

## RESPONSE OF LOCAL ORANGE TRANSPLANTS TO BIO AND ORGANIC FERTILIZERS AND SPRAYING WITH MORINGA LEAVES EXTRACT

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### ABSTRACT

This experiment was carried out in 2023 season of spring and fall growth cycle in a private canopy in Al-Radwanayah city, Baghdad Governorate, study included addition of bacterial biofertilizer at four treatments: no addition ( $A_1$ ), addition of *Stenotrophomonas maltophilia* bacteria ( $A_2$ ), addition of *Azospirillum brasilense* bacteria ( $A_3$ ) and addition of both types of bacteria ( $A_4$ ) and organic fertilizer (Decomposing buffalo manure) at three levels (0, 250 and 500 g.soil<sup>-1</sup>), which are symbolized by  $B_0$ ,  $B_1$ , and  $B_2$ , respectively Added once during the spring and fall growing cycle, and spray moringa extract at three levels (0, 20 and 40 g.L<sup>-1</sup>), which are symbolized  $M_0$ ,  $M_1$ , and  $M_2$ , respectively spray 5 times every 15 days. Treatments were replicated three times at factorial experiment in a RCBD and thus number of transplants used was 108. Experimental results showed that adding two types of bacteria ( $A_4$ ) and buffalo manure at a level of 500 g.soil<sup>-1</sup> ( $B_2$ ) and spraying moringa extract at a concentration of 40 g.L<sup>-1</sup> ( $M_2$ ) as interaction treatment  $A_4B_2M_2$  produced best results in shoot number, stem diameter, leaf potassium and iron percent %, and epidermal and spongy cells layers thickness, (25.00, 41.00) shoot.plant<sup>-1</sup>, (14.247, 5.283) mm, (2.980, 2.885) %, (215.8, 220.7) mg.kg<sup>-1</sup> for two growing seasons, respectively, and (48.60, 108.4) micrometer, respectively compared to control treatment.

Keywords: citrus, Bacteria, potassium, growth, anatomy, Buffalo

\*Part of Ph.D. Dissertation for 1<sup>st</sup> authors.

المرسومي والحديثي

مجلة العلوم الزراعية العراقية- 2025 :56 (عدد خاص):179-189

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### المستخلص

أجريت التجربة أثناء موسم النمو 2023 خلال دورتي النمو الربيعية والخريفية في ظللة خاصة في منطقة الرضوانية الواقعة في محافظة بغداد، تضمنت الدراسة إضافة السماد الحيوي البكتيري بأربعة مستويات هي: بدون إضافة ( $A_1$ )، إضافة بكتريا (*Stenotrophomonas maltophilia*) ( $A_2$ )، إضافة بكتريا (*Azospirillum brasilense*) ( $A_3$ ) وإضافة كلا نوعي البكتريا ( $A_4$ ) والسماد العضوي (مخلفات الجاموس المتحللة) بثلاث مستويات (0 و 250 و 500 غم.تربة<sup>-1</sup>) والتي يرمز لها بالرموز  $B_0$  و  $B_1$  و  $B_2$  على التوالي يضاف مرة واحدة خلال دورة النمو الربيعي والخريفي، ورش مستخلص المورينجا بثلاث مستويات (0 و 20 و 40 غم.لتر<sup>-1</sup>) والتي يرمز لها بالرموز  $M_0$  و  $M_1$  و  $M_2$  على التوالي، ترش 5 مرات بين رشة وأخرى 15 يوم. تكررت المعاملات ثلاث مرات في تجربة عاملية RCBD وبذلك يكون عدد الشتلات المستعملة في التجربة 108 شتلة. أظهرت نتائج الدراسة أن إضافة نوعي البكتريا معاً ( $A_4$ ) والسماد العضوي للجاموس بتركيز 500 غم.تربة<sup>-1</sup> ( $B_2$ ) والرش بمستخلص المورينجا بتركيز 40 غم.لتر<sup>-1</sup> ( $M_2$ ) متمثلة بمعاملة التداخل  $A_4B_2M_2$  أعطت أفضل النتائج في عدد الأفرع وقطر الساق والنسبة المئوية للبوتاسيوم وتركيز الحديد في الأوراق وسمك طبقتي البشرة والخلايا الإسفنجية بقيم بلغت (25.00 و 41.00) فرع.شتلة<sup>-1</sup>، (14.247 و 5.283) ملم، (2.980 و 2.885) %، (215.8 و 220.7) ملغم.كغم<sup>-1</sup> لموسمي النمو على التوالي، (48.60 و 108.4) مايكرومتر، على التوالي، قياساً بمعاملة المقارنة.

كلمات مفتاحية: الحمضيات، البكتريا، البوتاسيوم، النمو، التشريح، الجاموس

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Received:26/2/2024, Accepted:22/5/2024

## INTRODUCTION

The word "citrus" refers to a group of fruit trees that are characterized by presence of oil glands in their leaves, which give them a distinctive aromatic scent that distinguishes them from other types of fruit. Citrus fruits belong to Rutaceae family, which includes 13 genera and 65 species spread in tropical and subtropical regions, including Citrus genus, which is considered one of most important genera because it includes most species and genera of economic importance (12). Most historians and scientists believe that original homeland of different types of citrus is not precisely known and is likely to be tropical and subtropical regions of Southeast Asia, specifically Western India, China, Indonesia, some parts of Burma and some regions of Southwest Asia (11,34). Salah al-Din Governorate is ranked first in orange production in Iraq, followed by Baghdad Governorate, then Diyala. As for the Arab world, Egypt is the first country in orange production, followed by Algeria (17). Its cultivation has now spread in large areas of the world and has taken its global economic place because citrus trees are characterized by a special position among fruit trees, because their fruits are of great importance from a nutritional, economic, and even cosmetic standpoint, as they are included in human food in different shapes and forms, and may be eaten fresh, uncooked. Therefore, it retains the largest amount of vitamins and therefore has a high nutritional value. This may be due to its chemical composition, which includes many chemical elements, including carbohydrates, fats, proteins, and some organic acids, addition to fibers (19). Biofertilizers are known as preparations that contain microscopic organisms that have ability to supply plants with nutrients they need from natural sources, with the aim of reducing dependence on various chemical fertilizers (18), as these fertilizers are capable of continuously releasing nutrients. This makes it able to meet plants' need for chemical fertilizers. Biofertilizer consists of an organism or group of organisms that produce substances that help enrich soil with nutrients. It also helps plants absorb nutrients by interfering with root zone, which represents most active area in soil (24),

by accelerating certain vital processes that convert nutrients into forms and shapes available to the plant so that it can easily absorb and assimilate them (37). Plant nutrition is greatly affected by interaction that occurs between roots and soil microbes through direct and indirect mechanisms represented by manufacture of plant hormones, nitrogen fixation, organic phosphate mineralization, and others (35). There are some currently produced biofertilizers that are added scattered or mixed with soil or with seeds (29), most important of which are nitrogenous biofertilizers that fix atmospheric nitrogen in soil, such as *Stenotrophobacter* bacteria that fix nitrogen freely and *Azospirillum* that fix nitrogen freely association with plant (10,31). Many studies have proven role of biofertilizers in plant growth (3, 9), (23) Also found in his study on response of three citrus rootstocks to organic and biological fertilizers that addition of biological fertilizers, especially *A. brasilense* showed a significant superiority in most vegetative growth traits and leaves mineral content. Organic agriculture is a production system that maintains health of agricultural productivity and maintains soil health and ecosystem, as organic fertilization is one of important means and methods that provide plants with their need for nutrients without a negative impact on environment, and increasing it does not lead to damage to plant that occurs. When fertilizing with mineral fertilizers in large quantities, organic matter also has a role in plant growth and yield, whether it is added to soil or sprayed on shoots (6, 30). Among most important organic fertilizers are decomposed buffalo waste, which contains large quantities Available nitrogen and also contains large quantities of phosphorus and potassium. It is considered most potassium content among rest of waste and has a major role in conserving water and improving soil physical and chemical properties (21). As for plant extracts, they have recently gained attention of scientists and researchers by using them as alternatives or supplements to fertilizers and agricultural pesticides, as they can be added to soil or used as a spray on plant's shoots, because some of these extracts contain some nutrients and some substances that work action

of growth regulators is an alternative to them, in addition to containing some compounds that are used to combat diseases and insects, and among these extracts is moringa leaf extract (14). There are many studies that indicated positive effect of spraying moringa extract on plant growth. In a study (26) which included spraying moringa extract at concentrations of 0, 5, and 10%, it led to a significant increase in vegetative and fruiting traits of strawberry plants. So, among benefits of organic and biofertilizers are increasing soil nutrients availability, increasing tree productivity, improving fruit quality, in addition to improving soil traits and increasing microorganisms numbers in root zone, as well as improving plant tolerance to biotic and abiotic factors (5,35). Study aims to effect of biofertilizer and buffalos' manure and moringa extract spray in vegetative growth traits of local orange transplants.

#### MATERIALS AND METHODS

The experiment was carried out in 2023 season of spring growth cycle in a private canopy in Al-Radwanayah city, Baghdad Governorate to study effect of adding bacterial biofertilizer, buffalo organic fertilizer, and spraying moringa leaves extract on growth and leaves mineral content of local orange transplants of two years old planted in plastic pots, Atransplants of uniform in their vegetative growth, as far as possible, free of disease and insect infestations, study included addition of bacterial biofertilizer at four treatments: no addition ( $A_1$ ), addition of *Stenotrophomonas maltophilia* bacteria ( $A_2$ ), addition of *Azospirillum brasilense* bacteria ( $A_3$ ) and addition of both types of bacteria ( $A_4$ ) (3) and organic fertilizer (Decomposing buffalo manure) at three levels (0, 250 and 500 g.soil<sup>-1</sup>), which are symbolized by  $B_0$ ,  $B_1$ , and  $B_2$ , respectively and spray moringa extract at three levels (0, 20 and 40 g.L<sup>-1</sup>), which are symbolized  $M_0$ ,  $M_1$ , and  $M_2$ , respectively (32). Bacterial biofertilizer and buffalo organic fertilizer were added once during spring growth cycle in 15th January, and once during fall growth cycle in 15th September. Moringa leaves extract were sprayed five time period between one spraying and next is 15 days, as first spraying was done on 1<sup>st</sup> March and last spray was in 1<sup>st</sup> May of spring growth cycle,

while first spraying for fall cycle was done 10 September. Treatments were replicated three times at factorial experiment in a RCBD. The results of study were statistically analyzed and averages were compared according to (L.S.D) at 0.05 according to (16), The following parameters were determined:

1. Vegetative traits (shoots number, stem diameter).
2. Leaf Mineral Content: potassium (It was determined according to the method (38), Iron (It was determined according to the method (15)).
3. Anatomical characteristics: According to the method (7).

#### RESULTS AND DISCUSSION

shoots number and stem diameter: Tables (1 and 2) shows that experimental factors had a significant effect on studied vegetative traits, as treatment of adding two types of bacterial biofertilizers ( $A_4$ ) excelled in produced it highest increase in shoots number of (14.48, 25.11) shoot.plant<sup>-1</sup> for two growing seasons, respectively, compared to control treatment ( $A_1$ ), while ( $A_2$ ) treatment, which is addition of *S. maltophilia* bacteria, produced highest increase in stem diameter of 10.67 mm for Spring growing season, and ( $A_4$ ) treatment produced highest increase in stem diameter for fall growing season of 2.626 mm, compared to other treatments. Organic fertilization treatment ( $B_2$ ) also excelled in produced highest increase in shoots number and stem diameter of (14.97, 26.47) shoot plant<sup>-1</sup>, and (10.84, 3.209) mm for two growing seasons, respectively, compared to control treatment ( $B_0$ ). Moringa leaves extract spraying treatment ( $M_2$ ) also excelled it produced highest values for studied traits of (14.00, 23.42) shoot. Plant-1 and (10.77, 2.279) mm for two growing seasons, respectively, compared to control treatment ( $M_0$ ). As for twice interactions between experimental factors, they shows significant superiority in vegetative traits, as treatments ( $A_4B_2$ ), ( $A_4M_2$ ), and ( $B_2M_2$ ) excelled by produced them highest increase in shoot number and stem diameter of (19.11, 32.11 shoot plant<sup>-1</sup> and 12.07, 4.380 mm), (17.44, 28.78 shoot plant<sup>-1</sup> and 11.49, 3.166 mm), (18.17, 29.08 shoot plant<sup>-1</sup> and 12.25, 3.942 mm) for two growing seasons, respectively. Triple interactions

between study factors had a significant effect in these traits as treatment (A<sub>4</sub>B<sub>2</sub>M<sub>2</sub>) excelled by produced them highest increase in shoot number and stem diameter of (25.00, 41.00 shoot. plant<sup>-1</sup> and 14.247, 5.283 mm) for two growing seasons, respectively. Results show that adding bacteria to pots soil had a positive effect on studied vegetative growth traits. Increase in vegetative traits may be attributed to effect of these bacteria in improving soil's biological and physical traits as well as its chemical properties, which resulted in release of larger amounts of soil nutrients available for absorption by roots and thus increased influence on physiological processes such as increasing leaves photosynthesis efficiency (39) and increasing its products represented by carbohydrates, thus increasing vegetative growth of transplants. Reason may also be due to increased ability of these bacteria added to soil to produce plant growth regulators such as

auxins, cytokinins, and gibberellins, as auxin produced from these organisms increases vegetative growth of transplants due to its role in increasing divisions and thus increasing transplants growth, as (20) indicated spraying tangerine plants with tryptophan, which is initiating acid for building natural auxin (IAA), works to increase most of vegetative growth traits of transplants. Cytokinins resulting from these organisms added to soil also works to encourage buds formation on vegetative growths growing on transplants and to increase shoots number, in addition to about vital role of cytokinin in reducing inhibitory effect of auxins present in lateral shoots, and then encouraging these shoots to grow, which works to improve vegetative traits. Therefore, it affects increase in vegetative growth of orange transplants (13). These results are consistent with (1) on Pomelo transplants.

**Table 1. Effect of adding biofertilizer, buffalo manure and moringa leaves extract spray and their interaction on increase in shoots number (shoot. Plant<sup>-1</sup>) of local orange transplants (spring and fall season 2023).**

Biofertilizers (A)	Buffalo Manures (B)	Spring 2023				Fall 2023			
		Moringa Extract (M)			A × B	Moringa Extract (M)			A × B
		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	
A <sub>1</sub>	B <sub>0</sub>	4.33	5.67	7.00	5.67	10.33	12.33	14.67	12.44
	B <sub>1</sub>	8.67	9.33	11.33	9.78	20.00	20.67	21.00	20.56
	B <sub>2</sub>	9.33	7.00	12.33	9.56	21.33	21.67	21.67	21.56
A <sub>2</sub>	B <sub>0</sub>	8.00	12.00	12.00	10.67	15.33	19.00	19.67	18.00
	B <sub>1</sub>	11.00	14.00	14.33	13.11	20.00	22.67	23.00	21.89
	B <sub>2</sub>	13.00	17.00	18.67	16.22	24.67	26.00	28.33	26.33
A <sub>3</sub>	B <sub>0</sub>	7.33	10.33	10.67	9.44	15.00	16.67	18.33	16.67
	B <sub>1</sub>	11.33	13.33	12.67	12.44	21.33	22.00	22.67	22.00
	B <sub>2</sub>	11.67	16.67	16.67	15.00	24.00	28.33	25.33	25.89
A <sub>4</sub>	B <sub>0</sub>	8.33	9.33	11.00	9.56	16.33	20.67	20.67	19.22
	B <sub>1</sub>	11.67	16.33	16.33	14.78	23.00	24.33	24.67	24.00
	B <sub>2</sub>	11.33	21.00	25.00	19.11	25.00	30.33	41.00	32.11
LSD 5%		2.065			1.192	3.488			2.014
A × M		A			A × M			A	
A <sub>1</sub>	7.44		7.33	10.22	8.33	17.22	18.22	19.11	18.19
A <sub>2</sub>	10.67		14.33	15.00	13.33	20.00	22.56	23.67	22.07
A <sub>3</sub>	10.11		13.44	13.33	12.30	20.11	22.33	22.11	21.52
A <sub>4</sub>	10.44		15.56	17.44	14.48	21.44	25.11	28.78	25.11
LSD 5%		1.192			0.688	2.014			1.163
B × M		B			B × M			B	
B <sub>0</sub>	7.00		9.33	10.17	8.83	14.25	17.17	18.33	16.58
B <sub>1</sub>	10.67		13.25	13.67	12.53	21.08	22.42	22.83	22.11
B <sub>2</sub>	11.33		15.42	18.17	14.97	23.75	26.58	29.08	26.47
LSD 5%		1.033			0.596	1.744			1.007
M		9.67		12.67	14.00	19.69	22.06	23.42	
LSD 5%		0.596			1.007				

**Table 2. Effect of adding biofertilizer, buffalo manure and moringa leaves extract spray and their interaction on increase in stem diameter (mm) of local orange transplants (spring and fall season 2023).**

Biofertilizers (A)	Buffalo Manures (B)	Spring 2023				Fall 2023			
		Moringa Extract (M)			A × B	Moringa Extract (M)			A × B
		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	
A <sub>1</sub>	B <sub>0</sub>	6.010	6.360	8.147	6.839	0.327	0.357	0.543	0.409
	B <sub>1</sub>	9.177	9.167	8.847	9.063	1.317	1.370	1.390	1.359
	B <sub>2</sub>	9.427	6.767	10.72	8.971	1.440	1.537	1.563	1.513
A <sub>2</sub>	B <sub>0</sub>	8.140	10.77	11.35	10.08	0.703	1.083	0.507	0.764
	B <sub>1</sub>	9.74	10.93	10.81	10.49	1.197	1.717	1.993	1.636
	B <sub>2</sub>	10.11	11.99	12.18	11.43	2.467	3.157	4.387	3.337
A <sub>3</sub>	B <sub>0</sub>	8.387	8.753	9.637	8.926	0.537	0.747	0.953	0.746
	B <sub>1</sub>	9.907	10.10	11.26	10.42	1.540	1.610	1.977	1.709
	B <sub>2</sub>	9.870	10.96	11.86	10.90	2.420	3.870	4.533	3.608
A <sub>4</sub>	B <sub>0</sub>	8.310	9.257	9.417	8.994	0.723	1.277	1.300	1.100
	B <sub>1</sub>	10.43	11.25	10.81	10.83	2.033	2.243	2.913	2.397
	B <sub>2</sub>	9.837	12.13	14.24	12.07	3.033	4.823	5.283	4.380
LSD 5%			1.320		0.762		0.610		0.352
A × M					A		A × M		A
A <sub>1</sub>		8.204	7.431	9.238	8.291	1.028	1.088	1.166	1.094
A <sub>2</sub>		9.332	11.23	11.44	10.67	1.456	1.986	2.296	1.912
A <sub>3</sub>		9.388	9.942	10.92	10.08	1.499	2.076	2.488	2.021
A <sub>4</sub>		9.528	10.88	11.49	10.63	1.930	2.781	3.166	2.626
LSD 5%			0.762		0.440		0.352		0.203
B × M					B		B × M		B
B <sub>0</sub>		7.712	8.785	9.639	8.712	0.573	0.866	0.826	0.755
B <sub>1</sub>		9.815	10.36	10.43	10.20	1.522	1.735	2.068	1.775
B <sub>2</sub>		9.812	10.46	12.25	10.84	2.340	3.347	3.942	3.209
LSD 5%			0.660		0.381		0.305		0.176
M		9.113	9.872	10.77		1.478	1.982	2.279	
LSD 5%			0.381				0.176		

It was observed from results of Tables (1-2) an increase in vegetative growth indicators of orange transplants, reason for this increase may be due to role of organic manures in increasing proportion of elements in soil solution, which leads to an increase in cell expansion and division, which leads to an increase in stem diameter and an increase in growth strength in trees, thus improving vegetative growth. In addition, participation of major elements, especially nitrogen, which contributes to building plant's vegetative system and representation of vital chlorophyll pigment, thus increasing leaves mineral content and increasing their area and other vegetative characteristics, in addition to role of these dissolved elements in soil solution and ready for absorption in formation of compounds and some components of the basic processes of photosynthesis and respiration, in addition to its contribution to synthesis and increase in activity of a large number of

enzymes, which is reflected in increase in vegetative growth (40). The decomposition of organic manure in soil also leads to formation of organic acids such as humic and fulvic acids, and formation of natural chelates that contribute to release of potassium and other elements from soil elements in root system, this release of elements increases with increase in release of organic acids produced by decomposing organic materials, as these increase in elements coincided with an increase in levels of buffalo waste, which indicates an effect of these levels (22, 35). These results may be due to Moringa leaf extract containing plant hormone IAA and a number of amino acids, including Tryptophan, which is initiator in building natural auxin IAA, as latter works to increase vegetative growth due to its role in increasing divisions and thus its impact on growth. (20) Indicated that spraying tangerine transplants with tryptophan increases most of vegetative

growth traits. It is also due to fact that moringa leaf extract contains large amounts of zeatin, which natural cytokinin is found in plant. Cytokinin works to encourage buds formation on vegetative growths growing on plants and increase shoots number, in addition to vital role of cytokinin in reducing inhibitory effect of auxins present in buds then encouraged growing, which improves vegetative growth. The results are also attributed to fact that this extract contains some necessary nutritional elements, which may contribute to increasing plants vegetative growth (27, 33). These results are consistent with what was found by (8) on orange transplants, (2) on apple trees.

### Potassium and Iron

It was shows from Tables (3 and 4) that addition of bacterial biofertilizer had a significant effect in leaf potassium and iron concentration, as ( $A_4$ ) treatment produced highest leaf content of (2.109, 1.977)% and (204.2, 208.5)  $\text{mg.kg}^{-1}$  for Potassium and Iron for two growing seasons, respectively, compared to control treatment ( $A_1$ ), and organic fertilization treatment ( $B_2$ ) also produced highest concentration of (2.347, 2.216)% and (206.7, 210.4)  $\text{mg.kg}^{-1}$  for potassium and iron for two growing seasons, respectively, compared to control treatment ( $B_0$ ). spraying treatment of moringa leaves extract ( $M_2$ ) produced highest leaf concentration of (1.974, 1.931) % and (193.9, 198.6)  $\text{mg.kg}^{-1}$  for potassium and iron for two growing seasons, respectively, compared to ( $M_0$ ) treatment. twice interaction treatments between study factors also shows a significant superiority in leaves concentration of K and Fe as the treatments ( $A_4B_2$ ), ( $A_4M_2$ ), and ( $B_2M_2$ ) were they produced (2.665, 2.509% and 215.2, 218.6  $\text{mg kg}^{-1}$ ), (2.247, 2.065% and 206.3, 211.4  $\text{mg kg}^{-1}$ ), (2.465, 2.503%, and 209.2,

212.9  $\text{mg kg}^{-1}$ ) for potassium and iron for two growing seasons, respectively. The triple interactions between study factors had a significant effect in leaves concentration of K and Fe as the treatments ( $A_4B_2M_2$ ) of (2.980, 2.885%, 215.8, 220.7  $\text{mg.kg}^{-1}$ ) for two growing seasons, respectively, compared to control treatment ( $A_1B_0M_0$ ). As for increase in leaves mineral concentration as a result of adding bacteria, it is due to fact that adding these organisms to soil led to an increase in concentration of these elements in soil solution, thus increasing their availability and thus increasing their absorption by plant roots and increasing their transport, thus increasing concentration of these elements in leaves. Many researchers have also confirmed that increasing element concentration in soil solution leads to increased absorption by plant (28). Adding these organisms to soil leads to an increase in leaves mineral content as a result of increased growth and photosynthesis efficiency by increasing shoots number, diameter of the stem (tables 1 and 2) and increasing soil's content of these elements as a result of adding them to soil, which led to increased absorption (36). These results are consistent with (4) found on peaches, and with (25) on oranges, as they found that adding this bacterium led to significant differences in leaf mineral content compared to not adding it. As for effect of moringa leaf extract spray, tables (3 and 4) shows that there is an increase in leaf mineral content in orange transplants, and that this increase may be due to this extract containing macronutrient, especially nitrogen and potassium, in addition to micronutrient, including iron, which are absorbed directly, especially when spray it on leaves and thus increase its percentage in plant (32).

**Table 3. Effect of adding biofertilizer, buffalo manure and moringa leaves extract spray and their interaction on leaf Potassium percent (%) of local orange transplants (spring and fall season 2023).**

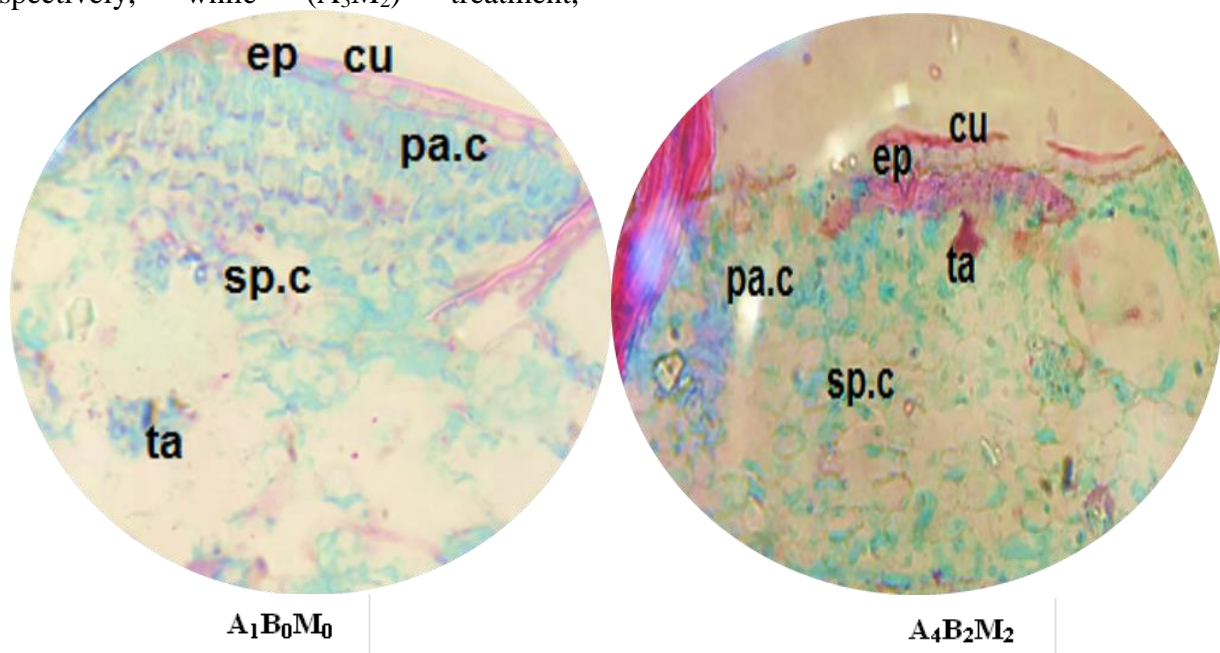
Biofertilizers (A)	Buffalo Manures (B)	Spring 2023				Fall 2023			
		Moringa Extract (M)			A × B	Moringa Extract (M)			A × B
		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	
A <sub>1</sub>	B <sub>0</sub>	1.365	1.420	1.455	1.413	0.848	0.938	1.245	1.010
	B <sub>1</sub>	1.735	1.740	1.760	1.745	1.485	1.620	1.710	1.605
	B <sub>2</sub>	2.100	2.125	2.150	2.125	1.755	2.003	2.400	2.053
A <sub>2</sub>	B <sub>0</sub>	1.530	1.530	1.540	1.533	1.323	1.344	1.861	1.509
	B <sub>1</sub>	1.790	1.825	2.015	1.877	1.582	1.708	1.750	1.680
	B <sub>2</sub>	2.185	2.380	2.420	2.328	1.925	2.128	2.296	2.116
A <sub>3</sub>	B <sub>0</sub>	1.460	1.473	1.510	1.481	1.700	1.328	1.410	1.479
	B <sub>1</sub>	1.755	1.780	1.790	1.775	1.643	1.755	1.875	1.758
	B <sub>2</sub>	2.193	2.300	2.310	2.268	2.040	2.093	2.430	2.188
A <sub>4</sub>	B <sub>0</sub>	1.545	1.625	1.690	1.620	1.333	1.346	1.385	1.354
	B <sub>1</sub>	2.025	2.035	2.070	2.043	1.762	2.522	1.924	2.069
	B <sub>2</sub>	2.480	2.535	2.980	2.665	2.184	2.457	2.885	2.509
LSD 5%			0.692		0.400		0.722		0.417
	A × M				A		A × M		A
A <sub>1</sub>		1.733	1.762	1.788	1.761	1.363	1.520	1.785	1.556
A <sub>2</sub>		1.835	1.912	1.992	1.913	1.610	1.727	1.969	1.769
A <sub>3</sub>		1.803	1.851	1.870	1.841	1.794	1.725	1.905	1.808
A <sub>4</sub>		2.017	2.065	2.247	2.109	1.759	2.108	2.065	1.977
LSD 5%			0.400		0.231		0.417		0.240
	B × M				B		B × M		B
B <sub>0</sub>		1.475	1.512	1.549	1.512	1.301	1.239	1.475	1.338
B <sub>1</sub>		1.826	1.845	1.909	1.860	1.618	1.901	1.815	1.778
B <sub>2</sub>		2.240	2.335	2.465	2.347	1.976	2.170	2.503	2.216
LSD 5%			0.346		0.200		0.361		0.208
M		1.847	1.897	1.974		1.632	1.770	1.931	
LSD 5%			0.200				0.208		

**Table 4. Effect of adding biofertilizer, buffalo manure and moringa leaves extract spray and their interaction on leaf Iron concentration (mg kg<sup>-1</sup>) of local orange transplants (spring and fall season 2023).**

Biofertilizers (A)	Buffalo Manures (B)	Spring 2023				Fall 2023			
		Moringa Extract (M)			A × B	Moringa Extract (M)			A × B
		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	
A <sub>1</sub>	B <sub>0</sub>	149.9	150.5	151.5	150.6	150.5	150.9	151.8	151.0
	B <sub>1</sub>	160.4	166.2	170.4	165.7	162.4	167.0	172.5	167.3
	B <sub>2</sub>	185.4	191.2	195.5	190.7	188.3	190.4	195.1	191.3
A <sub>2</sub>	B <sub>0</sub>	177.5	180.9	181.4	179.9	184.3	187.4	190.9	187.5
	B <sub>1</sub>	194.4	197.3	201.3	197.7	203.1	206.5	208.9	206.2
	B <sub>2</sub>	209.4	212.4	213.4	211.7	214.3	216.1	218.0	216.1
A <sub>3</sub>	B <sub>0</sub>	176.4	181.1	180.5	179.3	181.7	184.4	188.4	184.8
	B <sub>1</sub>	195.7	197.5	201.1	198.1	196.9	202.7	206.0	201.8
	B <sub>2</sub>	205.6	209.7	212.5	209.3	213.6	215.7	217.7	215.7
A <sub>4</sub>	B <sub>0</sub>	187.5	190.5	192.7	190.2	190.4	195.5	200.5	195.5
	B <sub>1</sub>	203.3	207.4	210.5	207.0	209.6	211.4	212.9	211.3
	B <sub>2</sub>	214.3	215.4	215.8	215.2	216.7	218.4	220.7	218.6
LSD 5%			1.103		0.637		1.214		0.701
	A × M				A		A × M		A
A <sub>1</sub>		165.2	169.3	172.5	168.9	167.1	169.4	173.1	169.9
A <sub>2</sub>		193.8	169.9	198.7	196.4	200.6	203.3	205.9	203.3
A <sub>3</sub>		192.6	196.1	198.0	195.6	197.4	200.8	204.1	200.8
A <sub>4</sub>		201.7	204.4	206.3	204.2	205.5	208.4	211.4	208.5
LSD 5%			0.637		0.368		0.701		0.404
	B × M				B		B × M		B
B <sub>0</sub>		172.8	175.7	176.5	175.0	176.7	179.5	182.9	179.7
B <sub>1</sub>		188.4	192.1	195.8	192.1	193.0	196.8	200.1	196.6
B <sub>2</sub>		203.7	207.2	209.2	206.7	208.2	210.1	212.9	210.4
LSD 5%			0.551		0.318		0.607		0.350
M		188.3	191.7	193.9		192.6	195.5	198.6	
LSD 5%			0.318				0.350		

It was shows from Tables (5) that addition of bacterial biofertilizer had a significant effect in epidermis and spongy cells layers thickness, as ( $A_4$ ) treatment produced highest thickness of 32.91 and 88.26 micrometer for the epidermis and spongy cells layers, respectively, compared to control treatment ( $A_1$ ), and organic fertilization treatment ( $B_2$ ) also produced highest thickness of 37.87 and 93.86 for micrometer for epidermis and spongy cells layers, respectively, compared to control treatment ( $B_0$ ). Spraying treatment of moringa leaves extract ( $M_2$ ) produced highest thickness of 31.48 and 86.76 micrometer for epidermis and spongy cells layers, respectively, compared to ( $M_0$ ) treatment. twice interaction treatments between study factors also shows a significant superiority in epidermis and spongy cells layers thickness as treatments ( $A_4B_2$ ) of (44.82, 100.3) , ( $A_4M_2$ ) of (36.64, 89.30), and ( $B_2M_2$ ) of (41.04, 101.7) micrometer for epidermis and spongy cells layers, respectively, while ( $A_3M_2$ ) treatment,

produced highest spongy cells layer thickness of (91.88, 29.91) micrometer. The triple interactions between study factors had a significant effect in anatomical traits as treatment ( $A_4B_2M_2$ ) produced highest thickness of (48.60, 108.4) micrometer for epidermis and spongy cells layers, respectively, compared to ( $A_1B_0M_0$ ) treatment. The combined effect of soil study factors represented by bacterial biofertilizers and organic fertilizers for buffalo can be increase nutrients availability in soil solution also the improving absorption of these nutrients by the plant roots and their joint effect with foliar nutrition, will be representing by spraying moringa leaf extract. In improving the traits of vegetative growth (Tables 1 and 2) and increasing leaf content of nutrients (Tables 3 and 4) has been reflected in improving leaves anatomical traits of local orange transplants, as increased epidermal layers and the spongy cells thickness (Figure 1)



**Figure 1. Effect of adding biofertilizer, buffalo manure and moringa leaves extract spray and their interaction on Anatomical characteristics (micrometer) of local orange transplants. appears in it: Cu= cuticle, Ep= epidermis , Ta= tannin cell Pa.c= palisade cell , sp.c= sponge cell**



**Table 5. Effect of adding biofertilizer, buffalo manure and moringa leaves extract spray and their interaction on epidermis and spongy cells layers thickness (micrometer) of local orange transplants**

Biofertilizers (A)	Buffalo Manures (B)	epidermis layer Thickness (micrometer)				spongy cell layer Thickness (micrometer)			
		Moringa Extract (M)			A × B	Moringa Extract (M)			A × B
		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>		M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	
A <sub>1</sub>	B <sub>0</sub>	13.29	17.61	18.39	16.43	58.46	59.99	61.62	60.02
	B <sub>1</sub>	19.45	23.68	26.18	23.10	61.59	70.64	76.48	69.57
	B <sub>2</sub>	31.15	31.65	36.16	32.99	76.74	86.32	88.15	83.74
A <sub>2</sub>	B <sub>0</sub>	21.43	23.59	25.20	23.41	61.30	69.77	72.44	67.84
	B <sub>1</sub>	26.55	26.97	30.76	28.09	78.70	82.74	95.63	85.69
	B <sub>2</sub>	32.47	36.92	41.46	36.95	87.18	97.18	103.2	95.86
A <sub>3</sub>	B <sub>0</sub>	19.26	21.35	24.77	21.79	66.56	76.71	78.25	73.84
	B <sub>1</sub>	25.21	25.26	27.05	25.84	88.52	82.56	90.19	87.09
	B <sub>2</sub>	35.43	36.84	37.93	36.73	86.63	92.72	107.2	95.51
A <sub>4</sub>	B <sub>0</sub>	21.38	21.74	29.69	24.27	69.76	74.71	67.68	70.72
	B <sub>1</sub>	27.63	29.64	31.63	29.63	91.70	97.70	91.81	93.73
	B <sub>2</sub>	42.82	43.05	48.60	44.82	93.73	98.85	108.4	100.3
LSD 5%			0.624	0.360		0.503		0.290	
	A × M				A		A × M		A
A <sub>1</sub>		21.29	24.31	26.91	24.17	65.59	72.32	75.42	71.11
A <sub>2</sub>		26.82	29.16	32.47	29.48	75.72	83.23	90.43	83.13
A <sub>3</sub>		26.63	27.82	29.91	28.12	80.57	83.99	91.88	85.48
A <sub>4</sub>		30.61	31.47	36.64	32.91	85.06	90.42	89.30	88.26
LSD 5%			0.360		0.208		0.290		0.167
	B × M				B		B × M		B
B <sub>0</sub>		18.84	21.07	24.51	21.47	64.02	70.29	70.00	68.10
B <sub>1</sub>		24.71	26.39	28.90	26.67	80.13	83.41	88.53	84.02
B <sub>2</sub>		35.47	37.12	41.04	37.87	86.07	93.77	101.7	93.86
LSD 5%			0.312		0.180		0.251		0.145
M		26.34	28.19	31.48		76.74	82.49	86.76	
LSD 5%			0.180				0.145		

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