Role of nano-selenium and irrigation water quality in some anatomical and chemical characteristics of some citrus rootstocks

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https://doi.org/10.59658/jkas.v11i4.2782

Received:
June 13, 2024

Accepted:
Sep. 15, 2024

Published:
Dec. 15, 2024

Abstract

The research was carried out in a lath-house at Karbala certified citrus nursery/Department of Horticulture/Kerbala Agriculture Directotae, Hindiyah District, for the season 2022, starting from 1/3 to 1/11, to study the effect of spraying with Nano-selenium and water types for irrigation water on some anatomical and chemical characteristics of three citrus rootstocks. The experiment was three factors Split-Split Plot Design in R.C.B.D. The main plot was two types of water: river water and well water, sub-plot was three Citrus rootstocks, Sour orange, Volcamariana and Lime seedlings, while the sub-sub-plot included Spraying Nano selenium at a concentration of 0, 1, and 2 mg L^{-1}. The result showed that the spraying with Nano-selenium at 2ml L^{-1} resulted in higher cuticle thickness, CHO%, proline acid, ABA hormone and Glutathione GSH. However, Nano-selenium and water quality did not show much effect on the average number of stomata in the leaves. For the water types, it showed that the irrigation with well water resulted in higher cuticle thickness, plant content of proline acid, ABA hormone and Glutathione GSH). Among the rootstocks, the Sour orange had the highest in plant content of proline acid, ABA hormone and Glutathione GSH, while Lime rootstock was the best in number of leaf stomata and CHO%, as the rootstocks didn't differ in leaf cuticle thickness. The best results for the most indicators under study were obtained in the presence of Nano-selenium 2 mg L^{-1} where interacted with well water and Sour orange. Lime rootstock seedlings performed better with river water. This study showed that applying Nano- selenium 2 mg L^{-1} can be the best choice to improve seedlings growth and to enhance seedling tolerance to well water salinity.

Keywords: Nano-minerals, salinity, leaf stomata, plant nutrition, citrus seedlings

Introduction

Citrus fruit trees belong to the Rutaceae plant family, which includes many genera, the most important of which from an economic standpoint is the genus Citrus, to which most citrus species are traced [1]. The cultivated area of citrus trees in the world amounted to 10,072,197 hectares, with a total production of 158,491,166 tons [2]. citrus trees in Iraq reached 10,355,596 million trees, and a production of 226,166 thousand tons, with an average production of 21.84 kg/tree, where Salah al-Din Governorate ranked first in production, followed by Baghdad and then Diyala [3]. Nowadays, there is an increase in the production and consumption of citrus fruits due to the nutritional and health importance of this fruit. This increase in agriculture and consumption also requires good quality, strong, fast-growing seedlings to be marketed in a short period to cover the need for establishing new orchards. Sour orange is one of the most important rootstocks used to propagate various citrus fruits due to its ease of propagation by seeds, as well as its complete compatibility with most of the grafts grown on it. It tolerates increased ground humidity and salinity to some extent, and the trees grafted on it are medium to strong in growth. Its fruits are of high quality, in addition to being a good rootstock suitable for the soils medium and heavy [4]. Also, there are other rootstocks, that are used for propagating citrus fruits, which is Volcameriana, which results from cross-breeding citron with lime, It is considered a suitable rootstock for grafting some citrus trees on it [5] and is characterized by its resistance to rapid deterioration and gum disease, and the fruits of the varieties grafted on it are characterized by high quality [6] As well as the rootstock of lime, which is that has been widely used in the past few years in Iraq due to its compatibility with most citrus fruits, as it is grown in light soils, but it is sensitive to the disease of rapid deterioration, gumming, and low temperature [7]. Nano-fertilization technology, is one of the modern methods, which use leads to an increase, in the quantity and quality of the yield, as it deals with extremely small particles with dimensions, ranging from 1-100 nanometers, where the length of 10 hydrogen atoms is approximately one nanometer, i.e. 1×10^{-9} meters [8]. among these elements is selenium, which is considered a rare element and has a major role in increasing the activity of enzymatic antioxidants, as it acts as a catalyst for these antioxidants, especially the enzyme glutathione peroxidase, which converts the toxic compound hydrogen peroxide, H_2O_2, resulting from the effect of salt stress, into water molecules, H_2O [9]. Selenium element, is also characterized by its association with amino acids, especially methionine acid and cysteine acid, forming selenium proteins, which have a superior ability to withstand cell membranes to salt stress and prevent the catabolism of protein plant metabolic compounds. This mechanism considered one of the most successful and effective means through which the plant can withstand salt stress in the presence of the selenium element [10]. Salt stress is one of the important abiotic environmental stresses that greatly affects the growth and productivity of plants, as the increase in soil salinity is considered one of the most important problems that reduce the areas allocated for agriculture in most regions of the world, especially in arid and semi-arid areas that have little rainfall and Relatively high temperatures, which leads to an increase in the rate of evaporation and a high rate of transpiration agricultural production [11].

The aim of this research is to determine the best concentration of Nano-selenium and the best rootstock of their effect on some anatomical and chemical characteristics under the influence of salinity stress.

Materials and Methods

The research was carried out in Karbala certified citrus nursery/Horticulture Department / Iraqi Ministry of Agriculture, located in Al-Hindiya District - Karbala Governorate, for the season 2022, starting from 1/3 to 1/11, As the seeds of three citrus rootstocks, Sour orange, Volcamariana and Lime, were planted Under 50% shade house. Three seeds of each citrus rootstocks planted in 10 Liter plastic pots filled 5:1 (V/V) Silt and peat moss. Soil and water samples taken for physical and chemical analysis (Table 1 and 2) in the laboratories of the Field Crops Department / College of Agriculture - University of Karbala [12]. The experiment was 2×3×3 split-split design factorial according a randomized complete block design (R.C.B.D) with three replications. The research included three factors, first factor was two types of water: river water (W1) and well water (W2) as main plot, the second factor (sub-plot) was three types of Citrus rootstocks: Sour orange(V1), Volcamariana (V2) and Lime (V3), while the third factor (sub-sub-plot) was spraying Nano-selenium at concentrations of 0, 1, and 2 mg L^{-1} symbolized S1, S2 and S3 respectively. The seedlings were irrigated as needed till grown seedlings reached 5 true leaves at which were abundant irrigated the day before spraying with Nano-selenium. Foliar spray with Nano-selenium was applied in the early morning using a 1.5-liter hand sprayer filled with Nano-selenium liquid to which a few drops of dish washing liquid were added as a surfactant. An appropriate distance also left between the treatments to avoid liquid splash to untargeted treatments. The spraying was applied four times with two weeks intervals, starting from 1/7 to 15/8. One month after the last spray treatment, on 1/11, data samples were collected taken from the seedlings of both groups to analyze and record the results.

Statistical analysis

The results were analyzed using Gen Stat package (GenStat version 12.1) [13], according to the analysis of variance ANOVA table, the means were compared according to the least significant difference test (LSD) $P \leq 0.05$ [14].

Studied traits

Number of stomata (stomata plant^{-1})

It calculate by counting the number of stomata present on the lower surface of the leaf after making longitudinal sections of the seedling leaves and making slides from them that placed under an optical microscope with 40X magnification. Several sections taken for each experimental unit, and the number of stomata in them was counted and then the average number of stomata was calculated and then the average number of stomata was extracted for each treatment.

Thickness of cuticle layer (micrometer)

it measured by taking a mature, fully developed leaves from the middle area of the seedling for each experimental unit, washing them to remove dust from them, then making slides from them by making cross-sections of them, then placing them under an optical microscope with 40X magnification, for this purpose, an ocular lens use. Several clips taken for each experimental unit to extract its rate. The rate for each treatment calculates by multiplying the rate x 2.5 (the calibration constant).

Leaf content of CHO (%)

It was estimated according to the method in [15].

Leaf content of proline acid (mg g^{-1})

It was determined according to the method of [16]

Leaf content of ABA (µmol g^{-1})

ABA (Abscisic acid) estimate based on the method presented in [17].

Leaf content of GSH (µmol g^{-1})

It was estimate according to the method used by [18].

Table (1): Some chemical and physical characteristics of the experiment soil

Properties	Value	Unit
Sand	745	g kg^{-1}
Silt	62	g kg^{-1}
Clay	193	g kg^{-1}
Soil texture	-------	Sandy loam
E.C	1.25	ds.m^{-1}
pH	8.10	--------
$N.NH_3$	45.3	mg kg^{-1}
$N.NO_3$	24.7	mg kg^{-1}
P	0.003	%
K	11.16	mg kg^{-1}
SO_4	17.91	p.p.m

Table (2): Some chemical characteristics of water used in the experiment

Water Types	E.C $ds.m^{-1}$	pH	dissolved ions						
			Na^{+} $mmol.l^{-1}$	K^{+} $mmol.l^{-1}$	Ca^{++} $mmol.l^{-1}$	Mg^{++} $mmol.l^{-1}$	Cl^{-} $mmol.l^{-1}$	HCO_3^{-} $mmol.l^{-1}$	SO_4^{-} $mmol.l^{-1}$
W1	1.7	7.91	7.72	0.20	0.45	0.18	3.87	1.53	3.10
W2	5.3	7.85	31.67	0.11	3.89	5.48	5.89	2.25	6.74

Results and Discussion

1-Number of stomata (stomata $plant^{-1}$)

Looking at the results shown in (Table 3), Nano-selenium did not show much effect on the average number of leaf stomata, regardless the concentration used. Again, the number of leaf stomata was not affected by types of water used, as the two water types did not differ.As the results of, while the three rootstocks had a clear significant effect represented by the superiority of the Lime rootstock (V3) over the other two rootstocks by recording the highest rate of 29.93 stomata $seedling^{-1}$, compared to the lowest rate recorded in Sour orange rootstock (V1) with an average of 25. 39 stomata $seedling^{-1}$. As for the interaction effect, there was a significant effect in there interaction water quality and rootstock as the interaction treatment W1 V3, recording an average of 30.11 stomata $seedling^{-1}$. On the other hand, the interaction of irrigation water type and Nano-selenium also led to significant increase especially the interaction treatment W1 S0 resulting in 31.10 stomata $seedling^{-1}$ (Table3). The same effect also recorded in the interactions V2 S0 and V3 S1 by recording the highest rates of 31.48 and 31.33 stomata $seedling^{-1}$, respectively. However, the highest numbers of leaf stomata was in the total interaction treatments of W1 V3 S0 resulted in 35.80 stomata $seedling^{-1}$, compared to the lowest rate in interaction treatment W2 V1 S0 that had 18.80 stomata $seedling^{-1}$.

when exposed to salt stress by helping to equalize the osmotic potential inside and outside cells and returning cell functions to normal by absorbing abundant water by the roots and getting rid of it through evaporation resulting from the transpiration process, this is consistent with what [19] found that the increase in the number of stomata per unit area results from the small leaf area of the plant, and this leads to their proximity to each other, as well as the plant’s attempt to equalize the osmotic potential of the soil solution by withdrawing the largest amount of water and excreting it to the outside through the process of transpiration.

Table (3): Effect of the experimental treatments on Number of stomata (stomata seedling^{-1})

Treatments		Nano-Selenium ml L^{-1}			W × V
Water types	**Variety**	**S0**	**S1**	**S2**	
W1	**V1**	25.40	24.53	22.73	24.22
	V2	32.09	24.80	28.00	28.30
	V3	35.80	27.13	27.40	30.11
W2	**V1**	18.80	26.87	34.00	26.56
	V2	30.87	25.73	21.20	25.93
	V3	20.00	35.53	33.73	29.75
Interaction		**L.S.D 0.05**			**L.S.D0.05**
		6.13			**4.40**
Nano-S.		27.16	27.43	27.84	**L.S.D0.05**
					n.s
					Water types
W× Nano-S.	**W1**	31.10	25.49	26.04	27.54
	W2	23.22	29.38	29.64	27.41
L.S.D 0.05		**4.27**			**L.S.D 0.05**
					n.s.
					Variety
V× Nano-S.	**V1**	22.10	25.70	28.37	25.39
	V2	31.48	25.27	24.60	27.12
	V3	27.90	31.33	30.57	29.93
L.S.D 0.05		**4.13**			**L.S.D 0.05**
					2.68

Values are means of three replications. The treatments are; two types of water (river water (W1) and well water (W2)), as main plot, three Citrus rootstocks: Sour orange (V1), Volcamariana (V2) and Lime (V3), Nano-selenium spray at 0, 1, and 2 mg L^{-1} S0, S1 and S2 respectiively.

2- **Thickness of cuticle layer (micrometer)**

As for the effect of experimental factors on cuticle layer thickness, Nano-selenium significantly affected the thickness of the cuticle layer in the leaves (Table4). Nano- selenium at 2 ml L^{-1} was distinguishable than other two concentrations recording 4.10 micrometers. The well water irrigation recorded a higher rate of 4.17 micrometers compared to irrigation with river water that resulted in 3.24 micrometers, while rootstocks did not differ among each other (Table4). The interaction treatments of the two factors W2V3 and W2 V2 resulted in higher values 4.58 and 4.44 micrometers over the other treatments. The interaction of irrigation water quality and Nano selenium also led to significant increase for this trait, as the interactions W2 S1 and W2 S2 recorded the highest rate of 4.44 micrometers. While the interaction between the rootstock and Nano selenium had a variation in their effects where the V2 S2 and V3 S2 interaction recorded the highest cuticle thickness that of 4.38 and 4.17 micrometers, respectively. As for the three factors interactions it is note that the interactions W2 V2 S2 and W2 V3 S2 distinguished by recording similar rates that amounted 5.42 and 5.00 micrometers, respectively compared with the other treatments. The increase in the thickness of the cuticle layer came from the effect of salinity in reducing the process of water absorption from the roots, which leads to a reduction in vegetative growth and inhibition of the process of cell division and expansion, thus reducing the size of the cells and reducing the surface area of the leaf [20].

Table (4): Effect of the experimental treatments on thickness of cuticle layer (micrometer)

Treatments		**Nano-Selenium ml L^{-1}**			**W × V**
Water types	**Variety**	**S0**	**S1**	**S2**	
W1	**V1**	2.92	3.33	4.58	3.61
	V2	2.92	2.50	3.33	2.92
	V3	2.92	3.33	3.33	3.19
W2	**V1**	2.92	4.58	2.92	3.47
	V2	3.33	4.58	5.42	4.44
	V3	4.58	4.17	5.00	4.58
Triple interaction		**L.S.D 0.05**			**L.S.D0.05**
		0.73			**0.45**
Nano-S.		3.26	3.75	4.10	**L.S.D0.05**
					0.31
					Water types
W× Nano-S.	**W1**	2.92	3.06	3.75	3.24
	W2	3.61	4.44	4.44	4.17
L.S.D 0.05		**0.37**			**L.S.D 0.05**
					0.20
					Variety
V× Nano-S.	**V1**	2.92	3.96	3.75	3.54
	V2	3.13	3.54	4.38	3.68
	V3	3.75	3.75	4.17	3.89
L.S.D 0.05		**0.55**			**L.S.D 0.05**
					n.s.

Values are means of three replications. The treatments are; two types of water (river water (W1) and well water (W2)), as main plot, three Citrus rootstocks: Sour orange (V1), Volcameriana (V2) and Lime (V3), Nano-selenium spray at 0, 1, and 2 mg L^{-1} S0, S1 and S2 respectively.

3- **Leaf content of CHO (%)**

The results in (Table 5) confirmed the significant effect of foliar Nano-selenium at 2 ml L^{-1}, river water and lime V3 seedlings on leaf content of total CHO, recording higher values as a single treatment, it was 20.77, 22.83 and 22.43% Respectively. Interactions of W1 V3, W1 S2, and V3 S2 were the best among the two factors interaction treatments by giving 25.93, 24.27 and 23.92% respectively. On the other hand, the highest leaf content of total CHO was recorded in the interaction treatment of W1 V3 S2 that gave 27.42% compared with other treatments. Decrease of CHO in seedlings exposed to salt stress is due to a decrease in the process of photosynthesis due to the effect of salinity, and thus a decrease in the production of chlorophyll in the leaves, accompanied by the closure of stomata in the process of gas exchange, or the reason may be due to the formation of the enzyme Chlorophylase as a result of the effect of salinity, which works to destroy the chlorophyll pigment. In addition, slow down the speed of their formation [21].

Table (5): Effect of the experimental treatments on leaf content of CHO (%)

Treatments		**Nano-Selenium ml L^{-1}**			**W × V**
Water types	**Variety**	**S0**	**S1**	**S2**	
W1	**V1**	18.30	18.78	19.68	18.92
	V2	21.74	23.46	25.72	23.64
	V3	23.51	26.85	27.42	25.93
W2	**V1**	11.36	11.82	12.69	11.96
	V2	14.82	16.48	18.70	16.67
	V3	16.53	19.87	20.41	18.94
Triple interaction		**L.S.D 0.05**			**L.S.D0.05**
		2.38			**1.51**
Nano-S.		17.71	19.54	20.77	**L.S.D0.05**
					0.99
					Water types
W× Nano-S.	**W1**	21.18	23.03	24.27	22.83
	W2	14.24	16.06	17.27	15.85
L.S.D 0.05		**1.47**			**L.S.D 0.05**
					1.70
					Variety
V× Nano-S.	**V1**	14.83	15.30	16.18	15.44
	V2	18.28	19.97	22.21	20.15
	V3	20.02	23.36	23.92	22.43
L.S.D 0.05		**1.68**			**L.S.D 0.05**
					1.07

Values are means of three replications. The treatments are; two types of water (river water (W1) and well water (W2)), as main plot, three Citrus rootstocks: Sour orange (V1), Volcamariana (V2) and Lime (V3), Nano-selenium spray at 0, 1, and 2 mg L^{-1} S0, S1 and S2 respectiively.

4- **Leaf content of proline acid (mg g^{-1})**

The results recorded in (Table 6) showed a significant effect of spraying with Nano-selenium increasing the leaf content of proline acid at both concentrations 1 and

2 ml^{-1} by giving17.84 and 18.84 mg g^{-1} sequentially, compared to the 0 ml L^{-1} which gave 16.64 mg g^{-1}. Higher content of proline acid were record in case of irrigation with well water compared with river water, it was 20.78 mg g^{-1}.The sour orange (V1) always had higher values of this indicator by recoding 19.99 mg g^{-1} compared to the other rootstocks. As for the interactions of two factors, highest values recorded in the interactions W2 V1, W2 S2, and V1 S2, it was 22.96, 21.84 and 21.35 mg g^{-1} respectively. In case of total interaction, highest value recorded in the interaction treatment W2 V1 S2 that gave24.31 mg g^{-1}. The accumulation of proline acid in plant tissues exposed to salt stress may be due to its rapid formation and slow effectiveness of the enzymes that oxidize it. In addition, the increase in its accumulation may be due to the increase in protein catabolism and its conversion into many amino acids, including proline acid[22].

Table (6): Effect of the experimental treatments on leaf content of proline acid(mg g^{-1})

Treatments		**Nano-Selenium ml L^{-1}**			**W × V**
Water types	**Variety**	**S0**	**S1**	**S2**	
W1	**V1**	15.46	17.18	18.39	17.01
	V2	12.34	13.57	14.44	13.45
	V3	13.11	13.75	14.72	13.86
W2	**V1**	21.46	23.12	24.31	22.96
	V2	18.34	19.58	20.54	19.49
	V3	19.15	19.86	20.67	19.89
Triple interaction		**L.S.D 0.05**			**L.S.D0.05**
		3.16			**2.06**
Nano-S.		16.64	17.84	18.84	**L.S.D0.05**
					1.30
					Water types
W× Nano-S.	**W1**	13.64	14.83	15.85	14.77
	W2	19.65	20.85	21.84	20.78
L.S.D 0.05		2.01			**L.S.D 0.05**
					2.41
					Variety
V× Nano-S.	**V1**	18.46	20.15	21.35	19.99
	V2	15.34	16.58	17.49	16.47
	V3	16.13	16.80	17.70	16.88
L.S.D 0.05		**2.20**			**L.S.D 0.05**
					1.40

Values are means of three replications. The treatments are; two types of water (river water (W1) and well water (W2)), as main plot, three Citrus rootstocks: Sour orange (V1), Volcamariana (V2) and Lime (V3), Nano-selenium spray at 0, 1, and 2 mg L^{-1} S0, S1 and S2 respectiively.

5- **Leaf content of ABA hormone (µmol g^{-1})**

From the results shown in (Table 7), it is note that spraying Nano-selenium especially at the highest concentration 2 ml L^{-1} significantly increased leaf content of ABA, compared to the other concentrations; it was 44.77-µmol g^{-1}. Higher ABA values were recorded in case of irrigation with well water by giving 46.16-µmol g^{-1} and Sour orange V1 seedlings was the higher content of the seedlings leaves of this hormone than the other rootstocks, as it reached 44.62 µmol g^{-1}. Higher values recorded in interaction of W2 V1 compared to other interactions of the two factors it record 49.93-µmol g^{-1}. Similar results were in interaction of W2 S2 and V1 S2 treatments, they given 50.03-µmol g^{-1} and 49.00-µmol g^{-1}. The highest rate for the leaf content of ABA hormone recorded in interaction treatment of W2 V1 S2 which gave 54.25-µmol g^{-1} compared to the other treatments These results are consistent with [23], who indicated that ABA accumulates when strawberry seedlings treated with Nano-selenium when irrigated with salt water, which is an indication of a stress state in the plant.

Table (7): Effect of the experimental treatments on leaf content of ABA(µmol g^{-1})

Treatments		**Nano-Selenium ml L^{-1}**			**W × V**
Water types	**Variety**	**S0**	**S1**	**S2**	
W1	**V1**	35.59	38.56	43.75	39.30
	V2	30.25	32.57	37.04	33.29
	V3	31.72	33.19	37.75	34.22
W2	**V1**	46.37	49.17	54.25	49.93
	V2	40.75	43.11	47.64	43.83
	V3	42.48	43.48	48.20	44.72
Triple interaction		**L.S.D 0.05**			**L.S.D0.05**
		3.96			2.60
Nano-S.		37.86	40.01	44.77	**L.S.D0.05**
					1.62
					Water types
W× Nano-S.	**W1**	32.52	34.77	39.52	35.60
	W2	43.20	45.25	50.03	46.16
L.S.D 0.05		2.46			**L.S.D 0.05**
					2.92
					Variety
V× Nano-S.	**V1**	40.98	43.87	49.00	44.62
	V2	35.50	37.84	42.34	38.56
	V3	37.10	38.33	42.98	39.47
L.S.D 0.05		**2.79**			**L.S.D 0.05**
					1.84

Values are means of three replications. The treatments are; two types of water (river water (W1) and well water (W2)), as main plot, three Citrus rootstocks: Sour orange (V1), Volcameriana (V2) and Lime (V3), Nano-selenium spray at 0, 1, and 2 mg L^{-1} S0, S1 and S2 respectively

6- **Leaf content of GSH (μmol g⁻¹)**

It can be seen (Table 8) that foliar Nano-selenium had a significant effect on the leaf content of GSH. The concentrations of 1 and 2 ml L^{-1} gave higher levels compared with seedlings sprayed with DW. This indicator was higher in seedlings irrigated with well water compared to river water irrigated ones. Rootstocks also differed significantly among each other this indicator; Sour orange (V1) had the highest value. Regarding the interaction of two factors, a clear significant effect appeared in the interaction treatment W2 V1 recording the highest leaf content of the GSH 28.06 μmol g^{-1}. Also, the treatment W2 S2 gave a high rate that reached 26.90 μmol g^{-1} with significant difference from the control. Similar results were recorded due in treatment V1 S2, where the value was 26.35 μmol g^{-1}. In general the highest leaf content of GSH was recorded in the total interaction treatment of W2 V1 S2 resulting in 29.38 μmol g^{-1}, which differed significantly from most the treatments. Spraying with Nano-selenium increased production of GSH in seedlings exposed to salt stress, as it played a major role in the plant's resistance to various stresses, including salt stress [24], compared with control. Sour orange rootstock was the best in its production compared to the other rootstocks; this indicates the importance of this acid in preventing oxidation of the compounds present inside the cells as it is one of the effective antioxidants, in addition to its role in removing toxins from plant cells. It also has a role in the pathways for building plant hormones and glutathione ascorbate. Jasmonic acid is also included as an essential substance in the path of formation of glutathione-S-transferase, as it helps protect the cell from the harmful effects of the oxidation [25].

Table (8): Effect of the experimental treatments on leaf content of GSH(μmol g^{-1})

Treatments		**Nano-Selenium ml L^{-1}**			**W × V**
Water types	**Variety**	**S0**	**S1**	**S2**	
W1	**V1**	20.46	22.15	23.33	21.98
	V2	17.23	18.41	19.38	18.34
	V3	18.16	18.79	19.74	18.90
W2	**V1**	26.54	28.26	29.38	28.06
	V2	23.45	24.63	25.65	24.57
	V3	24.37	24.72	25.66	24.92
Triple interaction		**L.S.D0.05**			**L.S.D0.05**
		3.17			2.02
Nano-S.		21.70	22.83	23.86	**L.S.D0.05**
					1.32
					Water types
W× Nano-S.	**W1**	18.62	19.78	20.82	19.74
	W2	24.79	25.87	26.90	25.85
L.S.D 0.05		1.99			L.S.D 0.05
					2.34
					Variety
V× Nano-S.	**V1**	23.50	25.21	26.35	25.02

	V2	20.34	21.52	22.51	21.46
	V3	21.27	21.76	22.70	21.91
L.S.D 0.05		2.22			L.S.D 0.05
					1.39

Values are means of three replications. The treatments are; two types of water (river water (W1) and well water (W2)), as main plot, three Citrus rootstocks: Sour orange (V1), Volcameriana (V2) and Lime (V3), Nano-selenium spray at 0, 1, and 2 mg L^{-1} S0, S1 and S2 respectively.

From this experiment, it conclude that treating citrus rootstock seedlings with Nano-selenium at two concentrations of 1 and 2 ml L^{-1} had a significant effect on most of the traits studied, but the concentration of 2 ml L^{-1} was the best in most of them. Irrigation with well water also led to damage to seedlings irrigated with it, as its negative effects appeared in the studied characteristics, whether anatomical or chemical, compared to seedlings irrigated with river water. Results also showed the superiority of Sour orange rootstock in most of the studied characteristics

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