatment, where (C2E1) and (C1E2 and C2E2) gave the highest values (3.09, 0.61 and 2.78%), respectively, the triple interaction indicated the significant excelled on the treatments (B1C2E1, B1C1E2 and B3C2E1) on the rest of the treatments by giving them the values (3.54, 0.70 and 3.22)%, respectively.

Discussion

The results in tables 1-5 show the high average of the studied traits above when using biofertilizers. The significant increase in the leaf area and the leaf content of chlorophyll may be due to the biofertilizer, whether it is fungal, bacterial, or a mixture of both, due to the secretion of these bacteria to the substances that stimulate and It activates growth, such as Auxin, gibberellins, and cytokines, in addition to its effectiveness in bio-resistance and stimulation of other beneficial organisms in the soil (El-Sayed, 2006). As for the fungi, they led to an increase in the roots volume through its contribution to dissolving insoluble forms of phosphorus and some minor elements, including iron, manganese, zinc and stimulating the absorption of potassium, which was reflected in improving plant growth and increasing the leaf area. Bio fertilization has a role in improving the biological and chemical properties of the soil, Which works to prepare many nutrients that are absorbable by the seedlings roots, which reflects positively on the physiological processes of seedlings such as improving photosynthesis in the leaves of the seedlings (Yu *et al.,* 2014). It was noted in the tables the high average of studied traits when using mineral fertilizers. The reason may be due to the ease

Table 4: Effect of bio and mineral fertilization and palm pollen grain extract on the percentage of Phosphorous(%) in leaves.

C × B	Palm pollen extract			Mineral	Bio fertil
	E 2	E 1	E 0	fertilization	-ization
0.32	0.39	0.33	0.26	C 0	B 0
0.40	0.42	0.44	0.35	C 1	
0.41	0.46	0.43	0.34	C 2	
0.41	0.44	0.44	0.37	C 0	B 1
0.57	0.70	0.56	0.45	C 1	
0.59	0.69	0.63	0.44	C 2	
0.41	0.44	0.47	0.34	C 0	B 2
0.60	0.64	0.69	0.47	C 1	
0.56	0.53	0.67	0.48	C 2	
0.42	0.49	0.44	0.33	C 0	B 3
0.61	0.68	0.69	0.45	C 1	
0.67	0.69	0.66	0.65	C 2	
	0.55	0.54	0.41	Average (E)	
B × C	E	B × C ×E		L.S.D$_{(0.05)}$	
0.023	0.012	0.041			
Average (B)	Interaction bio fertilization × Palm pollen extract				
0.38	0.42	0.40	0.31	B 0	B × E
0.53	0.61	0.54	0.42	B 1	
0.52	0.53	0.61	0.43	B 2	
0.56	0.62	0.60	0.48	B 3	
0.014	0.023				L.S.D$_{(0.05)}$
Average (C)	Interaction Mineral fertilization × Palm pollen extract				
0.39	0.43	0.42	0.31	C 0	C × E
0.54	0.61	0.60	0.43	C 1	
0.56	0.59	0.59	0.48	C 2	
0.012	0.020			L.S.D$_{(0.05)}$	

Table 5: Effect of bio and mineral fertilization and palm pollen grain extract on the percentage of potassium(%) in leaves.

C × B	Palm pollen extract			Mineral	Bio fertil
	E 2	E 1	E 0	fertilization	-ization
1.12	1.21	1.23	0.91	C 0	B 0
1.97	2.08	1.99	1.84	C 1	
1.73	2.08	1.91	1.19	C 2	
1.73	1.91	2.04	1.23	C 0	B 1
2.43	2.87	2.50	1.91	C 1	
2.46	3.11	2.48	1.81	C 2	
1.78	2.07	1.96	1.30	C 0	B 2
2.57	2.93	2.39	1.86	C 1	
2.66	2.93	2.97	2.07	C 2	
1.77	1.93	2.07	1.30	C 0	B 3
2.57	2.50	3.13	2.08	C 1	
2.76	2.99	3.22	2.07	C 2	
	2.39	2.37	1.63	Average (E)	
C × B	E	E × C × B		L.S.D$_{(0.05)}$	
0.061	0.031	0.106			
Average (B)	Interaction bio fertilization × Palm pollen extract				
1.60	1.79	1.71	1.32	B 0	E × B
2.21	2.63	2.34	1.65	B 1	
2.34	2.65	2.62	1.74	B 2	
2.37	2.48	2.81	1.81	B 3	
0.035	0.061				L.S.D$_{(0.05)}$
Average (C)	Interaction Mineral fertilization × Palm pollen extract				
1.60	1.78	1.82	1.19	C 0	E × C
2.39	2.60	2.64	1.92	C 1	
2.40	2.78	2.64	1.79	C 2	
0.031	0.053			L.S.D$_{(0.05)}$	

and speed of melting of mineral fertilizers. Thus, the availability of the nutrients in the soil solution increases and is absorbed by the seedlings roots (Saunders, 2001). The addition of nitrogen fertilizers causes a significant excelled in the leaf area (Belanger *et al.,* 2002). Peter and Rosen (2005) show that the percentage of nitrogen in the leaves can be estimated by their chlorophyll content, meaning that most of the nitrogen in the plant has its concentration in the leaf and the nitrogen component has a significant role in forming large-size leaves rich in chlorophyll, The results of the above tables also show that the leaves feeding with the extract of palm pollen extract has caused an increase in the number of leaves and the leaf area due to the nutrients it contains (Zn. Mn. Fe. K. Ca. Mg. P) and other important compounds such as organic acids, vitamins and Proteins in stimulating the process of photosynthesis and increasing the proportion of chlorophyll, respiration and other metabolic processes that lead to a high average of vegetative growth of seedlings through cell division, which reflects positively on the increase in the leaf area and the leaf content of chlorophyll. These results are consistent with (Abdul Hussain and Zaid, 2016; Al-Falahi and Al-Janabi, 2016). The increase in the percentage of nutrients (NPK) in the leaves when using biological fertilizers, whether bacterial or fungal, or a mixture of both may be due to the fact that beneficial microorganisms are characterized by their ability to secrete some enzymes such as Protease, Phosphatase, Dehydrogenase (Tirol-Padre *et al.,* 2007; Melero *et al.,* 2008), which increases the availability of the elements due to the metallic process and prevents their fixation by forming complexes with it (Agbede *et al.,* 2008). The secretion of some hormones, such as Auxin, gibberellins and cytokinins by bacterial genera, has a positive effect on nutrient uptake (Spaepen *et al.,* 2008; Bottini *et al.,* 2004; Timmusk *et al.,* 1999). The high percentage of phosphorus in seedlings pollinated with biofertilizers is due to the role played by the Mycorrhiza fungus in the absorption of phosphate ions that are located in areas far from the root system due to the prevalence of Hypha fungi (Smith and Read, 1997). Mineral fertilization has a role in increasing the proportion of nitrogen, phosphorous and potassium elements in the plant by enlarging the surface area of the roots, which has a role in increasing the absorption of mineral elements. That the palm pollen grains extract has a significant effect on increasing the percentage of nitrogen, phosphorus and potassium, and the reason may be due to its increased absorption by the seedlings leaves where a result of the convergence of the treatment periods, which caused an increase in the efficiency of photosynthesis and other biological processes inside the plant and thus increase the withdrawal of nutrients, including the important nitrogen component in the processes The plant bio and the synthesis of many plant materials and compounds as it enters into the construction of a chlorophyll molecule, which leads to an increase in the number of leaves formed on the plant and the total leaf area .The increase in the absorption of nutrients such as nitrogen encourages an increase in the absorption of potassium, which has the effect of stimulating the carbon building process and then the transmission of its products to other parts of the plant. It is also an ionic and enzymatic regulator of many physiological processes and thus improves growth average (Devlin and Wittam, 1998). Organic extracts are a mixture of Macro and Microelements and important components of growth such as nitrogen, phosphorus, potassium, iron, etc., and that their high concentration to a certain extent is positively reflected on their absorption by the plant and their content increases in the leaves (Osman *et al.,* 2010).

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