Effect of ground fertilization with Vermicompost and spraying with Moringa leaf extract on some vegetative growth traits of Lime saplings (*Citrus aurantifolia*).

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Abstract

The study was conducted in one of the private nurseries in Babylon province for the period from September 2020 to June 2021 to study the effect of fertilization with Vermicompost and spraying with Moringa leaf extract and the interaction between them on the growth of lime saplings. 144 sapling of homogeneous growth were selected as much as possible with age of one year. The experiment included adding Vermicompost fertilizer with four concentrations (0, 10, 20, 30) g. sapling^{-1}, and four levels of spraying with Moringa leaf extract (0, 50, 100, 150) ml.L^{-1}, the results can be summarized as follows:

The addition of Vermicompost treatment (V3) was excelled and gave the highest averages in the studied traits (increment in leaves number, increment in branches number, leaves area, chlorophyll content and carbohydrates) reached (226.20 leaf sapling^{-1}, 7.23 branch sapling^{-1}, 8266 cm^2 sapling^{-1}, 50.14 mg 100g^{-1} fresh weight, 6.41%).The treatment of Moringa extract (B2) excelled and gave the highest averages in (increment in leaves number, increment in branches number, leaves area, chlorophyll content and carbohydrates) (225.70 leaves.sapling^{-1}, 6.25 sapling. branch $^{-1}$, 8194 cm ^{2} sapling $^{-1}$, 45.20 mg 100 g^{-1} fresh weight, 6.25%).In terms of the best treatment between them, the combination of V3B2 has excelled and gave the highest averages for the above traits.

Introduction

lime (*Citrus aurantifolia* L.) is an evergreen fruit tree of the family Rutaceae, belonging to the genus Citrus. Its trees are characterized by being of medium to large size with many spreading branches, their leaves are small and alternate with an oval or ovate elongated shape, and the flowers are yellowish-white with a light purple tinge, The fruits are small spherical with a yellow apical bulge at maturity, but they are usually commercially selected green. The peel of the fruit is very thin, contains dense oil glands, and the pulp is juicy, clearly acidic (4). Its cultivation is spread in a number of countries around the world, such as India, Mexico, Italy and America and these countries are the original home of this plant, then its cultivation began to spread in several regions of the world, including the Middle East (14).lime propagate by seeds and cutting and cultivated in sandy and light clay sandy and have resistance to thirst because of its large roots and many hairs, Which goes deep into the soil, and the trees grafted on it are characterized by their large size, abundant yield, and resulting fruits have good qualities. One of its most important disadvantages is the severity of cold and lack of resistance to some viral diseases such as quick decline (Tresteza) (1). Agricultural concerns to use normal materials such as organic, vital fertilizers and plant extracts as an alternative to chemical fertilizers to minimize damage from the use of chemical fertilizers on soil and human health (15). Vermicompost Organic Fertilizer is a natural organic fertilizer produced by Earthworm's digestion of organic residues such as Household waste, animal waste and plant residues, then secretion of some enzymes on them within their digestive system, decomposing them and excreting them as a rapidly degrading fertilizer rich in humus and the macro-and micro-nutrients necessary for plants and beneficial microorganisms such as nitrogen-fixing and phosphate-dissolving bacteria, enzymes, vitamins, and growth hormones such as auxins,

gibberellins, and cytokinins (16). Scientific researches indicated that there are many plant extracts that have a role in encouraging the vegetative growth of many plants, and this is due to the fact that these plants contain many naturally occurring chemical compounds, Which differ quantitatively and qualitatively according to different species and plant parts, as well as different environmental conditions and stages of plant growth to which it is exposed (11). Among these plant extracts is Moringa leaf extract, which has important roles in the biological and physiological activities of the plant because it contains a high percentage of carbohydrates and fibers, is rich in proteins, amino acids, and vitamins, and is rich in macro and micronutrients and antioxidants (8).It also contains a high percentage of plant hormones, especially the plant hormone Zeatin (12). Based on what was mentioned above and due to the lack of studies on lemon saplings in the conditions of Babylon province, the study aims to:

Studying the effect of all Vermicompost fertilizer and Moringa leaf extract solely or in combination between them on vegetative and chemical growth traits in order to obtain strong-growing of lime saplings.

Materials and Methods

The research was conducted in one of the private nurseries in Babylon province for the period from September 2020 to June 2021 to study the effect of fertilization with Vermicompost and spraying with Moringa leaf extract and the interaction between them on the growth of lime saplings. One-year-age lime saplings were brought from the certified citrus production nursery in Al-Hindiya District - Holy Karbala province, which belongs to the Iraqi Ministry of Agriculture - General Directorate of Horticulture and Forests. The saplings were transferred to the study site and were cultivated in plastic bags of 1 kg, then they were transferred to a larger pot of 5 kg capacity and filled with the medium containing a mixture of riverine and peat moss in a ratio of (1:3). The saplings were placed in the lath house covered with saran as shown in the table (1).

Table 1 Some physio-chemical characteristics of the experimental soils.

traits		**units**	**values**
availability nitrogen		$mg.kg^{-1}$ soil	21.00
availability phosphorous		$mg.kg^{-1}$ soil	11.18
availability potassium		$mg.kg^{-1}$ soil	30.00
pH		-	7.8
E.C . electrical conductivity		$Ds.m^{-1}$	1.51
Organic matter		$g.kg^{-1}$ soil	2.01
CaCo3		$g.kg^{-1}$ soil	13.3
Soil Separators	Sand	$g.kg^{-1}$ soil	820
	Silt		85
	clay		95
Soil Texture		Sandy Loam	

Two factors were used in this experiment

The first-factor "Vermicompost" which include the following concentration:

1- control (without addition) which is symbolized by V0

2- Adding 10 $g.sapling^{-1}$, which is symbolized by V1

3- Adding 20 g.sapling^{-1} , which is symbolized by V2

4- Adding 30 g. sapling^{-1} , which is symbolized by V3

The process of adding vermicompost was conducted twice, According to the manufacturer's recooendations, the first on 9/1/2020 and the second on 3/1/2021

The second factor "Moringa leaf extract"

It included spraying the vegetative growth with Moringa leaf extract prepared in the Horticulture and Landscaping Laboratory - College of Agriculture / Al-Qasim Green University. The extract was prepared with a weight of 100 g of moringa leaf powder and placed in a beaker. One liter of distilled water was added to it and placed on an electric vibrator for one hour. The solution was filtered using several layers of medical gauze and completed the volume to a liter. The result was a concentration of 100%, which is the original (crude) concentration from which the final concentrations used were made. The extract was placed in plastic bottles and kept in the refrigerator until use. Its components are shown in Table (2).

Table 2 Some components of dry Moringa leaves (100 g) (Witt, 2013)

8.2	Niacin	-5	**Mineral composition (mg)**		**No.**
Amino acids (g 100g^{-1})		**No.**	297	phosphorous	-1
0.891	Lysine	-1	1467	potassium	-2
0.196	Histidine	-2	473	magnesium	-3
0.487	Phenyl alanine	-3	1897	Calcium	-4
0.123	Methionine	-4	220	Sodium	-5
0.140	Cysteine	-5	-	manganese	-6
0.517	Glycine	-6	32.5	Iron	-7
1.035	Glutamic acid	-7	2.4	zinc	-8
0.920	Aspartic acid	-8	0.9	copper	-9
0.411	Threonine	-9		**other compounds**	**No.**
0.532	Arginine	-10	24 g	Protein	-1
0.705	Alanine	-11	36 g	carbohydrates	-2
0.611	Valine	-12	216.93 mg	IAA	-3
0.791	Leucine	-13	170.07 mg	phenol compounds	-4
0.451	Isoleucine	-14	**Vitamins (mg 100 g^{-1})**		**No.**
0.347	Tyrosine	-15	172	Vit.C	-1
0.414	Serine	-16	113 – 56	Vit.E	-2
0.451	Proline	-17	2.4	Vit.B6	-3
0.144	Tryptophan	-18	1.29	Riboflavin	-4

This factor included spraying with the following concentrations:

1- control (spraying with distilled water only) which was symbolized by B0

2- Spraying with 50 ml L^{-1} , which was symbolized by B1

3- Spraying with 100 ml L^{-1} , which was symbolized by B2

4- Spraying with 150 ml L^{-1} , which was symbolized by B3

spraying was carried out early in the morning till run- off with 0.1% tween 20 as a wetting agent at an average of 7 sprays (4 sprays in the fall season and 3 sprays in the spring season) between one spray and another one month according to the following times: 15/9/2020 and 15/10/2020 and 15/11/2020, 15/12/2021 _15/3/2021, 15/4/2021 and 15/5/2021. A factorial experiment (4*4) was conducted with three replications, and each experimental unit included 3 saplings. Randomized Complete Block Design (RCBD) was used, the data were analyzed statistically and the arithmetic averages were compared with the LSD test at the 0.05 probability level (2).

The following traits were measured:

1 - increment in leaves number (leaf sapling $^{-1}$)

In The number of leaves for each sapling was calculated at the beginning and also at the end of the experiment, and the difference between the two readings is the average of increment.

2- increment in branches number (branch sapling $^{-1}$)

The number of lateral branches on the main stem of each sapling was calculated at the beginning and at the end of the experiment, and the difference between the two readings is the average of increment.

3 -leaf area (cm^2 $sapling^{-1}$)

The leaf area was calculated on the basis of the dry weight of the leaf according to (7) by taking 5 full-width leaves from each experimental unit and the leaf petioles were separated from them.Then 10 circles with an area of 1.5 cm2 were taken from the cut leaves and the circles were dried separately after placing them in perforated paper bags in an oven at a temperature of 70 °C until the weight was stable.

Then the average leaf area per sapling was measured according to the following equation:

The leaf area for each sapling was calculated according to the following equation:-

$$\text{Leaf area } (cm^2) = \frac{disk\ area\ (cm\ 2) \times Dry\ weight\ for\ leaves\ seedling(g)}{Dry\ weight\ of\ disk\ area\ (g)}$$

leaves area of the sapling (cm^2) = the average leaf area (cm^2) x the average number of leaves per sapling.

4- Total chlorophyll content (mg $100g^{-1}$ fresh weight)

total chlorophyll in leaves was estimated by taking a sample of the fifth leaf from the growing tip of each treatment, weighing 0.5 g Placed in ceramic mortar, 10 ml of 85% acetone was added, mashed, and filtered to separate the dye solution from the paper tissue using Whatman No.1 filter paper. Then the resulting filtrate was collected and the volume was completed to 10 ml using acetone and it was read using a spectrophotometer. The optical absorption readings were taken at two wavelengths of 645 and 663 nm, then the total amount of chlorophyll was calculated in units of (mg 100 g-1 fresh weight) by applying the following equation (9).

Total chlorophyll=20.2*D (645) +8.02*D (663) (V/W *1000) *100

Where:

D (663) = optical absorption reading at a wavelength of 663 nm

D (645) = optical absorption reading at a wavelength of 645 nm

V = final volume of extract (10 ml)

W = weight of plant tissue (0.5 g)

5- Total Carbohydrate (mg g^{-1} dry weight)

The amount of total carbohydrates in the leaves was estimated according to the method approved by (10),Where 0.25 g of the dried sample was crushed with 10 ml of distilled water and then centrifuged at 1500 rpm for 10 minutes, the filtrate was taken and the volume was completed to 10 ml with distilled water.1 ml of the filtrate was taken and 1 ml of phenol reagent 5% concentration and 5 ml of sulfuric acid of 80% concentration were added to it, then the sample was left to cool for 25 minutes. Then the light absorption intensity was measured using a spectrophotometer with a wavelength of 490 nm.

Results and Discussion

1- increment in leaves number (leaf sapling $^{-1}$)

The results in (Table 3)showed that there is a significant effect of fertilizing with Vermicompost on the average increase in the number of leaves, where treatment V3 gave the highest increment of 226.20 leaf sapling^{-1} compared to control treatment V0 which gave 181.80 leaf sapling^{-1}.Spraying with Moringa leaf extract caused a positive effect, where treatment B2 was significantly excelled with the highest increase of 225.70 leaf sapling^{-1} compared to the control treatment B0, (179.90 leaf sapling^{-1}) The interaction between them had a significant effect where the treatment V3B2 excelled by recording the highest value (267.30 leaf sapling^{-1}) compared to the control treatment V0B0, which gave (113.30 leaf sapling $^{-1}$).

Table 3 Effect of ground fertilization with Vermicompost and spraying with Moringa leaf extract and interaction between them on the increment in leaves number (leaf sapling $^{-1}$)

Vermicompost g.sapling^{-1}	Moringa Leaf Extract ml.L^{-1}				V average
	B0	B1	B2	B3	
V0	113.30	248.00	201.00	164.70	181.80
V1	228.30	193.00	200.00	248.00	217.30
V2	212.00	187.30	234.30	232.00	216.40
V3	166.00	219.30	267.30	252.00	226.20
average B	179.90	211.90	225.70	224.20	
LSD 0.05		V=41.32	B=41.32	V*B=82.64	

2- increment in branches number (branch sapling $^{-1}$)

The results in Table 4 showed a significant effect of fertilizing with Vermicompost on the increment in branches number, where treatment V3 gave the highest value (7.23 branches sapling $^{-1}$), compared to the control treatment V0, (4.95 branch sapling^{-1}).Spraying with Moringa leaf extract had a positive effect, where the treatment B2 was significantly superior to the highest value (6.25 branch sapling $^{-1}$) compared to the control treatment B0, which gave (5.12 branch sapling $^{-1}$).The interaction between them had a significant effect where the treatment V3B2 was superior by recording the highest value (8.33 branches sapling $^{-1}$) compared V0B0, which gave the lowest value (3.16 branch sapling $^{-1}$) .

Table 4 Effect of ground fertilization with Vermicompost and spraying with Moringa leaf extract and interaction between them on increment in branches number (branch sapling $^{-1}$)

Vermicompost g.sapling^{-1}	Moringa Leaf Extract ml.L^{-1}				V average
	B0	**B1**	**B2**	**B3**	
V0	3.16	5.16	6.33	5.16	4.95
V1	4.11	4.89	5.22	5.44	5.08
V2	5.22	5.83	5.11	5.66	5.45
V3	7.33	6.83	8.33	6.44	7.23
average B	5.12	5.68	6.25	5.68	
LSD 0.05	V=0.5641 B=0.5641 V*B=1.1282				

3- leaf area (cm^2 sapling^{-1})

The results in (Table 5) showed that there was a significant effect on leaf area when treated with Vermicompost fertilizer, where treatment V3 gave the highest value(8266 cm^2 sapling^{-1}) compared to control treatment V0, which amounted to 6844 cm^2 sapling^{-1}. While spraying with Moringa leaf extract had a positive effect, where treatment B2 was significantly excelled with the highest value of 8194 cm^2 sapling^{-1} compared to control treatment B0 which gave 6669 cm^2 sapling^{-1}.The interaction them had a significant effect on the average leaf area. The treatment V3B2 excelled by recording the highest value of 9758 cm^2 sapling^{-1} compared to the control treatment V0B0 which gave the lowest value of 5154 cm^2 sapling^{-1}.

Table 5 Effect of ground fertilization with Vermicompost and spraying with Moringa leaf extract and interactions between them on leaf area (cm^2 sapling^{-1})

Vermicompost g.sapling^{-1}	Moringa Leaf Extract ml.L^{-1}				V average
	B0	**B1**	**B2**	**B3**	
V0	5154	7983	7034	7205	6844
V1	6522	6844	7922	7984	7318
V2	8247	6295	8065	7411	7504
V3	6751	9065	9758	7489	8266
average B	6669	7547	8194	7522	
LSD 0.05	V=1284.60 B=1284.60 V*B=2569.20				

4- Total chlorophyll content (mg.100gm^{-1} fresh weight)

The results in (Table 6) showed that there was a significant effect on the total chlorophyll when treated with Vermicompost fertilizer. treatment V3 excelled on the rest of the treatments by giving it the highest value 50.14 mg 100 gm^{-1} fresh weight compared to the control treatment V0, which gave 39.70 mg 100 gm^{-1} fresh weight. As for spraying with Moringa leaf extract, treatment B2 was significantly excelled with the highest value 45.20 mg 100 g^{-1} fresh weight compared to control treatment B0, which gave 41.68 mg 100 g^{-1} fresh weight. The

interaction between them had a significant effect. when treatment V3B2 excelled by recording the highest value 54.79 mg 100 g^{-1} fresh weight compared to the control treatment V0B0, which gave 32.07 mg 100 g^{-1} fresh weight.

Table 6: Effect of fertilizing with Vermicompost and spraying with Moringa leaf extract and interaction between them on the total chlorophyll content (mg 100g^{-1} fresh weight)

Vermicompost g.sapling^{-1}	Moringa Leaf Extract ml.L^{-1}				V average
	B0	**B1**	**B2**	**B3**	
V0	32.07	41.90	42.90	41.93	39.70
V1	40.05	39.95	38.10	42.20	40.07
V2	42.88	40.40	45.01	43.63	42.98
V3	51.71	45.22	54.79	43.85	50.14
average B	41.68	41.87	45.20	44.15	
LSD 0.05		V=2.462	B=2.462	V*B=4.923	

5- Percentage of carbohydrates in leaves (%)

The results in (Table 7) showed a significant increase in total carbohydrate content of leaves when treated with Vermicompost fertilizer. treatment V3 gave the highest value 6.41 % compared to the control treatment V0 which gave 5.79%, while Moringa leaf extract had no significant effect on that. The interaction them had a significant effect. Treatment V3B2 excelled by recording the highest average of 7.30% compared to treatment V0B0, which gave the lowest value of 4.88%.

Table 7: Effect of ground fertilization with Vermicompost and spraying with Moringa leaf extract and interaction between them on the percentage of carbohydrates (%)

Vermicompost g.sapling^{-1}	**Moringa Leaf Extract ml.L^{-1}**				**V average**
	B0	**B1**	**B2**	**B3**	
V0	**4.88**	**5.77**	**5.84**	**6.67**	**5.79**
V1	**6.17**	**6.09**	**5.77**	**5.68**	**5.93**
V2	**6.32**	**6.28**	**6.08**	**6.82**	**6.37**
V3	**6.56**	**6.13**	**7.30**	**5.67**	**6.41**
average B	**5.98**	**6.07**	**6.25**	**6.21**	
LSD 0.05		**V=0.3817**	**B=0.3817**	**V*B=0.7633**	

These results are due to vermicompost, which improves the physical and chemical properties of the soil and increases the availability of the necessary nutrients for the plant, which positively affects the increase in photosynthesis and thus increases the construction of carbohydrates in the leaves (13).Also, Vermicompost fertilizer contains some plant hormones such as auxins, gibberellins and cytokinins, which have an effective role in increasing vegetative growth by stimulating cell division and activating vital processes within the plant. Which causes an increase in the efficiency of photosynthesis, which is positively reflected in the traits of vegetative growth such as the number of leaves, branches, leaf area, and the increase in the leaf area leads to an increase in the products of photosynthesis, which was positively reflected on the increase in the construction of carbohydrates in the leaves (13).

Moringa leaf extract had an effective role in increasing vegetative growth because it contains nutrients (Table 2) necessary for photosynthesis and respiration. the various metabolic processes that have a major role in the process of cell division and elongation, which is positively reflected on the traits of vegetative growth, number of leaves, number of branches, and leaf area (6). it contains auxins and a number of amino acids, including the amino acid Tryptophan (Appendix 2), which is the initiating compound in the formation of auxins that have a role in increasing cell division and elongation and thus its reflection on vegetative and root growth. A large amount of zeatin, a natural cytokinin found in plants that works to regulate and distribute the products of photosynthesis and nutrients towards growth in the plant (5).

References

1- Al-Khafaji, M. A., Suhail A and Alaa A. R. A. 1999. The evergreen fruit. Ministry of Higher Education and Scientific Research. Baghdad University. The Republic of Iraq.

2- Alrawi, k. M and Abdel Aziz K. 1980. Design and analysis of agricultural experiments. Dar Al-Kutub Press for Printing and Publishing - University of Mosul - Ministry of Education and Scientific Research - Republic of Iraq.

3- Bhat, S. A., Singh, J and Vig, A. P. 2018. Earthworms as organic waste managers and biofertilizer producers.Waste and biomass valorization, 9(7): 1073-1086.

4- Bhatt B.B and Tomar Y.K. 2011. Effect of IBA and growing conditions on vegetative performance of Citrus aurantifolia (Swingle) cuttings. Journal of Hill Agriculture, 2(1): 98-101.

5- Blackman, P . G and Davies, W. J. 1984. Age-related changes in stomata response to cytokinins and abscisic acid. Annals of Botany. 54: 121-125.

6- Devlin, Robert M. and Francis H. widham 1985. Plant Physiology, translated by Muhammad Fawzi Abd al-Hamid, Muhammad Sharaki, Abd al-Hadi Khader, Nadia Kamel and Ali Saad al-Din Salama. Arab House for Publishing and Distribution, Arab Republic of Egypt.

7- Dvorinic , V. 1965. Lacarali pactic de amelo graphic , Ed. Dielactict Spedagogica . Bucureseti . R. S. Romania .

8- El-Sohaimy, S. A., Hamad, G. M., Mohamed, S. E., Amar, M. H and Al-hindi, R. R. 2015. Biochemical and functional properties of Moringa oleifera leaves and their potential as a functional food. Global Advanced Research Journal of Agricultural Science, 4(4): 188-199.

9- Goodwin , T. W. 1976 .Chemistry and Biochemistry of plant Pigment . 2nd Ed. Academic Press, N. Y., Sanfrancisco .USA.pp. 373.

10- Herbert, D., Philips, P.J and Strange, R.E. 1971. Determination of Total Carbohydrates ,(C.F. Methods in Microbiology . Norris J.R. and D.W. Robbins (Eds) Acad ., Press , London

. 5B, Chap. England.

11- Omran, W. H. Hassoun. 2004. Effect of some plant extracts on the growth and yield of Cucumis sativum L. in heated greenhouses. Master's Thesis, College of Agriculture, University of Baghdad, Republic of Iraq. p. 98.

12- Iqbal, M.A., Hussain, M., Ur Rehman, M.W., Ali, M., Rizwan, M and fareed, M.I. 2013. Allelopathy of *Moringa*. A review. Scientia Agriculture, 3(1): 9-12.

13- Joshi, R., Singh, J and Vig, A.P. 2015. Vermicompost as an effective organic fertilizer and biocontrol agent: effect on growth, yield and quality of plants. Reviews in Environmental Science and Bio Technology, 14 (1): 137-159.

14- Morton, J. 2013. Mexican lime. In: Julia F. Morton, Fruits of warm climates. Miami. 168-172.

15- Nasab, K., Kalat, S and Haghighi, R. 2013. The study effects of bio-fertilizer and foliar spray with complete fertilizer on yield and yield components of sunflower (*Helianthus annuus* L.). International Journal of Agronomy and Plant Production, 4(12): 3404-3409.

16- Sinha, R. K., Agarwal, S., Chauhan, K and Valani, D. 2010. The wonders of earthworms & its vermicompost in farm production: Charles Darwin's 'friends of farmers', with potential to replace destructive chemical fertilizers. Agricultural Sciences, 1(2): 76–94.

17- Witt, K.A. 2013. The Nutrient Content of *Moringa oleifera* Leaves. Messiah College Department of Nutrition and Dietetics:1-6.