

Effects of Agricultural Sulfur and Salicylic Acid on Soil Nutrient Availability and Leaf Nutrient Concentration of Bitter Orange Seedlings under Water Stress

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Received (08/01/2026), Received in revised form (02/03/2026)
Accepted (14/03/2026), Available online (19/03/2026)

FJIAS 2026, 2(1): 63-78

Abstract Agriculture in Iraq is increasingly challenged by water scarcity, which negatively affects plant growth, particularly evergreen species like sour orange (*Citrus aurantium*). Agricultural sulfur and salicylic acid are known to improve soil fertility and enhance plant tolerance to environmental stresses; however, their combined effect on sour orange seedlings under water stress has not been sufficiently studied. This experiment, conducted at the Department of Soil and Water Technologies, Al-Mussaib Technical College, during the 2025 season, aimed to evaluate the effects of sulfur application (0, 1000, 2000 kg ha⁻¹), salicylic acid spraying (0, 200, 400 mg L⁻¹), and water stress (50, 75, 100% field capacity) on soil nutrient availability and leaf nutrient concentration. One-year-old uniform seedlings were planted in pots containing a soil–peat moss mixture (1:2). Results showed that the highest sulfur level (S2) significantly improved all measured traits. Water stress at 75% field capacity (W2) enhanced soil nutrient availability, while full irrigation (W3) increased leaf nutrient concentration. Spraying with salicylic acid at 200 mg L⁻¹ (L1) significantly increased leaf nutrient concentrations. Two-factor interactions revealed that S2W2, S2L0, and W2L0 treatments maximized soil nutrient availability, whereas S2W3, S2L1, and W3L1 enhanced leaf nutrient concentrations. The three-factor interaction indicated that S2W2L0 was superior for soil nutrient availability, and S2W3L1 was most effective in increasing nutrient concentration in leaves. These findings highlight the potential of combining sulfur application and salicylic acid spraying under controlled water conditions to improve nutrient dynamics in sour orange cultivation under water-limited environments.

Keywords: Agricultural sulfur, Salicylic acid, Soil nutrients, Water stress

1. INTRODUCTION

The use of sulfur as one of the critical and invaluable nutrients in the growth of plants and important physiological functions has its roots back to 1860. It is also significant in the

improvement of the supply of various nutrients such as nitrogen, phosphorus, and potassium. The major source of sulfur in soil is the organic matter followed by the acid rain and the volcanic activities as the other sources of sulfur to the soils. Other causes are the fertilizers that contain sulfur [1]. Normal plant tissues contain between 0.1-1 percent of sulfur, reduction of the concentration below 0.1 percent leads to noticeable deficiency symptoms, such as chlorosis of leaves [2]. The lack of this element results in disruption of the synthesis of proteins. Sulfur is a very important element in the production of pyridoxine one of the basic components needed in the growth of plants because of its capacity to serve as hydrogen donor and also because it is involved in reduction of nitrates. Sulfur is also involved in amino acid synthesis in plants alongside nitrogen in the production of sulfur containing amino acids like cysteine and methionine, which play a role in protein structure. This occurs by taking place within chloroplasts whereby sulfate is reduced to sulfide and later on, it is incorporated in cysteine [3]. Water stress has been regarded as one of the most apparent forms of environmental stress and also one of the most significant on plant growth. It can be either due to the lack of water to plants or the inability of the plants to absorb it, resulting in the increasing levels of reactive oxygen species (ROS), plasma membrane permeability increase, and the alterations in enzymatic and non-enzymatic antioxidants in addition to the inhibition of plant growth and photosynthesis [4]. Under drought stress, many systems, including physiological, biochemical, and morphological systems, are influenced in plants [5]. Examples of these physiological systems are stomatal activity, photosynthesis, osmotic balance, transpiration, water content in the leaves and water transport. The biochemical systems encompass the content of antioxidant, chlorophyll, proline accumulation, hormone and secondary metabolites. Morphological systems are reduced leaf area, reduced leaf number, increased root length, leaf senescence, early maturity, and growth stage differentiation [6].

Salicylic acid has been regarded as one of the key bioactive plant compounds and significant in regulating plant responses to abiotic stresses including drought, salinity, heat, through interaction with other compounds including auxins and ethylene. It assists in sustaining plant homeostasis, and stabilization of salicylic acid concentration in plants is among the factors that enhance antioxidant resistance [7]. This compound is critical in triggering physiological and biochemical processes in the life cycle of the plants including the stimulation of root and vegetative growth and flowering, control of stomatal opening and closing and enhanced nutrient uptake and photosynthetic efficiency. It thereby boosts synthesis of pigments like chlorophyll and carotenoids as well as presence of activity of essential enzymes and amino and nucleic acids [8].

Sour orange (*Citrus aurantium* L.) is a citrus plant in the family Rutaceae and has been ranked as one of the key groups in the citrus genus. It contains important bioactive compounds, including flavonoids, which are natural substances characterized by health benefits, such as naringenin and hesperidin, as well as essential oils and vitamin C, which exhibit important biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects [9].

The study aimed to evaluate the effect of adding agricultural sulfur and salicylic acid on the availability of NPK elements in the soil and their concentrations in the leaves of sour orange seedlings under water stress conditions

2. MATERIALS AND METHODS

The experiment was conducted in Babil Governorate, Iraq, at latitude 32.785506° N and longitude 44.293929° E during the 2025 season, following a factorial experiment using a Completely Randomized Design (CRD) with three replicates. Each replicate included 27 experimental units, with each unit containing three seedlings. One-year-old sour orange seedlings were obtained, and 243 seedlings as uniform as possible in size and growth were selected.

The planting soil used in the experiment was prepared by mixing sandy loam soil with peat moss at a ratio of 1:2, respectively. The components were thoroughly mixed to ensure uniform distribution, then filled into agricultural pots with a capacity of 8 kg, and the seedlings were transplanted into the pots. Standard cultural practices were applied uniformly to all treatments, and the seedlings were placed under a shade covered with polyethylene to start the experiment on 15/3/2025. Soil samples were collected randomly from the growing medium, mixed to obtain a representative homogeneous sample, and subjected to chemical, physical, and fertility analyses in the laboratories of the Department of Soil and Water Technologies, Al-Mussaib Technical College / Middle Euphrates University of Technology. The analysis results are presented in Table.(2)

2.1. Experimental factors:

1. Agricultural sulfur addition at levels of (0, 1000, 2000) kg ha⁻¹, coded as S0, S1, and S2, respectively, applied during soil preparation.
2. Water stress at three levels of field capacity (50, 75, 100)%, coded as W1, W2, and W3, respectively, estimated using the gravimetric method as described by [10].
3. Foliar spraying of seedlings with salicylic acid at concentrations of (0, 200, 400) mg L⁻¹, coded as L0, L1, and L3, respectively, applied three times during the experiment on 1/4, 1/5, and 1/9 of 2025.

2.2. Studied traits in soil and plants:

1. Available nitrogen (mg kg⁻¹): determined using the Micro-Kjeldahl apparatus according to [11].
2. Available phosphorus (mg kg⁻¹): determined using a spectrophotometer according to [11].
3. Available potassium (mg kg⁻¹): determined using a flame photometer according to [12].
4. Leaf nitrogen concentration (%): determined using a Kjeldahl apparatus according to [13].
5. Leaf phosphorus concentration (%): determined using a spectrophotometer according to [14].
6. Leaf potassium concentration (%): determined using a flame photometer according to [15].

Table (1): Some properties of agricultural sulfur used in the study

pH 1:1	Ec 1:1 dSm ⁻¹	Sulfur %	Ca ⁺⁺ mg kg ⁻¹	CaCO ₃ %	CaSO ₄ %	C %	Clay %	Diameter Mesh
3.7	4.4	95	64	-	0.0036	0.12	1.5	325

Table (2): Some chemical, physical, and fertility properties of the soil before planting

<i>The attribute</i>	<i>Value</i>	<i>unit of measurement</i>	
<i>Soil reaction (pH)</i>	7.58	-	
<i>Electrical conductivity (ECe)</i>	2.01	<i>ds m⁻¹</i>	
<i>Cation exchange capacity (CEC)</i>	11.7	<i>cmol_c kg⁻¹ soil</i>	
<i>Organic matter</i>	6.8	<i>g kg⁻¹</i>	
<i>Available nitrogen</i>	18.7	<i>mg kg⁻¹</i>	
<i>Available phosphorus</i>	6.9		
<i>Available potassium</i>	143.2		
<i>Bulk density</i>	1.45	<i>Mg m⁻³</i>	
<i>Particle density</i>	2.65		
<i>Soil texture</i>	<i>sand</i>	70.12	<i>%</i>
	<i>silt</i>	17.68	
	<i>clay</i>	12.20	
<i>Soil texture type</i>	<i>Loamy sand</i>		

3. RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1 Available nitrogen in soil (mg kg⁻¹): The results presented in Table (3) indicate significant differences among the levels of the studied factors in soil nitrogen availability. Treatment S2 (agricultural sulfur addition) was superior, recording the highest mean of 37.98 mg kg⁻¹, with an increase of 20.15% compared to the control treatment S0, which recorded the lowest mean of 31.61 mg kg⁻¹. Regarding the water stress factor, treatment W2 was superior, recording the highest mean of 36.75 mg kg⁻¹, with an increase of 12.24% compared to treatment W1, which recorded the lowest mean of 32.74 mg kg⁻¹. For salicylic acid, treatment L0 was superior, recording the highest mean of 36.03 mg kg⁻¹ compared to treatment L1, which recorded the lowest mean of 34.19 mg kg⁻¹.

The interaction of the two factors, where the agricultural sulfur and water stress were involved, it was observed that treatment S2W2 was significantly better, with the highest mean of 39.25 mg kg⁻¹ with an increase of 35.06 being compared with S0W1 which had the lowest mean of 29.06. The two factor interaction between farming sulfur and salicylic acid revealed that treatment S2L0 was by far superior as it had the highest mean of 38.53 mg kg⁻¹ with an increment of 25.95 per cent as compared to treatment S0L1 that had the lowest mean of 30.59 mg kg⁻¹. The interaction of the two factors of water stress and salicylic acid indicated that

treatment W2L0 was better, with the highest mean of 37.53 mg kg⁻¹ with an increase of 19.44 percent as compared to treatment W1L1 which had the lowest mean of 31.42mg kg⁻¹.

The results of the three-factor interaction among the studied factors indicated that treatment S2W2L0 was significantly superior, recording the highest mean of 39.97 mg kg⁻¹, with an increase of 43.46% compared to treatment S0W1L1, which recorded the lowest mean of 27.86 mg kg⁻¹.

Table (3). Effect of agricultural sulfur addition, water stress, and salicylic acid on available nitrogen in soil (mg kg⁻¹)

Agricultural sulfur (kg ha ⁻¹)	Field capacity (%)	Salicylic acid (mg L ⁻¹)			Average
		L0	L1	L2	
S0	W1	30.27	27.86	29.06	29.06
	W2	34.55	32.60	33.23	33.46
	W3	33.82	31.31	31.76	32.29
S1	W1	34.30	31.40	34.07	33.26
	W2	38.07	36.87	37.70	37.54
	W3	37.71	35.80	36.63	36.71
S2	W1	36.86	35.01	35.80	35.89
	W2	39.97	38.20	39.60	39.25
	W3	38.75	38.65	38.96	38.79
LSD0.05		0.90			0.52
S0		32.88	30.59	31.35	31.61
S1		36.69	34.69	36.13	35.84
S2		38.53	37.29	38.12	37.98
LSD0.05		0.52			0.30
W1		33.81	31.42	32.98	32.74
W2		37.53	35.89	36.84	36.75
W3		36.76	35.25	35.79	35.93
LSD0.05		0.52			0.30
Average		36.03	34.19	35.20	
LSD0.05		0.52			

3.1.2. Available phosphorus in soil (mg kg⁻¹):The results presented in Table (4) indicate important differences in the levels of the factors studied on the availability of phosphorus in soil. Best treatment was treatment S2 (agricultural sulfur addition) because it had the highest mean of 16.08 mg kg⁻¹ and an increase of 76.31% relative to the control treatment S0 which had the lowest mean of 9.12 mg kg⁻¹. Treatment W2 was better as far as the water stress factor is concerned whereby the highest mean was 14.27 mg kg⁻¹ with a percentage increase of 27.98% as opposed to treatment W1 where the highest mean stood at 11.15 mg kg⁻¹. In the case of salicylic acid, treatment L0 performed better since it gave the highest mean of 13.64 mg kg⁻¹ as compared to the lowest mean of 12.69 mg kg⁻¹ in treatment L1.

The results of the two-factor interaction between agricultural sulfur and water stress revealed that treatment S2W2 was significantly better in which the mean of 17.55 mg kg⁻¹ was recorded, and its increase was 110.9% as compared to the lowest mean of 8.32mg kg⁻¹ recorded in treatment S0W1. The two factor interaction between agricultural sulfur and salicylic acid

presented the fact that the treatment S2L0 was much better giving the highest mean of 16.75 mg kg⁻¹ whereby an increase of 89.90% was experienced with treatment S0L1 giving the lowest mean of 8.82 mg kg⁻¹. The two factor interaction of the water stress with salicylic acid revealed that treatment W2L0 was best with the highest mean of 14.69 mg kg⁻¹ and the treatment W1L1 recorded the lowest mean of 10.76 mg kg⁻¹.

The outcome of the three factor interaction of the factors under study revealed that treatment S2W2L0 was by far the best with the highest mean of 18.15mg kg⁻¹ with an increase percentage of 124.9 compared to the treatment S0W1L1 with the lowest mean of 8.07mg kg⁻¹.

Table (4). Effect of agricultural sulfur addition, water stress, and salicylic acid on available phosphorus in soil (mg kg⁻¹)

Agricultural sulfur (kg ha ⁻¹)	Field capacity (%)	Salicylic acid (mg L ⁻¹)			Average
		L0	L0	L0	
S0	W1	8.59	8.07	8.29	8.32
	W2	9.90	9.23	9.65	9.59
	W3	9.86	9.15	9.30	9.44
S1	W1	12.19	11.10	11.50	11.60
	W2	16.01	15.26	15.75	15.67
	W3	16.00	15.11	15.44	15.52
S2	W1	13.96	13.12	13.57	13.55
	W2	18.15	17.05	17.43	17.55
	W3	18.13	16.12	17.17	17.14
LSD0.05		0.35			0.20
S0		9.45	8.82	9.08	9.12
S1		14.73	13.82	14.23	14.26
S2		16.75	15.43	16.06	16.08
LSD0.05		0.20			0.12
W1		11.58	10.76	11.12	11.15
W2		14.69	13.85	14.28	14.27
W3		14.67	13.46	13.97	14.03
LSD0.05		0.20			0.12
Average		13.64	12.69	13.12	
LSD0.05		0.12			

3.1.3. Available potassium in soil (mg kg⁻¹): Table (5) results show that there are significant differences between the levels of the studied factors in the availability of potassium in soil. The treatment S2 (agriculture addition of sulfur) was the best treatment as it captured the highest average of 200.16mg kg⁻¹ and an improvement of 19.87% over the control treatment S0 which had the lowest average of 166.97mg kg⁻¹. On the water stress factor, treatment W2 was better in terms of the highest mean of 195.73 mg kg⁻¹, where the mean of W2 was 14.75 percent higher than the mean of W1 which was 170.56mg kg⁻¹. In the case of salicylic acid,

treatment L0 had better treatment than treatment L1 with the highest mean value standing at 192.57 mg kg⁻¹ and the lowest standing at 181.92 mg kg⁻¹ respectively.

The outcome of the interaction between the two factors, agricultural sulfur and water stress indicated that treatment S2W2 was far more superior and it had the highest mean of 213.00mg kg⁻¹ with a percentage increment of 38.70 as compared to S0W1 which had the lowest mean of 153.56mg kg⁻¹. The interaction between the two factors of the agricultural sulfur and salicylic acid revealed that the treatment of S2L0 was significantly better with the highest mean of 213.08mg kg⁻¹ and increased by 29.49 percent as compared to the treatment S0L1 which had the lowest mean of 164.55mg kg⁻¹. The two-factor interaction of water stress and salicylic acid revealed that W2L0 was better as the treatment had the highest mean of 204.92 mg kg⁻¹ with an increase of 22.2% compared to treatment W1L1 with the lowest mean of 167.68mg kg⁻¹.

The outcomes of the three-factor interaction between the factors under study showed that treatment S2W2L0 was significantly better as it had the highest mean of 234.00 mg kg⁻¹, with an increase of 55.43 percent over treatment S0W1L1 which had the lowest mean of 150.55 mg kg⁻¹.

Table (5). Effect of agricultural sulfur addition, water stress, and salicylic acid on available potassium in soil (mg kg⁻¹)

Agricultural sulfur (kg ha ⁻¹)	Field capacity (%)	Salicylic acid (mg L ⁻¹)			Average
		L0	L1	L2	
S0	W1	157.28	150.55	152.85	153.56
	W2	178.66	172.54	173.34	174.85
	W3	175.33	170.54	171.67	172.51
S1	W1	180.93	174.26	176.33	177.17
	W2	202.10	196.43	199.46	199.33
	W3	199.61	194.75	196.33	196.90
S2	W1	185.24	178.24	179.31	180.93
	W2	234.00	201.00	204.00	213.00
	W3	220.00	199.00	200.67	206.56
LSD0.05		6.44			3.72
S0		170.43	164.55	165.95	166.97
S1		194.22	188.48	190.71	191.13
S2		213.08	192.75	194.66	200.16
LSD0.05		3.72			2.15
W1		174.48	167.68	169.50	170.56
W2		204.92	189.99	192.27	195.73
W3		198.31	188.10	189.56	191.99
LSD0.05		3.72			2.15
Average		192.57	181.92	183.77	
LSD0.05		2.15			

3.1.4. Leaf nitrogen concentration (%): According to the data shown in Table (6), there is a significant variance between the levels of the factors under study in nitrogen content of leaves. The best treatment S2 (addition of sulfur in agriculture) had the best mean of 2.77 with an increase of 16.87 as compared to the control treatment S0 which had the lowest mean of 2.37. On the water stress factor, treatment W3 was the best with the highest mean of 2.80 which increased by 23.43 in comparison with treatment W1 which had the lowest mean of 2.27. In the case of salicylic acid, L1 treatment was the best with the highest mean of 2.67 with an increment of 7.66 being the maximum in comparison with L0 with the lowest mean of 2.48. The interaction of two factors, agricultural sulfur and water stress revealed that treatment S2W3 was by far better and the highest mean of 3.07 was obtained with a percentage increase of 42.12 against treatment S0W1 which had the lowest mean of 2.16. The two factor interaction between agricultural sulphur and salicylic acid revealed that the treatment S2L1 is much better and presented the highest mean of 2.88, and grew by 26.31 compared to a treatment S0L0 which had the lowest mean of 2.28. The interaction of the two factors water stress and salicylic acid indicated treatment W3L1 was best with a mean of 2.89, and when the meaning of the increased by 31.96, the treatment W1L0 had the lowest mean of 2.19.

Table (6). Effect of agricultural sulfur addition, water stress, and salicylic acid on leaf nitrogen concentration

Agricultural sulfur (kg ha ⁻¹)	Field capacity (%)	Salicylic acid (mg L ⁻¹)			Average
		L0	L1	L2	
S0	W1	2.05	2.26	2.19	2.16
	W2	2.38	2.47	2.43	2.43
	W3	2.41	2.61	2.51	2.51
S1	W1	2.26	2.37	2.32	2.32
	W2	2.61	2.79	2.72	2.70
	W3	2.72	2.92	2.80	2.81
S2	W1	2.27	2.38	2.34	2.33
	W2	2.71	3.10	2.95	2.92
	W3	2.93	3.15	3.12	3.07
LSD0.05		0.06			0.03
S0		2.28	2.45	2.38	2.37
S1		2.53	2.69	2.61	2.61
S2		2.64	2.88	2.80	2.77
LSD0.05		0.03			0.02
W1		2.19	2.34	2.28	2.27
W2		2.57	2.79	2.70	2.68
W3		2.69	2.89	2.81	2.80
LSD0.05		0.03			0.02
Average		2.48	2.67	2.60	
LSD0.05		0.02			

The outcome of the three factor interaction between the factors under study showed that treatment S2W3L1 was much better as it had the highest mean of 3.15 and the increase of 53.65 over the treatment S0W1L0 which had the lowest mean of 2.05.

3.1.5. Leaf phosphorus concentration (%): The findings in Table (7) have shown that there are strong variations among the levels of the researched factors in the leaf phosphorus concentration. Treatment S2 (agricultural sulfur addition) was best with the highest mean of 0.34, and a growth of 70 percent as compared to the control treatment S0 which had the lowest mean of 0.20. Treatment W3 was found to be better in terms of the water stress factor whereby the highest mean was 0.33% with an increment of 65 percent as compared to treatment W 1 which had the lowest mean of 0.20 percent. In the case of salicylic acid, treatment L1 was better since it had the highest mean of 0.30 and an increment of 20 percent as compared to L0 which had the lowest mean of 0.25.

Table (7). Effect of agricultural sulfur addition, water stress, and salicylic acid on leaf (%) phosphorus concentration

Agricultural sulfur (kg ha ⁻¹)	Field capacity (%)	Salicylic acid (mg L ⁻¹)			Average
		L0	L1	L2	
S0	W1	0.14	0.17	0.16	0.16
	W2	0.19	0.23	0.22	0.21
	W3	0.22	0.26	0.24	0.24
S1	W1	0.18	0.22	0.20	0.20
	W2	0.27	0.34	0.31	0.31
	W3	0.30	0.37	0.34	0.34
S2	W1	0.22	0.26	0.24	0.24
	W2	0.34	0.39	0.37	0.37
	W3	0.36	0.48	0.41	0.42
LSD0.05		0.02			0.01
S0		0.18	0.22	0.21	0.20
S1		0.25	0.31	0.29	0.28
S2		0.31	0.38	0.34	0.34
LSD0.05		0.01			0.008
W1		0.18	0.22	0.20	0.20
W2		0.26	0.32	0.30	0.30
W3		0.29	0.37	0.33	0.33
LSD0.05		0.01			0.008
Average		0.25	0.30	0.28	
LSD0.05		0.01			

The interaction of the two factors of the agricultural sulfur and water stress indicated that treatment S2W3 was much superior as it recorded the highest mean of 0.42, associated with an increment of 162.5 per cent between the treatment S 0W 1, which recorded the least mean of 0.16. The interaction between agricultural sulfur and salicylic acid as a pair of factors indicated that treatment S2L1 was much better with the highest mean being 0.38 with an increasing

percentage of 111 in contrast to treatment S0L0 which had the lowest mean of 0.18. Two factor interaction between water stress and salicylic acid indicated that treatment W3L1 was better and the highest mean was 0.37 with an increase of 105.5 percent when compared to treatment W1L0 which recorded the lowest mean 0.18 percent.

The output of the interaction between three factors that had been studied showed that S2W3L1 was significantly better and registered the best mean of 0.48 with an incremental percentage of 242.85 in contrast to S0W1L0 which registered the worst of 0.14.

3.1.6. Leaf potassium concentration (%): Table 8 results show that the levels of the factors studied in leaf potassium concentration have significant differences between levels. The agricultural sulfur treatment S2 had best mean of 2.17% with an increase of 19.23% than agricultural sulfur treatment S0, which had the lowest mean of 1.82%. On the factor of water stress, the treatment W3 was the best in terms of mean of 2.34, with an 80.0 percent increase as compared to treatment W1, which had the lowest mean of 1.30. Treatment L1 was also superior as it recorded the highest mean of 2.05 with an increment of 8.46 compared to treatment L0 which recorded the lowest mean of 1.89.

Table 8 Effect of agricultural sulfur addition, water stress, and salicylic acid on potassium (%) concentration in leaves

Agricultural sulfur (kg ha ⁻¹)	Field capacity (%)	Salicylic acid (mg L ⁻¹)			Average
		L0	L1	L2	
S0	W1	1.15	1.26	1.24	1.22
	W2	2.01	2.15	2.11	2.09
	W3	2.13	2.17	2.16	2.15
S1	W1	1.21	1.32	1.28	1.27
	W2	2.08	2.28	2.16	2.17
	W3	2.20	2.36	2.26	2.27
S2	W1	1.36	1.48	1.44	1.43
	W2	2.39	2.58	2.46	2.48
	W3	2.50	2.78	2.53	2.60
LSD0.05		0.07			0.04
S0		1.76	1.86	1.84	1.82
S1		1.83	1.99	1.90	1.90
S2		2.09	2.28	2.14	2.17
LSD0.05		0.04			0.02
W1		1.24	1.35	1.32	1.30
W2		2.16	2.34	2.24	2.25
W3		2.28	2.44	2.31	2.34
LSD0.05		0.04			0.02
Average		1.89	2.04	1.96	
LSD0.05		0.02			

The outcome of the two-factor interaction between agricultural sulfur and water stress indicated that the treatment S2W3 was more superior as it had the highest mean of 2.60 with an increase of 113.1 percent compared to the lowest mean of 1.22 of the treatment S0W1. The analysis of the binary interaction between the agricultural sulfur and the salicylic acid showed that the

treatment S2L1 was much better showing the highest mean of 2.28 with an increase of 29.54 when compared with the other treatment, S0L0 which showed the lowest mean of 1.76. Regarding the binary interaction between the stress of water and salicylic acid, treatment W3L1 was the best with a mean of 2.44 and increase by 96.77 compared with the lowest mean of 1.24 in treatment W1L0.

The outcomes of the three-fold interaction between the factors under study indicated that the treatment S2W3L1 was significantly better, and the highest mean was 2.78, with the increase of 141.7 compared with the lowest mean of 1.15 in the treatment S0W1L0.

3.2. DISCUSSION

It can be observed from the results of Tables (3, 4, 5) that the addition of agricultural sulfur to the soil led to an increase in the availability of nitrogen, phosphorus, and potassium in the soil. This could be attributed to the fact that the oxidation of sulfur in the soil produces sulfuric acid, which lowers the soil's pH, thus increasing the availability of nutrients. It contributes to the release of nitrogen and the dissolution of some precipitated phosphorus compounds, freeing phosphorus from them. The addition also led to an increase in the availability of potassium by dissolving certain potassium-containing materials in the soil. Furthermore, it increased the effectiveness of the hydrogen ions released due to the oxidation process, which competed with the potassium ion adsorbed on the exchange complex surfaces, thus releasing potassium into the soil solution. These results are consistent with those reported by [1], [16], [17], [18].

Table (3, 4, 5) results also indicated that water level increased with the availability of nutrients in the soil. It is said that soil moisture is one of the determinants that increase the availability of nutrients because it enables mineral and organic compounds to dissolve in the soil solution hence making the nitrogen, phosphorus, potassium and other elements in the soil available to the plants. Wetness also favors the presence of microorganisms that help to break down organic materials and liberate elements thereby enhancing their availability in soil [19]. In recent research, it is observed that the nutrient availability and the microbial activity depend on the soil water content. A decline in the moisture of the soil below 60 percent of the field capacity causes the elements to be dissolved less, as well as reduces the capacity of the plant to absorb them. Also, microbial activity that leads to the decomposition of organic matter and the release of nutrients reduces. Conversely, a high level of moisture causes an unsuitable condition of anaerobiosis that decreases microorganism activity [20].

The physiological health of the plant determines the level of absorption of the available nutrients by the plants. The healthier the plant is the better the root system thus making it more efficient in the absorption of water and nutrients in the soil. Consequently, uncultivated soils will not exhaust their nutrients with use as cultivated soils do; hence, they will supply the plant with larger amounts of nutrients as they will be absorbed by the roots and translocation to other components of the plant [22]. The application of salicylic acid will enhance the ability of the biomass to absorb nutrients into the plant because the uncultivated soils will provide the nutrients in the large amount as they will be absorbed by the roots and transferred to other parts of the plant. This eventually leads to soil degradation in terms of nutrient levels. As the data in Tables (3, 4, 5) demonstrate, salicylic acid at the concentration of 200, 400 mg/L, when sprayed on the vegetatively growing plant, resulted in the significant decrease of the levels of the available nitrogen, phosphorus, and potassium in the soil. This is attributable to the fact that it leads to a greater uptake of nutrients in the soil because of foliar application of salicylic acid.

As the root growth would be enhanced during the initial stages under the influence of salicylic acid, the level of nutrient uptake in the plant increases, and the level of mineral salts in the soil is lessened. It will then, ultimately cause the loss of nutrient reserves in the soil, which could be the reason behind the reported loss in available levels of nitrogen, phosphorus, and potassium levels in the soil reservoir [23], [24].

Table (6, 7, 8) indicated that agricultural sulfur added led to the rise in concentration of nitrogen, phosphorus and potassium in the plant leaves. This is attributed to the fact that sulfur is vital in enhancing the action of plant enzymes that are involved in plant metabolism and thus improve capacity of roots to absorb the soil nutrients and convey them to the leaves. Moreover, sulfur is also involved in production of amino acids, proteins and enzymes that help enhance the overall nutritional efficiency of the plant. These findings are in line with the results of [25]. The outcome of the interaction between agricultural sulfur and water stress revealed that the introduction of sulfur alleviated the effect of the water stress. Sulfur compounds are either antioxidants or have a modulation effect on antioxidant defense system. One of the best antioxidants and stress protectants is glutamine (GSH) of them. Sulfur reaction with other biological molecules enables to signal stress response and confer protection against stress in the environment as shown in [25], [26].

The findings of the experiment depicted in Tables (6, 7, 8) revealed that the concentration of nitrogen, phosphorus, and potassium in the leaves of the orange seedlings were significantly higher when sprayed with salicylic acid at the levels of 200 and 400mg/L than it was in the control treatment. This is congruent with the findings [27], [28]. This improving change in nutrient levels in the leaves may be attributed to the fact that salicylic acid is a plant hormone that activates the enzymes involved in the metabolism of energy in the plant resulting in an increase in nutrient levels in the plant hence enhancing the growth of vegetative and root characteristics of the plant [30]. The results however indicated that the 200mg/L concentration was a better performance as compared to the 400mg/L concentration and this may be as a result of the fact that spraying the acid at the concentration of 400mg/L suppressed some of the physiological processes which included closing the stomata of the leaves of the orange seedlings more as compared to the 200mg/L concentration. This closing helps in a drastic increase in the concentration of carbon dioxide between the leaf cells, a decline in the intake of carbon in photosynthesis, and augmented photorespiration dealings. The decreased transpiration can cause the shortage of minerals in the leaves. These findings are consistent with the past results that show the effect of salicylic acid to be dose-dependent with over doses sometimes increasing the growth-promoting activity of salicylic acid [23], [32].

The findings also indicated that the nutrient concentration increased in the leaves of the plant as a result of an increase in the field capacity. The high increase in the nutrient level in the leaves in the treatment (W3) relative to the other treatments can be explained by the fact that the water content in the soil is adequate to sustain the hydraulic process in the plant and also serves as a carrier of nutrients on the soil to the green tissues of the plant [33].

The findings also showed that the interplay of salicylic acid and various concentrations of field capacity was also involved in enhancing nutrient levels despite the water stress conditions. Salicylic acid usage enhances the enzyme activities in SOD and APX, the total phenolic content,

and antioxidant activity and reduction of oxidative stress, enhanced photosynthetic growth, and development in plants under moderate drought stress [34]. This might be due to the fact that the absorption of nutrients is reduced and the amount of free radicals that build up in the roots becomes high when the plants are subjected to water stress, and this compromises growth. Extracellular salicylic acid triggers the response of the plant including enhancement of antioxidant enzyme activity, osmotic balance, stomata opening, and closing that minimizes water loss and overall preserves the nutrition in the tissues. Hence, the key factor that made salicylic acid treated plants to have higher performance and tolerance to water shortage than untreated ones is that salicylic acid aided important physiological functions and preserved the efficiency of growing under water stress [35].

4. CONCLUSIONS

The findings of the work suggest that the nutrient availability of soil and the increment of nutrient levels in bitter orange seedling leaves can be achieved by the application of the agricultural sulfur and salicylic acid and by the regulation of the levels of soil moisture. The results indicated that sulfur promotes the enzyme activity, which allows the nutrient uptake and translocation to the leaves, and salicylic acid promotes the efficiency of nutrient uptake and reduces the impact of water stress. The experiment also revealed that the correct one of the salicylic acid concentration (200 mg L⁻¹) was more effective, compared to the one with higher concentration (400 mg L⁻¹) hence the significance of finding the right dosing to obtain the best results. These findings prove that the relationship among sulfur, salicylic acid and moisture level can be an effective approach that can sustain growth and nutrition of bitter orange seedlings in the presence of water-stress condition.

ACKNOWLEDGEMENT

The authors would also like to acknowledge the support in facilities and technical assistance of the Department of Soil and Water Technologies and Al-Mussaib Technical College Al-Furat Al-Awsat Technical University. The laboratory personnel who helped the authors with their work on soil and plant analysis are also appreciated by the authors.

DECLARATION OF COMPETING INTEREST

None

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