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# Influence of Water Stress and Ortho Salicylic Acid on Sweet Potato Plants (*Ipomoea batatas L.*) Under Environment and Climate Changes

Ebtessam A. Youssef and Hany K. Abdelaal



Water Relations and Field Irrigation Department, Agricultural and Biological Institute, National Research Centre, 33 EL Bohouth St., Dokki, Giza, Egypt, Postal Code: 12622

#### Abstract

This investigation was conducted over two consecutive seasons (2021 and 2022) for testing ortho salicylic acid influence on sweet potato plants (Ipomoea batatas L.) under the water deficiency effect. It was aimed to reinforce sweet potato plants growth process and yield parameters by using different concentrations of ortho salicylic acid (4, 6 and 8%) as well as the control under different levels of water supply (100, 75 and 50% of ETc i.e. evapotranspiration) in sandy loam soil conditions at Belbeis region – El Sharkia Governorate, Egypt.

The data obtained during the two experimental seasons clearly disclosed that, all the applied treatments enhanced both plants growth process and yield parameters as compared to the control. In particular, spraying with ortho salicylic acid (6 and 8 %) in combination with water supply (100 or 75 of ETc) enhanced vegetative growth and increased the tuber yield and quality as compared to the control, but the most effective treatment for water productivity was gained by ortho salicylic acid 8 % combination with water supply ETc 75 %, which produced 6.77 and 8.09 kg m-3 in the first and the second seasons, respectively.

Keywords: sweet potato plants, irrigation, evapotranspiration, ortho salicylic acid.

# 1. Introduction

Sweet potato (*Ipomoea batatas* L.) is a perennial plant, dicotyledonous, belongs to the family Convolvulaceae and counted as one of the essential crops in many developing nations; specifically, in Africa and Asia. Sweet potato ranks seventh among the world's most important crops and fifth ranking in developing countries. Moreover, it ranked as the second important tuber root crop after cassava in many tropical countries [1, 2]. In Egypt, the area of sweet potatoes reached 13154 hectares with a production amount of 450,985 tons [3]. In 2022, Egypt's exports of sweet potatoes amounted to 38,653 tons, occupying the fifth place in Egypt's agricultural exports after citrus, potatoes, onions, and beans (https://www.youm7.com/story/2022/6/13/10 -

5798126). Sweet potato is significant source of carbohydrates, vitamins A and C, fiber, iron, potassium, and protein.

Drought is one of the most frightening natural phenomena in the world. It destroys farmland, livelihoods and causes untold suffering. They occur when an area suffers from a lack of water supply due to a lack of rainfall or lack of surface or groundwater. It may last for weeks, months, or years. It is abiotic stress increased mainly in arid and semi-arid regions like Egypt, causing a direct decline in crop growth may be across either decrease in cell elongation, cell turgor or cell volume due to covering of xylem and phloem vessels thus obstructing any translocations [4]. In semi-arid and arid regions sweet potato plants

\*Corresponding author e-mail: dr.ebtessamyoussef@yahoo.com.; (Youssef, Ebtessam A.).

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are grown under high day and night temperatures combined with a dry atmosphere, irrigation is essential for good crop production [5,6].

Salicylic acid is an organic compound with the formula HOC6H4CO2H. A colorless, bitter-tasting solid. The name is from the Latin salix for willow tree. Salts and esters of salicylic acid are known as salicylates. The structural composition of ortho salicylic acid molecules is as shown in Figure (1) (https://en.wikipedia.org/wiki/Salicylic\_acid)

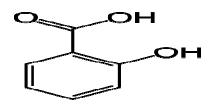


Figure (1): The structural composition of ortho salicylic acid molecules

The increasing number of studies in crop plants revealed a potential role of salicylic acid (SA) as a growth regulator and in the activation of abiotic stress tolerance apart from their role in biotic stress resistance. Evidently, the endogenous level of salicylic acid in plants increased during abiotic stress [7].

This study aimed to gained good plants growth process and yield parameters of sweet potato by using different concentrations of ortho salicylic acid (4, 6 and 8%) as well as the control under different levels of water supply (100, 75 and 50% of ETc i.e. evapotranspiration).

# 2. Material and Methods

The present investigation has been carried out throughout two successive seasons (2021 and 2022) on sweet potato plants cv. Beauregard (Ipomoea batatas L.) to enhance growth process and yield parameters by using different concentrations of ortho salicylic acid (4, 6 and 8%) as well as the control under different levels of water supply (100, 75 and 50% of ETc i.e. evapotranspiration) in sandy loam soil conditions at Belbeis region – El Sharkia Governorate, Egypt, (30°20'42.3"N 31°37'24.5"E).

This experiment included 12 treatments, which were the interactions between three rates of evapotranspiration (100, 75 and 50% of ETc i.e. evapotranspiration) and four concentrations of ortho salicylic acid (0, 4, 6 and 8%). The experimental layout was a split plot system in a complete randomized block design with five replicates. Spraying with ortho salicylic acid was randomly

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arranged in the main plots while evapotranspiration rates were randomly distributed in the sub plots.

The area of the experimental unit was  $17.5 \text{ m}^2$  containing 5 rows with a length of 5.0 m and a width of 0.7 m, as well as the distance between the stem cuttings within the row was 50 cm, so the experimental unit contained 50 plants, that is the Feddan contains 12,000 plants (Hectare is equivalent to 2.4 Feddan). Stem cuttings of 25 cm in length were planted on the 1st of June in 2021 and 2022 seasons, respectively and the normal agriculture practices for growing sweet potato plants were applied whenever required.

After twenty days from planting, sweet potato plants were subject to three levels of water supply [50, 75 and 100 % of evapotranspiration (ETc)]. These treatments reflect conditions achieved as severe water stress, moderate and optimum levels of water supply, respectively. The plants in every treatment were irrigated every 3 days, while the spraying of ortho salicylic acid was applied at 30 and 60 days from planting.

The tested irrigation levels are based on different rates of irrigation water i. e. 2684.64, 2014.74 and 1343.16 m<sup>3</sup>/fed./season, which resulted from the FAO – Penman -Moteith equation using meteorological data of the region and characteristics of the experimental trees as in Table (1).

# The tested treatments were evaluated throw the following parameters:

#### 2.1. Vegetative growth:

Five plants were selected randomly from each replicate at 90 days after planting to measure plant height (m), leaves area per plant (cm<sup>2</sup>), and foliage fresh weight (g).

# 2.2. leaf area

Leaf area (cm<sup>2</sup>) was determined using discs of the leaf blades according to [8].

#### 2.3. Yield and its components:

At harvest, (after approximately 130 days from planting) plants that were produced from the inner rows of each plot were harvested and data were recorded for the following traits: the number of tubers per plant, tuber fresh weight (g), tuber yield per plant (g) and total tubers yield (ton fed<sup>-1</sup>) was recorded as the total weight of harvested tuber per plot and converted into ton per feddan.

Table	1
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Reference crop evapotranspiration rate (ETo) calculated with CROPWAT V.8.00 computer program from meteorological data under Sharkia Governorate conditions using FAO – Penman – Moteith equation (Average of two years 2019&2020)

Month	Day	Stage	Number	ETo	Kc	ETc	ETc	ETc	W. R.	W. R.	*W. R.
			of days	100%		100%	75%	50%	for ETc	for ETc	for ETc
									100%	75%	50%
									m <sup>3</sup> /fed.	m <sup>3</sup> /fed.	m <sup>3</sup> /fed
	1-10	Initial	10	6.46	0.50	3.23	2.42	1.62	135.66	101.64	68.04
June	11-20	Initial	10	6.72	0.50	3.36	2.52	1.68	141.12	105.84	70.56
	21-30	Development	10	6.60	0.53	3.50	2.63	1.75	147.00	110.46	73.50
	1-10	Development	10	6.40	0.72	4.61	3.46	2.31	193.62	145.32	97.02
July	11-20	Development	10	6.30	0.93	5.86	4.40	2.93	246.12	184.80	123.06
	21-30	Mid-season	10	6.07	1.11	6.74	5.06	3.37	283.08	212.52	141.54
	1-10	Mid-season	10	5.84	1.13	6.60	4.95	3.30	277.20	207.90	138.60
August	11-20	Mid-season	10	5.61	1.13	6.34	4.76	3.17	266.28	199.92	133.14
	21-30	Mid-season	10	5.33	1.13	6.02	4.52	3.01	252.84	189.84	126.42
	1-10	Late season	10	5.02	1.13	5.67	4.25	2.84	238.14	178.50	119.28
September	11-20	Late season	10	4.76	1.03	4.90	3.68	2.45	205.80	154.56	102.9
	21-30	Late season	10	4.32	0.90	3.89	2.92	1.95	163.38	122.64	81.90
October	1-10	Late season	10	4.10	0.78	3.20	2.40	1.60	134.40	100.80	67.20
Total			130						2684.64	2014.74	1343.1

<sup>\*</sup>W. R. = water requirements

# 2.4. Water productivity:

Water productivity (W.P.) values were calculated according to the following equation [9].

W.P. = [Yield (Kg per feddan) / seasonal ETc  $(m^3 per feddan)$ ].

## 2.5. Carotene:

Carotene content was determined as  $\beta$  carotene, using the method described by [10]. A Milton Roy spectrophotometer-601 at 440 nm, was used.

# 2.6. Canopy total water content

Sweet potato plants aerial biomass was cut above the ground for all studying plots. Thereafter, a representative subsample was placed in an oven at 70 C for 24 hours. Samples were weighted before and after drying to determine canopy water content. The percentage canopy water content was calculated using the following equation: Canopy water content =100 [(fresh weight - dry weight)/dry weight].

#### 2.7. Leaf proline content:

The proline content of fresh leaves ( $\mu$  moles/g fresh weight) was determined following the method adopted by [11].

# 2.8. Leaf total chlorophyll:

The leaf total chlorophyll was recorded in fresh leaves per plant using a portable chlorophyll meter SPAD 502 according to [12].

# 2.9. Statistical analysis:

The experimental design was a split-plot system in a complete randomized block design with five replicates. Ortho salicylic acid was randomly arranged in the main plots while evapotranspiration rates were randomly distributed in the subplots. This experiment included 12 treatments, which were the interactions between three rates of evapotranspiration (ETc) and four ortho salicylic acid concentrations (control, 4,6 and 8%). The data obtained were statistically analyzed using the analysis of variance method as reported by [13].

The differences between means were differentiated by using Duncan's range test [14].

# 3. RESULTS AND DISCUSSION

As shown in Table (2), ortho salicylic acid concentrations, different amount of water evapotranspiration and their interaction have a great positive effect on vegetative growth parameters of sweet potato plants in both seasons. Data tabulated in Table (1) cleared that, there are statistically differences values for the three tested spraying concentrations as compared to the control. This came true in the two seasons.

For ortho salicylic acid effect, the highest significant value for plant height in the first season was 2.74 m with ortho salicylic acid (8%) compared to the control which was 2.39 m. For the

evapotranspiration effect, the highest significant values were 3.02 m for ETc 100% compared to 2.19 m for ETc 50%. Moreover, the interaction between ortho salicylic acid and ETc amount effect indicated that the highest significant values were found with ortho salicylic acid (6 or 8%) combination to ETc 100%, which were 3.17 and 3.21m, respectively.

The total leaves area and foliage fresh weight per plant parameters showed a nearly similar trend as the plant height parameter. These results are agreeable with those reported by [6, 15, 16, 17, 18, 19, 20, 21, 22, 23].

Particularly, it can be noticed that ortho salicylic acid gained the highest significant values compared with the control. In this connection, this increase may be due to the role of salicylic acid in activating cell division, biosynthesis of organic foods and availability and movement of mineral nutrients toward the leaves [18, 20].

Table 2

Effect of water stress and ortho salicylic acid of	on vegetative growth parameters of s	weet potato plants (2021-2022 seasons)

Factors	Treatments		Plant l	ength (m)		Total l	eaves	area cm²/ pla	Foliage fresh weight (kg)				
Ц		First se	ason	Second s	eason	First sea	son	Second sea	First sea	ason	Second se	eason	
	OSA 0 %	2.39	D	2.63	D	366.42	D	362.07	D	0.447	D	0.480	С
	OSA 4 %	2.54	С	2.73	С	376.29	С	373.33	С	0.481	С	0.524	В
А	OSA 6 %	2.66	В	2.85	В	393.91	в	385.68	В	0.514	В	0.571	А
	OSA 8 %	2.74	А	2.95	А	401.66	А	392.46	А	0.546	А	0.589	А
	100% ETc	3.02	А	3.14	А	421.72	А	415.64	А	0.607	А	0.659	А
В	75% ETc	2.54	В	2.81	В	386.15	в	381.11	В	0.499	В	0.542	В
	50% ETc	2.19	С	2.42	С	345.84	С	338.40	С	0.385	С	0.421	С
	OSA 0 %X100 ETc%	2.71	с	2.97	с	401.96	с	399.57	с	0.544	с	0.579	с
	OSA 0 %X75 ETc%	2.43	d	2.70	d	374.11	d	360.58	d	0.452	d	0.504	d
	OSA 0 %X50 ETc%	2.05	f	2.21	f	323.18	f	326.05	f	0.346	f	0.356	g
	OSA 4 %X100 ETc%	2.99	b	3.12	b	416.04	b	412.27	b	0.593	b	0.632	b
u	OSA 4 %X75 ETc%	2.48	d	2.74	d	379.11	d	373.12	d	0.479	d	0.533	d
ctio	OSA 4 %X50 ETc%	2.14	f	2.34	f	333.73	f	334.60	f	0.370	f	0.406	f
Interaction	OSA 6 %X100 ETc%	3.17	а	3.22	а	432.47	а	422.70	а	0.633	а	0.709	а
In	OSA 6 %X75 ETc%	2.53	d	2.81	d	386.63	d	389.32	d	0.504	d	0.548	d
	OSA 6 %X50 ETc%	2.28	e	2.53	e	362.61	e	345.02	e	0.406	e	0.456	e
	OSA 8 %X100 ETc%	3.21	а	3.24	а	436.40	а	428.04	а	0.657	a	0.718	а
	OSA 8 %X75 ETc%	2.73	с	2.99	с	404.75	с	401.40	с	0.563	с	0.584	с
	OSA 8 %X50 ETc%	2.30	e	2.61	e	363.84	e	347.94	e	0.416	e	0.464	e

ETc = evapotranspiration and OSA = ortho salicylic acid

Mean followed by the same letter\s within each column are not significantly different from each other at 0.5% level.

In this respect, the present data in Table (3) showed that, all ortho salicylic acid concentrations under different rates of water evapotranspiration and their interaction treatments had a great statically influence on yield and water use efficiency in both seasons. As shown in Table (3), there are statistically different values for the three tested ortho salicylic acid concentrations as compared to the control. This came true in the two seasons.

For the ortho salicylic acid effect in Table (3), the highest significant values for total tuber yield in the first season were 12.70 ton fed<sup>-1</sup> with ortho salicylic acid (8%) as compared to the control which was 8.48 ton/fed. For the evapotranspiration effect in Table (3), the highest significant value was15.79 ton fed<sup>-1</sup> for ETc 100% as compared to ETc 50% which was

6.18 ton fed<sup>-1</sup>. Additionally, the interaction between spraying ortho salicylic acid and ETc amount effect, it was cleared that the highest significant values were 18.16 and 17.12 ton fed<sup>-1</sup> for ortho salicylic acid 8% combined with ETc 100% and ortho salicylic acid 6% combined with ETc 100%, respectively.

It seems that, the tuber yield per plant (kg) parameters cleared a nearly similar trend as the total yield parameter.

Moreover, the highest significant values for ortho salicylic acid with water productivity was recorded by ortho salicylic acid (8%), which produced 6.32 kg m-3 as compared to the control which gained 4.22 kg m-3. The highest significant value for water supply was ETc 100%, which produced 5.88 kg/m<sup>3</sup> as compared to ETc 50%, which produced 4.60 kg/m<sup>3</sup>. For the interaction, the highest significant values

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were 6.77 & 6.76, which gained by ortho salicylic acid (8%) with ETc 75% or ETc 100%, respectively. In this scenario, these finding are in line with those of with [18, 20, 24, 25].

This improvement in the yield and its component could be the role of salicylic acid which acts as

cofactors for some specific enzymes, i.e., dismutases, catalases, peroxidases, which results in breakdown of the toxic (H2O2), (OH), (O-2) radicals, Also, SA decreasing generation of ROS), inhibits of auxin oxidation [20].

#### Table 3

Factors	Treatments	Τι		oot yield per nt (Kg)		То		per root yield on fed <sup>-1</sup> )		Water productivity (Kg m <sup>-3</sup> )				
Ц		First sea	First season		Second season		ason	Second se	ason	First se	ason	Second s	Second season	
	OSA 0 %	0.706	D	0.825	D	8.48	D	9.90	D	4.22	D	4.92	D	
	OSA 4 %	0.828	С	0.946	С	9.94	С	11.35	С	4.95	С	5.64	С	
А	OSA 6 %	0.948	В	1.114	В	11.38	В	13.36	в	5.67	В	6.72	в	
	OSA 8 %	1.058	А	1.203	Α	12.70	Α	14.44	А	6.32	А	7.27	А	
	100% ETc	1.316	А	1.500	А	15.79	Α	18.00	А	5.88	А	6.70	А	
В	75% ETc	0.891	В	1.061	в	10.69	В	12.73	В	5.31	В	6.32	В	
	50% ETc	0.515	С	0.593	С	6.18	С	7.11	С	4.60	С	5.29	С	
	OSA 0 %X100 ETc%	1.092	с	1.305	с	13.10	с	15.66	c	4.88	e	5.83	e	
	OSA 0 %X75 ETc%	0.705	d	0.858	d	8.46	f	10.29	f	4.20	f	5.11	e	
	OSA 0 %X50 ETc%	0.402	f	0.426	f	4.83	j	5.11	j	3.60	g	3.80	g	
	OSA 4 %X100 ETc%	1.263	b	1.490	b	15.16	b	17.88	b	5.65	c	6.66	c	
ų	OSA 4 %X75 ETc%	0.824	d	0.927	d	9.89	e	11.12	e	4.91	e	5.52	e	
ctio	OSA 4 %X50 ETc%	0.479	f	0.530	f	5.75	i	6.36	i	4.28	f	4.74	f	
Interaction	OSA 6 %X100 ETc%	1.427	а	1.600	а	17.12	а	19.20	а	6.38	b	7.15	b	
In	OSA 6 %X75 ETc%	0.911	d	1.131	d	10.93	d	13.58	d	5.43	с	6.74	с	
	OSA 6 %X50 ETc%	0.582	e	0.701	e	6.98	h	8.41	h	5.20	d	6.26	d	
	OSA 8 %X100 ETc%	1.514	а	1.610	а	18.16	а	19.32	a	6.76	а	7.20	b	
	OSA 8 %X75 ETc%	1.136	с	1.357	с	13.64	с	16.29	c	6.77	а	8.09	а	
	OSA 8 %X50 ETc%	0.607	e	0.731	e	7.28	g	8.77	g	5.42	c	6.53	c	

Mean followed by the same letter\s within each column are not significantly different from each other at 0.5% level.

\* Hectare is equivalent to 2.4 Feddan

Table (4), summarizes mainly the a major statically impact of ortho salicylic acid concentrations under different rates of water evapotranspiration and their interaction on water content, tuber root carotene content, leaf chlorophyll content and proline content in both seasons. Generally, there are statistically differences values for the three tested ortho salicylic acid concentrations as compared to the control. This came true in the two seasons.

For ortho salicylic acid effect, the highest significant values for total water content in the first season were 77.16% with ortho salicylic acid 8% as compared to the control which was 75.29%. For evapotranspiration effect, the highest significant values were 78.29% for ETc 100 as compared to 74.08 for ETc 50%. Regarding the interaction between spraying ortho salicylic acid and ETc rates effect, it was cleared that the highest significant values were found with ortho salicylic acid (6 or 8%)

combination to ETc 100%, which were 78.73% and 78.82%, respectively.

The leaf chlorophyll content parameters showed a nearly similar trend as the total water content parameter. On the contrary, the tuber root carotene content parameter did affect by ortho salicylic acid concentrations under different rates of water evapotranspiration or their interaction.

For ortho salicylic acid effect on proline content, the lowest significant values were 11.33 µ moles/g fresh wt with ortho salicylic acid (8%) as compared to the control which was  $13.22 \mu$  moles/g fresh wt. For evapotranspiration on proline content, the lowest significant values were 10.15 µ moles/g fresh wt for ETc 100% as compared to ETc 50%, which was 14.60 µ moles/g fresh wt. Regarding the interaction between spraying ortho salicylic acid and ETc rates effect, it was clear that the lowest significant values were found with ortho salicylic acid (6 and 8 %) combination to ETc 100%, which was 9.54 and 9.40 µ moles/g fresh wt, respectively. This came true in the two seasons.

In this respect, our results are in agreement with those obtained by other researchers [6,, 18, 19, 20, 26].

photosynthesis, antioxidant capacity and ion homeostasis processes.

Moreover, according to [20, 27], the influence of salicylic acid du to activating changes in

# Table 4

Effect of water stress and spraying with ortho salicylic acid on total water, chlorophyll and proline contents of sweet potato leaves (2020-2021seasons)

Factors	Treatments	W			e conten 100g)	ıt	lea		orophyll tent		Proline content (μ moles/g fresh wt.)						
Fac		First season		Second season		First season		Second season		First season		Second		First season		Second season	
	OSA 0 %	75.29	С	74.38	С	4.06	Α	4.02	Α	10.59	D	11.21	D	13.22	Α	13.49	А
	OSA 4 %	76.13	в	75.20	В	4.12	А	4.11	А	11.12	С	11.58	С	12.71	в	12.66	В
А	OSA 6 %	76.90	в	76.04	Α	4.22	А	4.18	А	11.65	в	12.14	в	11.65	С	12.06	С
	OSA 8 %	77.16	Α	76.57	А	4.26	А	4.22	А	11.83	А	12.40	А	11.33	D	11.79	D
	100% ETc	78.29	Α	77.97	А	3.93	Α	3.94	Α	12.71	А	13.15	Α	10.15	С	10.59	С
В	75% ETc	76.74	в	75.51	в	4.19	А	4.11	А	11.38	в	11.96	в	11.93	в	12.58	в
	50% ETc	74.08	С	73.16	С	4.38	А	4.35	А	9.81	С	10.38	С	14.60	А	14.32	А
	OSA 0 %X100 ETc%	77.42	b	76.44	с	3.82	а	3.83	а	11.79	с	12.44	с	11.26	d	12.02	d
	OSA 0 %X75 ETc%	75.78	с	74.61	e	4.09	а	4.05	а	10.74	d	11.66	d	12.56	с	13.26	с
	OSA 0 %X50 ETc%	72.67	d	72.10	g	4.28	а	4.18	а	9.26	e	9.54	e	15.84	а	15.18	а
	OSA 4 %X100 ETc%	78.21	а	77.89	b	3.88	а	3.92	а	12.41	b	12.94	b	10.41	e	10.69	f
u	OSA 4 %X75 ETc%	76.53	b	75.16	d	4.12	а	4.07	а	11.35	с	11.74	c	12.20	с	12.81	d
Interaction	OSA 4 %X50 ETc%	73.64	d	72.55	g	4.35	а	4.33	а	9.60	e	10.07	e	15.52	а	14.47	b
tera	OSA 6 %X100 ETc%	78.73	а	78.63	а	3.99	а	4.00	а	13.21	а	13.52	а	9.54	f	9.84	g
In	OSA 6 %X75 ETc%	77.04	b	75.63	d	4.24	а	4.10	а	11.60	с	11.98	c	11.76	d	12.44	d
	OSA 6 %X50 ETc%	74.92	с	73.86	f	4.42	а	4.43	а	10.15	d	10.93	d	13.65	b	13.89	с
	OSA 8 %X100 ETc%	78.82	а	78.93	a	4.01	а	4.00	а	13.44	а	13.71	а	9.40	f	9.80	g
	OSA 8 %X75 ETc%	77.58	b	76.65	с	4.30	а	4.20	а	11.82	с	12.48	с	11.19	d	11.82	e
	OSA 8 %X50 ETc%	75.08	c	74.13	e	4.46	a	4.46	a	10.24	d	11.00	d	13.40	b	13.75	с

ETc = evapotranspiration and OSA = ortho salicylic acid

Mean followed by the same letter\s within each column are not significantly different from each other at 0.5% level.

# 4. CONCLUSION

It can be concluded that sweet potato plants (*Ipomoea batatas L*.) treated by using ortho salicylic acid (6 and 8 %) in combination with water supply (100 or 75 of ETc) enhanced vegetative growth and increased the tuber yield and quality as compared to the control, but the most effective treatment for water use efficiency was gained by ortho salicylic acid 8 % combination with water supply ETc 75 %, which produced 6.77 and 8.09 kg m<sup>-3</sup> in the first and the second seasons, respectively, this means obtaining a high quality and quantity crop and at the same time saving a percentage of the quantities of water used in irrigation that may reach 25% without causing any collateral damage to the plant.

## 5. Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

# 6. Author's contribution

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Youssef, Ebtessam A. and Abdelaal, H. K.: conceptualization, formal analysis, investigation, methodology, resources, supervision, validation, writing (original draft, review, and editing).

#### 7. Conflict of interest:

The authors declare that there was no conflict of interest in carrying out this work.

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