Effect of rootstocks, Vermicompost and biofertilization on the growth of *Citrus aurantifolia* L. seedling.

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Abstract

The experiment was conducted in lath house of the College of Technology , Al-Musaib during the period from September 2023 to June 2024 to study the effect of rootstock type, organic and bio fertilization on the growth of *Citrus aurantifolia* L. seedling, according to a completely randomized block design. The results showed that there were significant differences between the rootstocks. sour orange rootstock gave the highest averages in some traits, including (leaves area, number of secondary roots, dry weight of the root system, iron content of leaves, zinc content of leaves) respectively, reaching (1642 cm^{-2} sapling, 22.56 root $sapling^{-1}$, 11.29 g, 46.74%, 37.36 % Compared to the volkameriana rootstock, the volkameriana rootstock excelled in (the fresh weight of the root system) reaching (42.50 g. $seedling^{-1}$). The results showed that the second concentration of vermicompost (10%) was excelled, where the sour orange rootstock gave the highest averages in all traits, including (leaves area, number of secondary roots, fresh weight of the root system, dry weight of the root system, iron content of leaves, zinc content of leaves) respectively. It reached (1689 cm^2 $sapling^{-1}$, 23.16 root $sapling^{-1}$, 42.15 g , 12.07 g , 49.62%, 37.69%) compared to control treatment. The results showed the excelled of biofertilization at the third concentration (mycorrhizal fungi), as it gave the highest averages in some traits, including (leaves area, number of secondary roots, fresh weight of the root system, dry weight of the root system, and iron content of the leaves), respectively reaching (1707 cm^2, 24.50 root $sapling^{-1}$, 42.70 g , 12.22 g , 49.07% (compared to control treatment). The second concentration (bacterial) was excelled (zinc content of leaves) by (38.75%) compared to control treatment. As for bi and triple interactions between roots, vermicompost, and biofertilization, there are significant differences in some of the studied traits.

ISSN 2072-3857

Introduction

Citrus aurantifolia is classified as a member of a certain group. Lime is a term used to describe the citrus genus Citrus, which is part of the Rutaceae family. These trees are found in the tropical and subtropical regions of Southeast Asia [23,25]. The number of fruit-bearing lemon trees in Iraq is estimated at about (291,487) trees, and Iraq's production of Lemon fruits are about (5212) tons, while the average production of one tree is (18.4) kg [16]. Citrus aurantifolia, which is known locally in Iraq as (Nomi Basra), is one of the fruit trees whose fruits are used for dry consumption. It has great nutritional and medicinal importance because it contains a high percentage of phenols, flavonoids, carotenoids, minerals and vitamins. Its dry fruits are used in folk medicine for viruses and fungi, and Citrus aurantifolia oil is used which is distilled from the peel as a stomach tonic and antibiotic, and is mainly used to flavor and taste foods. It is also used in cosmetics and perfumes [21]. Excessive use of mineral fertilizers in fruit orchards may have a negative impact on the growth of trees and the surrounding environment. Causing environmental pollution and deteriorating the biomass of fungi and bacteria that are important in improving soil properties, which led to resorting to searching for safer and more environmentally friendly alternative methods using organic and biofertilizers [4,6]. Biofertilizers, which are organic substances derived from living organisms, have the capacity to enhance both soil fertility and crop yield. In sustainable agriculture, they play a critical role by enhancing soil health, augmenting nutrient accessibility, and diminishing reliance on synthetic fertilizers. The implementation of bio fertilizers by farmers can have a dual-pronged effect: enhance crop quality, reduce ecological harm, and foster enduring sustainability within the agricultural sector [11,12]. Biofertilizers are one of them. It is one of the important pillars of sustainable agriculture for regulating production, protecting the environment, and producing crops free of pollutants, as microbial vaccines supply the plant with the nutrients it needs and facilitate their absorption by converting the elements from the unready form to the form available for absorption by the plant. Biofertilizers also provide some Plant growth regulators and fixation of atmospheric nitrogen through their symbiotic and non-symbiotic living, as well as protecting the plant from some pathogens, thus reducing production costs and reducing environmental pollution [8,10]. Vermicompost is a natural organic fertilizer resulting from the various biological activities of earthworms, which is characterized by being rich in humus,

ISSN 2072-3857

macro- and micro-nutrients, and beneficial soil microbes such as nitrogen-fixing and phosphate-dissolving bacteria [2,5].

Materials and Methods

The experiment was conducted in lathhouse of Al-Musaib Technical College during the period from September 2023 until June 2024 to study effect of Rootstock type, organic fertilization, and biofertilization on the growth of *Citrus aurantifolia* seedling. 216 lime seedling as homogeneous as possible, planted in 5 kg bags at one year of age, were selected from certified citrus multiplication nurseries located in Al- Hindiyah District/Holy Karbala province, and they were transferred to 8 kg anvils for pot. It was used on 216 seedling of *Citrus aurantifolia* grafted on two types of Rootstocks (sour orange, Volkameriana) according to a three-factor experiment within a completely randomized block design with three factors (2*3*4) and three replicates. The experiment contains 24 experimental units with 3 seedling for each. Experimental unit in one replicate, and the factors were as follows:

- The first factor: includes two types of Rootstocks: (sour orange and Volkameriana) and is symbolized as A1, A2, respectively.

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- The second factor: It includes adding vermicompost at three levels: (0, 10%, 20%) of the weight of the anvil and is symbolized by (V0, V1, V2) respectively.
- The third factor: includes adding biofertilization at four levels: (Without biological vaccine (control), *Azospirillum brasilense* bacterial vaccine, *Glomus mossa* mycorrhizal fungal vaccine, a mixture of bacterial and fungal vaccine) and are symbolized (B3, B2, B1, B0) respectively.

The seedling were placed in the canopy covered with green net cover , and experimental treatments were applied and service operations were conducted on them, which included continuous watering, manual removal of weeds, and combating leaf miners with Bactin insecticide and a spray was used on the seedling in concentration 2 ml per liter

ISSN 2072-3857

Table 1. chemical and physical traits of pot soil utilized.

Traits	Values	Units
sand	76.3	$g.kg^{-1}$
Clay	13.6	$g.kg^{-1}$
silt	10.1	$g.kg^{-1}$
Soil texture	sandy loam	-
pH	7.4	-
Electrical conductivity Ec	2.24	$DS.m^{-1}$
Organic matter M.O	2.47	$g.kg^{-1}$
Nitrogen	1.15	$mg.kg^{-1}$
Phosphorus	12.3	$mg.kg^{-1}$
Potassium	0.40	$mg.kg^{-1}$
Iron	0.84	$mg.kg^{-1}$
Calcium	14.03	$mg.kg^{-1}$

Studied traits:

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

The leaves area was measured at the end of the experiment for each sapling in the replicate, and the measurement was based on the dry weight of the leaf according to what was stated in[18] by taking 5 fully expanded leaves from each experimental unit and the petioles were separated from them, then circles with an area of 1 cm^2 were taken from The cut leaves were dried separately after placing them in perforated paper bags in an oven at 70°C for 48 hours. Then the average leaf area per sapling was measured according to the following equation :

$$\text{Leaf area } (cm^2 = \frac{Dry\ weight\ of\ sapling\ leaves\ (g)\ x\ area\ of\ cut\ circle\ (cm2}{Dry\ weight\ of\ area\ traveled\ (g)}$$

The leaves area of each sapling was calculated according to the following equation:

Leaf area per sapling (cm2) = Average leaf area (cm2) x Average number of leaves per sapling.

ISSN 2072-3857

2-fresh weight of the root system (g.seedling $^{-1}$)

The root system was cut from the bottom of the sapling stem, then the weight of the leaves and vegetative branches was measured using a sensitive electric balance.

3-Dry weight of the root system (g.seedling $^{-1}$)

The root system was cut from the bottom of the sapling stem, then placed in perforated paper bags in an electric oven, for the purpose of getting rid of moisture, at a temperature of 70 degrees Celsius for 48 hours until the weight was constant, and the dry weight was taken using a sensitive electric balance[9] .

4-Number of secondary roots (root sapling^{-1})

The number of secondary roots was counted for each plant.

•Digestion of plant samples

Leaf samples were collected from Citrus aurantifolia seedling for each experimental unit. The mature leaves on the growing shoots in the same year were taken, then washed and dried in an electric oven at a temperature of 70°C for 48 hours until the weight was constant, after which they were ground using an electric grinder. 200 mg were taken for each experimental unit and digested in Pyrex flasks by adding 3 ml of concentrated sulfuric acid for 24 hours according to the method suggested by [17] after which 1 ml of concentrated sulfuric acid and 1 ml were added. Per and chloric acid , the digestion flask was heated, and the vapors rose until a clear, transparent solution was obtained. The liquid was then cooled and the volume was brought to 50 ml, after which the solution was filtered and the nutrients in the digestion solution were estimated according to the established estimation methods.

5- Iron content of leaves (mg.kg^{-1}.dry weight)

It was estimated using an Atomic Absorption Spectrophotometer according to the method of [3]

6-Zinc content of leaves (mg.kg^{-1}.dry weight)

It was estimated using an Atomic Absorption Spectrophotometer according to the method of[3]

Results and discussion

Leaves area(cm^2)

The results of Table (2) showed that there were significant differences between rootstocks in the trait of leaves area, as the sour Orange rootstock gave the highest average in leaves area, amounting to (1642 **cm^2**), compared to the Volkameriana rootstock, which gave the lowest average for that trait, amounting to (1455 **cm**^{2}). The reason is that the choice Each citrus growing region in the world has the rootstock that is most compatible with the climatic conditions prevailing in that region, and the sour orange rootstock is considered the most compatible with the conditions, and this is consistent with [19].Table (2) revealed that there are significant differences in vermicompost concentrations in the leaves area traits. The second concentration, V1 (10%), excelled and gave the highest average of (1689 **cm**^{2}), compared to control treatment, which gave the lowest average in this trait of (1444 **cm**^{2}). In one of the experiments on the use of vermicompost in organic agriculture and its effects on the soil, it improves the porosity, aeration, and ability of the soil to retain water. It also reduces the pH and electrical conductivity, as well as increasing the readiness of the necessary plant elements, organic matter, and microbial activity beneficial to the soil, which led to increased growth and productivity. Leaves area, . It is clear from Table (2) that there are significant differences between the concentrations of biofertilization in the leaves area trait, as the third concentration, B2 (mycorrhizal fungus), gave the highest average in leaves area, amounting to (1707 **cm**^{2}), compared to the treatment. The comparison that gave the lowest average for that trait was (1412 **cm**^{2}). The reason is due to the physiological activity of the mycorrhizae, including an increase in the content of chlorophyll and reducing sugars, and it stimulates the rate of carbon synthesis by increasing the bundles of plates in the chloroplasts and cells of the mesophyll layer and increasing the veins of the leaves, thus increasing the leaf area. This is in agreement with [1]. As for the binary interaction between rootstock and vermicompost, it was significant in the leaves area trait, as shown in Table (2), as the combination (A1V1) gave the highest average for leaves area, amounting to (1825 **cm**^{2}), while the combination (A2V0) gave the lowest average for that trait, amounting to (1392). The interaction between rootstock and biofertilization was significant in the leaves area trait, where the combination (A1B2) gave the highest average leaves area, amounting to (1816 **cm**^{2}), while the combination (A2B0) gave the lowest average for that trait, amounting to (1336 **cm**^{2}). As for bi-interaction between vermicompost and biofertilization, it was significant in terms of leaves area, as the combination (V1 B2) gave the highest average of (1852 **cm**^{2}) compared to the combination (V0 B0), which gave the lowest average of (1301 **cm**^{2}). The triple interactions between rootstock and Vermicompost and biofertilization significantly affected leaves area, as the combination (A1V1B2) gave the highest average of (2016 **cm^2**) cm compared to the combination (A2V0B0) which gave the lowest average of (1273 **cm^2**).

Table (2) The effect of rootstock type, vermicompost, and biofertilization and their interactions on leaves area of Citrus aurantifolia

× rootstock Vermicompost (V ×A)	Biofertilization				Vermicompost	rootstock
	B3	B2	B1	B0		
1495	1552	1661	1437	1330	V0	A1 sour orange
1825	1870	2016	1761	1653	V1	
1607	1620	1771	1558	1480	V2	
1392	1416	1546	1334	1273	V0	A2 Volkameriana
1553	1594	1689	1494	1435	V1	
1420	1446	1562	1373	1300	V2	
34.74	69.48				0.05 L.S.D	
rootstock average (A)	(B×A)Biofertilization × rootstock					
1642	1681	1816	1585	1487	A1	
1455	1485	1599	1400	1336	A2	
20.06	40.12				0.05 L.S.D	
average Vermicompost (V)	(B×V) Biofertilization × Vermicompost					
1444	1484	1604	1385	1301	V0	
1689	1732	1852	1628	1544	V1	
1514	1533	1667	1465	1390	V2	
24.57	49.13				0.05 L.S.D	
	(B) Biofertilization average					
28.37	1583	1707	1493	1412	0.05 L.S.D	

ISSN 2072-3857

Number of secondary roots

The results of Table (3) showed that there were significant differences between rootstocks in the character of the number of secondary roots, as the sour Orange rootstock gave the highest average in the number of secondary roots, amounting to (22.56), compared to the Volkameriana rootstock, which gave the lowest average for that trait, amounting to (20.72), and the reason is that the root has an effect. In the vegetative, root and chemical growth traits, when orange seedlings were grafted onto three citrus rootstocks, which are orange, volkameriana, and mandarin , the results showed that there were significant differences in the orange rootstock in increasing the number of roots, and it agrees with what was mentioned [18]. Table (3) revealed There were significant differences between vermicompost concentrations in the character of the number of secondary roots, as the second concentration, V1 (10%), was superior and gave the highest average of (23.16), compared to control treatment, which gave the lowest average in this trait of (20.44). The reason is that vermicompost improves the porosity of the soil by digging channels with earthworms while they are performing their vital activity, as these channels help the penetration of fertilizers into the soil and thus increase the number of secondary roots, and this is consistent with[20] .This is shown in Table (3). There were significant differences between the concentrations of biofertilization in the character of the number of secondary roots, as the third concentration, B2 (mycorrhizal fungus), gave the highest average in the number of secondary roots, amounting to (24.50), compared to control treatment, which gave the lowest average for that trait, amounting to (18.77). The reason is due to the occurrence of morphological and anatomical changes in the host root due to symbiotic living between plant roots and fungi. These changes are evident in the ectotrophic mycorrhiza that infect plants, and this agrees with [19]. As for bi-interaction between the rootstock and vermicompost, It was significant in the number of secondary roots, as shown in Table (3), as the combination (A1V1) gave the highest average in the number of secondary roots, amounting to (24.45), while the combination (A2 V0) gave the lowest average for that trait, amounting to (19.72). The interaction between rootstock and biofertilization was significant in terms of the number of secondary roots, as the combination (A1 B2) gave the highest average in the number of secondary roots, amounting to (25.55), while the combination (A2B0) gave the lowest average for that trait, amounting to (17.84). As for bi-interaction between vermicompost and biofertilization, it was significant in terms of the number of secondary roots, as the combination (V1B2) gave the highest average of (25.55) compared to the combination (V0B0), which gave the lowest average of (16.71). The triple interactions between rootstock and vermicompost affected Biofertilization was significantly related to the number of secondary roots, as the combination (A1V1B2) gave the highest average, amounting to (27.00), compared to the combination (A2V0B0), which gave the lowest average, amounting to (15.53).

Table (3) Effect of rootstock type, vermicompost, biofertilization, and their interactions on the average number of secondary roots of grafted Citrus aurantifolia seedling.

× rootstock Vermicompost (V ×A)	Biofertilization				Vermicompost	rootstock
	B3	B2	B1	B0		
21.16	22.63	24.56	19.56	17.90	V0	A1 sour orange
24.45	25.13	27.00	23.53	22.13	V1	
22.08	23.00	25.10	21.16	19.06	V2	
19.72	20.73	23.63	19.00	15.53	V0	A2 Volkameriana
21.88	22.10	24.10	21.33	20.00	V1	
20.56	21.66	22.60	20.00	18.00	V2	
0.475	0.950				0.05 L.S.D	
rootstock average (A)	(B×A)Biofertilization × rootstock					
22.56	23.58	25.55	21.42	19.70	A1	
20.72	21.50	23.44	20.11	17.84	A2	
0.274	0.548				0.05 L.S.D	
average Vermicompost (V)	(B×V) Biofertilization × Vermicompost					
20.44	21.68	24.10	19.28	16.71	V0	

ISSN 2072-3857

23.16	23.61	25.55	22.43	21.06	V1
21.32	22.33	23.85	20.58	18.53	V2
0.336				0.672	0.05 L.S.D
	(B) Biofertilization average				
0.388	22.54	24.50	20.76	18.77	0.05 L.S.D

fresh weight of root system (g)

The results of Table (4) showed that there were significant differences between the rootstocks in the fresh weight trait of the rootstock, where Volkameriana rootstock gave the highest average in the fresh weight of the rootstock, amounting to (42.50 g), compared to the sour orange rootstock, which gave the lowest average for that trait, amounting to (36.97), and the reason for this rootstock It is fast growing and has a stimulating effect on the growth of budding. It is adapted to a wide range of soils, especially sandy soils. This is consistent with [29]. Table (4) revealed that there were significant differences between the concentrations of vermicompost in the fresh weight of the root system, as the second concentration exceeded V1 (10%) and gave the highest average of (42.15g), compared to control treatment that It gave the lowest average in this trait, amounting to (37.38g). The reason is that vermicompost is the type of fertilizer that contains the least organisms that cause plant diseases, and is richest in organisms beneficial to the plant. The components of vermicompost dissolve in water, which makes it easier for the plant through its roots to absorb and benefit from it. This agrees with [26] It is clear from Table (4) that there are significant differences between the concentrations of biofertilization in the fresh weight of the root system, as the third concentration, B2 (mycorrhizal fungus), gave the highest average in the fresh weight of the root system, amounting to (42.70 g), compared to control treatment that gave The lowest average for this trait was (36.85 g). The reason is that the fungus, through its symbiosis, colonizes the root tissues of high-end plants and the place surrounding the roots. This method has a positive role in nourishing the plant and increasing the absorption of macro- and micro-elements, so plants infected with

ISSN 2072-3857

the fungus are better than those that are not infected. Recent research has tended to introduce biological factors in general and mycorrhizal fungi in particular as a modern, advanced technology to improve agricultural production, as explained [23]. As for the binary interaction between rootstock and vermicompost, it was significant in terms of the fresh weight of the root system, as well as Shown in Table (4), the combination (A2 V1) gave the highest average in the fresh weight of the root system, amounting to (44.76 g), while the combination (A1 V0) gave the lowest average for that trait, amounting to (34.39 g).

The interaction between rootstock and biofertilization was significant in the fresh weight of the root system, as the combination (A2 B2) gave the highest average in the fresh weight of the root system, amounting to (45.87 g), while the combination (A1 B0) gave the lowest average for that trait, amounting to (34.90 g). As for the binary interaction between vermicompost and biofertilization, it was significant in the fresh weight of the root system, as the combination (V1B2) gave the highest average (45.70 g) compared to the combination (V0B0), which gave the lowest average (34.90 g). The triple interactions between rootstock, vermicompost, and biofertilization significantly affected the fresh weight of the root system, as the combination (A2V1B2) gave the highest average of (48.53g) compared to the combination (A1V0B0), which gave the lowest average of (32.60 g).

Table (4) The effect of rootstock type, vermicompost, biofertilization, and their interactions on the fresh weight of the Citrus aurantifolia root system.

× rootstock Vermicompost (V ×A)	Biofertilization				Vermicompost	rootstock
	B3	B2	B1	B0		
34.39	35.00	36.43	33.53	32.60	V0	A1 sour orange
39.54	40.73	42.86	38.26	36.30	V1	
37.00	38.03	39.26	36.06	34.63	V2	
40.38	41.46	43.56	39.30	37.20	V0	A2 Volkameriana
44.76	45.56	48.53	43.66	41.30	V1	
42.37	43.20	45.53	41.10	39.10	V2	
0.426	0.852				0.05 L.S.D	
rootstock average	(B×A)Biofertilization × rootstock					

ISSN 2072-3857

(A)					
36.97	37.92	39.52	35.95	34.90	A1
42.50	43.41	45.87	41.54	39.20	A2
0.246				0.492	0.05 L.S.D
average Vermicompost (V)					(B×V) Biofertilization × Vermicompost
37.38	38.23	40.00	36.41	34.90	V0
42.15	43.15	45.70	40.96	38.80	V1
39.68	40.62	42.40	38.86	36.86	V2
0.301				0.602	0.05 L.S.D
					(B) Biofertilization average
0.347	40.66	42.70	38.75	36.85	0.05 L.S.D

Dry weight of root system (g)

The results of Table (5) showed that there were significant differences between rootstocks in the dry weight of the rootstock, as the sour orange rootstock gave the highest average in the dry weight of the rootstock, amounting to (11.29g), compared to the Volkameriana rootstock, which gave the lowest average for that trait, amounting to (10.21 g). The reason is that the type rootstock had a significant effect on the root growth traits of the resulting seedling, and this agrees with Ismail and Salman, 2014). Table (5) revealed that there were significant differences for vermicompost concentrations in the dry weight of the root system, as the second concentration V1 (10%) was superior and gave the highest average of (12.07g), compared to control treatment, which gave the lowest average for this trait, amounting to (9.60 g). The reason is that vermicompost has a role in sustainable agriculture in order to encourage the use of environmentally friendly fertilizers and reduce mineral fertilizers. It also speeds up biological processes 2-5 times

ISSN 2072-3857

compared to mineral fertilizers, and this agrees with [27]. It is clear from Table (5) that there are significant differences between the concentrations Biofertilization in the dry weight of the root system, as the third concentration, B2 (mycorrhizal fungi), gave the highest average in the dry weight of the root system, amounting to (12.22 g), compared to control treatment, which gave the lowest average for that trait, amounting to (9.30). This is due to the symbiotic relationship between the organisms Each of them belongs to a different kingdom, namely fungi and plant roots. The fungus, through its symbiosis, colonizes the tissues of the roots of higher plants and the place surrounding the roots, and this agrees with [23] As for bi-interaction between rootstock and vermicompost, it was significant in its capacity. The dry weight of the root system, as shown in Table (5), as the combination (A1 V1) gave the highest average in the dry weight of the root system, amounting to (12.58) g, while the combination (A2V0) gave the lowest average for that trait, amounting to (9.05 g). It was The interaction between rootstock and biofertilization is significant in the dry weight of the root system, as the combination (A1B2) gave the highest average in the dry weight of the root system, amounting to (12.87 g), while the combination (A2B0) gave the lowest average for that trait, amounting to (8.78 g). As for bi-interaction between vermicompost and biofertilization, it was significant in terms of the dry weight of the root system, as the combination (V1 B2) gave the highest average of (13.53 g) compared to the combination (V0 B0), which gave the lowest average of (7.78 g). The triple interactions affected Rootstock, vermicompost, and biofertilization significantly affected the dry weight of the root system, as the combination (A1V1B2) gave the highest average, amounting to (14.06 g), compared to the combination (A2V0B0), which gave the lowest average, amounting to (7.26 g).

ISSN 2072-3857

(5) Effect of rootstock type, vermicompost, and biofertilization and their interactions on the dry weight of the root system of Citrus aurantifolia seedling

× rootstock Vermicompost (V ×A)	Biofertilization				Vermicompost	rootstock
	B3	B2	B1	B0		
10.16	10.90	11.83	9.63	8.30	V0	A1 sour orange
12.58	13.06	14.06	12.06	11.13	V1	
11.14	10.60	12.73	11.23	10.00	V2	
9.05	9.76	10.50	8.66	7.26	V0	A2 Volkameriana
11.56	12.03	13.00	11.06	10.16	V1	
10.03	10.33	11.23	9.63	8.93	V2	
0.464	0.929				0.05 L.S.D	
rootstock average (A)	(B×A)Biofertilization × rootstock					
11.29	11.52	12.87	10.97	9.81	A1	
10.21	10.71	11.57	8.78	8.78	A2	
0.268	0.536				0.05 L.S.D	
average Vermicompost (V)	(B×V) Biofertilization × Vermicompost					
9.60	10.33	11.16	9.15	7.78	V0	
12.07	12.55	13.53	11.56	10.65	V1	
10.58	10.46	11.98	10.43	9.46	V2	
0.328	0.656				0.05 L.S.D	
	(B) Biofertilization average					
0.379	11.11	12.22	10.38	9.30	0.05 L.S.D	

ISSN 2072-3857

Iron content of leaves

The results of Table (6) showed that there were significant differences between rootstocks in the trait of iron content of leaves, as the sour orange rootstock gave the highest average in the iron content of leaves, amounting to (46.74), compared to the Volkameriana rootstock, which gave the lowest average for that trait, amounting to (43.89), and the reason is that the rootstock A significant effect on the content of leaves of various mineral elements from a study of the effect of rootstock type on the ionic content of ordinary orange leaves grafted on four roots, and this agrees with [19] Table (6) revealed the presence of significant differences for vermicompost concentrations in the iron content of the leaves. The second concentration, V1 (10%), excelled and gave the highest average of (49.62), compared to control treatment, which gave the lowest average in this trait of (41.80). The reason is that vermicompost affects the physical and mineral traits of the soil and leads to an increase in the availability of nutrients in it, increases the activity of microorganisms and the amount of organic matter, improves the composition of the soil and the movement of water and nutrients, and also improves the porosity in it by digging channels while they are performing their vital activity, as these channels help to The penetration of fertilizers into the soil, and this is consistent with [26]. It is clear from Table (6) that there are significant differences between the concentrations of biofertilization in the content of the leaves of plant iron, as the third concentration, B2 (mycorrhizal fungi), gave the highest average in the content of the leaves of iron. Iron reached (49.07) cm, compared to control treatment that gave the lowest average for that trait, which amounted to (41.57). The reason that the endomycorrhizal fungus is considered one of the most economical types is because it infects most agricultural crops and works to increase the availability of phosphorus, nitrogen, and other nutrients such as K, Ca, Mg, Cu, Zn, and Fe, and this agrees [21] As for the binary interaction between rootstock and vermicompost, it was significant in terms of leaf iron content, as shown in Table (6). The combination (A1 V1) gave the highest average in leaf iron content, amounting to (51.32), while the combination gave (51.32). A2 V0) had the lowest average for this trait, reaching (40.61). The interaction between rootstock and biofertilization was significant in terms of leaf iron content, as the combination (A1 B2) gave the highest average in leaf iron content, reaching (50.58), while the combination (A2 V0) gave the highest average in leaf iron content, reaching (50.58). A2 B0) The lowest average for this trait was

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(40.00). As for bi-interaction between vermicompost and biofertilization, it was significant in terms of the iron content of the leaves, as the combination (V1 B2) gave the highest average amounting to (54.45) cm compared to the combination (V0 B0), which gave the lowest average amounting to ((39.14). The triple interactions affected There was a significant difference between rootstock, vermicompost, and biofertilization in terms of the iron content of leaves, as the combination (A1V1B2) gave the highest average, amounting to (56.39), compared to the combination (A2V0B0), which gave the lowest average, amounting to (37.65).

Table (6): The effect of rootstock type, vermicompost, and biofertilization and their interactions on the iron content of Citrus aurantifolia leaves.

× rootstock Vermicompost (V ×A)	Biofertilization				Vermicompost	rootstock
	B3	B2	B1	B0		
42.99	43.00	45.48	42.85	40.63	V0	A1 sour orange
51.32	53.57	56.39	49.15	46.16	V1	
45.92	46.77	49.87	44.43	42.61	V2	
40.61	41.30	43.83	39.67	37.65	V0	A2 Volkameriana
47.92	49.70	52..50	46.33	43.14	V1	
43.14	44.50	46.33	42.50	39.22	V2	
1.098	2.196				0.05 L.S.D	
rootstock average (A)	(B×A)Biofertilization × rootstock					
46.74	47.78	50.58	45.48	43.13	A1	
43.89	45.17	47.56	42.83	40.00	A2	
0.634	1.268				0.05 L.S.D	
average Vermicompost (V)	(B×V) Biofertilization × Vermicompost					
41.80	42.15	44.65	41.26	39.14	V0	
49.62	51.64	54.45	47.74	44.65	V1	

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44.53	45.63	48.10	43.47	40.91	V2
0.776	1.553				0.05 L.S.D
	(B) Biofertilization average				
0.897	46.47	49.07	44.15	41.57	0.05 L.S.D

Zinc content of leaves

The results of Table (7) showed that there were significant differences between the rootstock in the trait of zinc content of leaves, as the sour orange rootstock gave the highest average in the zinc content of leaves, reaching (37.36), compared to the Volkameriana rootstock, which gave the lowest average for that trait, amounting to (34.27). The reason is that spraying orange trees grafted onto sour orange rootstock with Azotobacter bacteria caused a significant increase in the leaves' content of zinc and other nutrients, and this agrees with [27] Table (7) revealed that there were significant differences for vermicompost concentrations in the leaves' zinc content. The second concentration, V1 (10%), excelled and gave the highest average of (37.69), compared to control treatment, which gave the lowest average in this trait of (34.16). The reason is that earthworms secrete vermicompost, which is a nutritional organic fertilizer rich in humus and macro- and micro-nutrients such as N, P, K, Zn, Fe (and others necessary for plants and beneficial microorganisms such as nitrogen-fixing and phosphate-dissolving bacteria. This is in agreement with [28] This is clear from Table (7) There are significant differences between the concentrations of biofertilization in the zinc content of leaves. The third concentration, B1 (bacteria), gave the highest average in the zinc content of leaves, amounting to (38.75), compared to control treatment, which gave the lowest average for that attribute, amounting to (33.02). The reason is that adding bacteria leads to an increase in the number of lateral roots and enhances the formation of root hairs to provide a good root surface area that helps absorb the nutrients present in the soil. This agrees with [29] As for bi-interaction between rootstock and vermicompost. It was significant in the zinc content of the leaves, as shown in Table (7), as the combination (A1 V1) gave the highest average in the zinc content of the

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leaves, amounting to (39.05), while the combination (A2V0) gave the lowest average for that trait, amounting to (32.44). The interaction between rootstock and biofertilization was significant in the zinc content of leaves, as the combination (A1B1) gave the highest average in the zinc content of leaves, amounting to (40.66), while the combination (A2 B0) gave the lowest average for that trait, amounting to (32.04). . As for bi-interaction between vermicompost and biofertilization, it was significant in terms of the zinc content of the leaves, as the combination (V1 B1) gave the highest average of (40.30) compared to the combination (V0 B0), which gave the lowest average of (30.48). The triple interactions affected Rootstock, vermicompost, and biofertilization significantly affected the zinc content of leaves, as the combination (A1V1B1) gave the highest average of (42.30) compared to the combination (A2V0B0), which gave the lowest average of (29.43).

Table. (7) The effect of rootstock type, vermicompost, and biofertilization and their interactions on the zinc content of Citrus aurantifolia leaves.

× rootstock Vermicompost (V ×A)	Biofertilization				Vermicompost	rootstock
	B3	B2	B1	B0		
35.88	34.53	37.96	39.50	31.53	V0	A1 sour orange
39.05	38.03	39.26	42.30	36.60	V1	
37.15	36.20	38.33	40.20	33.86	V2	
32.44	31.53	33.46	35.33	29.43	V0	A2 Volkameriana
36.33	35.50	37.03	38.30	34.50	V1	
34.03	32.93	34.14	36.86	32.20	V2	
0.501	1.003				0.05 L.S.D	
rootstock average (A)	(B×A)Biofertilization × rootstock					
37.36	36.25	38.52	40.66	34.00	A1	
34.27	33.32	34.88	36.83	32.04	A2	
0.289	0.579				0.05 L.S.D	
average Vermicompost	(B×V) Biofertilization × Vermicompost					

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(V)					
34.16	33.03	35.71	37.41	30.48	V0
37.69	36.76	38.15	40.30	35.55	V1
35.59	34.56	36.23	38.53	33.03	V2
0.354				0.709	0.05 L.S.D
	(B) Biofertilization average				
0.409	34.78	36.70	38.75	33.02	0.05 L.S.D

Conclusions and recommendations

The grafted sour orange rootstock outperformed Citrus aurantifolia seedling and gave the highest averages in some of the studied traits, including (leaves area, number of secondary roots, dry weight of the root system, iron content of the leaves, and zinc content of the leaves). The Volkameriana rootstock excelled in the trait (fresh weight of the rootstock) root) compared to control treatment. The results showed that the concentration (10%) of vermicompost was superior in all the traits studied above. The results showed that the biofertilization was superior in concentrations (10 grams of mycorrhizal fungi), (20 grams of a mixture between bacteria and mycorrhizal fungi), and (10 grams Bacteria) gave the highest averages in some of the studied traits compared to control treatment. Therefore, we recommend conducting a similar experiment and using several different citrus roots and some concentrations of vermicompost and biofertilization.

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