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Effect of Irrigation Water Quality and Spraying with Tocopherol on Na,

K, K⁺/Na⁺ and Cl⁻ and Chemical Components of Okra (Abelmoschus

esculentus L. Moench)

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Abstract The experiment was carried out in a private orchard at Shat-Al-Arab District, Basrah Province during the summer seasons of 2011and 2012 in order to study the effect of irrigation water quality and spraying with Tocopherol on growth and yield of okra plants tolerance to salinity resulted from irrigation by salinity water and its effect on the leaves of chemical parameters. Treatments included three sources of water (i.e. tap water, tap water + river water 1:1 and river water) and spraying plants with Tocopherol at four concentrations 0, 50, 100 and150mg. 1-1). Treatments of this experiment were distributed in Split Plot Design with three replicates, which were the main Plots represented water sources, spraying with tocopherol represented sub-plots. Means were compared according to L.S.D. Test at significant level of 0.05. Result could be summarized as follows: Plants irrigated with tap water had a significantly higher parameters (N, K, K⁺/Na⁺ ratio), carbohydrate, leaves chlorophyll concentration, abscisic acid contents in leaves for both seasons, Foliar application with Tocopherol at 150 mg.l⁻¹ caused an increase in N,K, K⁺/Na⁺, total soluble carbohydrate and chlorophyll contents in leaves while resulted decrease in proline and abscisic acid contents in leaves. The interactions between the studied factors were significant in all studied parameters for both seasons.

Keywords: Okra, Irrigation water quality, Tocopherol, Leaves, Chemical composition.

Introduction

Okra (*Abelmoschus esculentus* Moench) is one of an important summer Malvaceae family plants in the world. Its production in Iraq in 2010 about 151219 ton as range1.935 ton.donum⁻¹.The productivity lowed in Iraq compare with another countries as Saudi Arabia, Egypt and India which their productivity were 3.37, 3.131,2.657 ton.donum⁻¹ respectively (FAO, 2010).

Okra classified as moderately tolerant to salinity (Unlukara *et al.*, 2008). Salinity effect on chemical composition and Ion concentrations of leaves. Tropics and subtropical regions of the world depend on salt water. Salinity causes decrease nutrient availability and this effect on bio- chemical growth parameters (Carter *et al.*, 2005). Mbagwu & Adesipe (1987) found that water salinity on flowering and yield caused decrease in N,P,K, Ca and Mg concentrations.

Shahid *et al.* (2011) found that salt stress effect on physiological characteristics of Okra at salinity water 25, 50 and 75 mM caused accumulation Na and Cl with reduction on K concentration in leaves and roots. Habib *et al.* (2012) found that salt stress at 150 mM NaCl caused increase glycine betaine and proline on leaves and Na, Cl on leaves and roots but reduced K, Ca and K⁺/Na⁺ in okra plants.

One of the ways to improve salt tolerance either by decreasing salinity or by using antioxidents such as Tocopherol (Vitamin E) which naturally found in plant and protect them from oxidative stress(Munne-Bosch, 2005; Hussein *et al.*, 2007).

The study aims to decrease salt stress by suitable water quality and spraying with Tocophrol under salt stress and to study the interaction between water quality and foliar application with Tocopherol.

Materials & Methods

The experiment was conducted in Alfaiha Shatt-Al-Arab, Basrah Province in silty-clay soil. Table (1) explained some of physical and chemical soil characteristics and which were analyzed in soil and water Department laboratory, College of Agriculture, University of Basrah.

Experiment has been studied the effect water quality and Tochopherol on leaves chemical composition and ion concentrations. The treatments included three water quality: tap water, tap +river (1:1) and river water with using drip irrigation system. Table (2) explained the chemical structure for every water quality. The second factor was the spraying with Tochopherol at four concentrations 0, 50,100 and 150 mg.l⁻¹. Plants had been sprayed four times as rate two weeks between one and another spray. The treatment started after three weeks from planting.

Randomized Complete Block Design (R.C.B.D) is used as Split- Plot Design.Water quality presented main plots while tochopherol sub-plot. The soil prepared and organic fertilizers were added 10 ton.donum⁻¹. The soil divided into 12 divisions length 24 m (lines) space between 40 cm. P_2O_5 45% added about 35 kg.donum⁻¹.

Field planting and agriculture processes

The field was irrigated before two days from seeds planting to increase soil moisture The seeds had been planted in the experimental units on 1/3 both seasons with an average three seeds for all pore after that seeds thinning was done into one plant after germination stage. The length of the experimental unit was two meters and the number of plants was10 into every experimental unit. Plants were irrigated with treatments after 10 days of germination.

All agriculture is processed for all treatments starting with fertilization NPK 20-20-20 100kg.donum⁻¹on tow additions first at thinning and second at flowering. A protection program to protect the field from the insects and diseases during both experiment seasons was by using super methrin 25%.

Tocopherol solution was prepared as stock solution by using 6 capsules and were solved in hexane produced in Canada Jamieson laboratories, capsule weight 270 mg after

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Table (1). Son physic-che	incal characteristics at tw	vo seasons zorranu zorz
soil characteristics	2011	2012
E.C. (ds.m ⁻¹⁾	6.87	4.06
рН	7.2	7.2
Total nitrogen(mg.kg ⁻¹)	0.6	0.4
Phosphorus (mg.kg ⁻¹).	30.61	33.48
Potassium(mg ⁻ .1 ⁻¹)	13.48	17.39
Organic matter (%)	0.28	0.3
Sand (%)	12.49	14.76
Silt (%)	36.51	34.51
Clay (%)	51.00	50.73
Texture Class	silty clay	silty clay

Table (1): Soil physic-chemical characteristics at two seasons 2011and 2012

 Table (2): Chemical analyze of water samples at two seasons 2011and 2012.

characteristic		2011		2012							
	Irrigat	tion water	quality	Irrigation water quality							
	Тар	Tap+ river	river	Тар	Tap+ river	river					
EC (ds.m ⁻¹)	1.85	6.66	11.64	3.07	6.62	10.22					
pН	7.4	6.9	6.8	7.5	7.6	7.7					
SAR	2.11	2.29	4.16	2.46	2.55	3.79					
Ion (mM)											
Ca++	6.8	10.8	22	11.00	18.0	20.6					
Mg++	10	22.2	30.2	11.00	30.0	35.0					
Na+	6.35	10.26	20	10.00	10.22	20.43					
Cl	20.28	24.16	23.53	5.00	23.00	34.00					
SO4	3.5	12.7	23.5	5.20	15.7	18.3					
HCO3 ⁻	0.5	1.0	1.5	0.4	1.5	2.0					

adding hexane to be solved and complete to 6 litres water, dilute 925,9 ml of stock solution

was to completed to 5 litres to prepare the first concentration $50 \text{ mg} \cdot l^{-1}$.

Stock solution at 1851.8 ml of was taken and completed to 5 litres to prepare the second concentration 100 mg.l⁻¹. 2777.8 ml of stock solution completed to 5 litres to prepare the third concentration 150 mg.l⁻¹. Add several drops from tween 20 0.1% as spread material.

Results & Discussion:

The results showed (Table 3) that water quality had significantly effect in N % in the first season only and K% and K⁺/Na⁺ ratio in both seasons, plants irrigated with tap +river were the best compared with the other treatments while the plants irrigated with river water were better than plants irrigated with tap water.

Tocopherol treatment caused significantly effect in both seasons in N %,K%,K⁺/Na⁺ ratio in both seasons, plants treated were better than plants untreated ,however plants were treated with Tocopherol 150 mg.l⁻¹ were the best compared with 50 and 150 mg.l⁻¹ which not differ significantly. Second season plants treated with 150 mg.l⁻¹were better than untreated plants significantly while there were no significant differences between other treatments.

The interaction between both factors caused significant effect in both seasons, in the first season, plants irrigated with river with water and sprayed 150 mg.l⁻ ¹Tocopherolgave highest N 4.47% compared the lowest N% which was 3.65 for plants irrigated with tap water and untreated with Tocopherol, however in the second season, plants irrigated with tap+river gave the highest N 2.72% compared with the lowest N% which was 2.48% for plants irrigated with river water and treated with 50 mg.l⁻ ¹Tocopherol.

The results exhibited (Table 3) that water quality had significantly effect in Na % and Cl % in both seasons, plants irrigated with river water higher than other treatments, while the plants irrigated with tap +river water were not differ significantly compared with plants irrigated with tap in first season, but in the second season were higher than plants irrigated with tap water.

Tochopherol treatment caused significantly effect in both seasons in this characteristic, Tochopherol was reduced Na% and the effect increase with increasing the Tochopherol concentration.

The interaction between both factors caused significant effect in both seasons, plants which irrigated with river water and not sprayed with Tocopherol gave the highest Na% 0.738 and 1.328%.in both seasons respectively compared to lowest Na% 0.317 and 0.616% for plants irrigated with tap water and treated with Tocopherol at 150 mg.l⁻¹ in both seasons, respectively.

The table (3) explained that plants irrigated with tap water accumulated N and K in their leaves while reduced both Na and Cl with high K⁺/Na⁺ ratio in cytoplasm however, in opposite Na and Cl accumulated in river water and river + tap water. The reduction in N % may be due to proteins reduction resulted from salt conditions which due to reduce nitrate reductase enzyme activity (Jabeen & Ahmad, 2011; Undovenko, 1971).

The increase in activity and quantity of this enzyme caused an increase in protein, amino and total acids synthesis nitrogen assimilation.(Barneix & Causin, 1996; Lopez-Cantarero et al., 1997), or the reduction in protein content under salt stress conditions may bedue to reduction in water availability or induction protease activity (Reddy &Vora, 1985). The reduction in N % maybe due to the direct competition between chloride ion and nitrate which may inhibited nitrate absorption and its movement by nitrate transporters or may the transporters inactive because of the toxic effects of salty ions (Lin et al., 1997) caused high accumulation to

Water quality	Spraying	N%		K %		Na%		K ⁺ /Na		Cl %	
	with tocopherol (mg.l ⁻¹)	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Tap water	0	3.65	2.55	5.02	6.05	0.519	0.759	10.60	8.56	3.78	4.17
	50	4.04	2.63	5.19	6.62	0.436	0.717	13.64	10.03	3.67	4.04
	100	3.98	2.64	5.22	6.81	0.387	0.669	14.87	11.77	3.41	3.98
	150	4.08	2.69	5.61	7.14	0.317	0.616	19.93	16.17	3.28	3.73
Tap+ river	0	4.29	2.52	4.36	3.61	0.482	0.859	10.67	4.81	4.12	4.46
water	50	4.31	4.31	4.45	4.12	0.439	0.810	11.90	7.3	3.73	4.15
	100	4.17	4.17	4.52	4.44	0.407	0.753	12.86	6.8	3.80	4.10
	150	4.35	4.35	4.80	4.79	0.361	0.665	14.83	7.7	3.47	3.99
River water	0	3.77	3.77	3.52	2.61	0.738	1.328	4.97	2.36	5.53	4.50
	50	4.05	4.05	3.65	3.26	0.664	1.070	5.85	3.49	5.32	4.29
	100	4.30	4.30	3.68	3.72	0.607	0.917	6.35	4.65	4.98	4.16
	150	4.47	4.47	3.89	4.42	0.559	0.746	7.27	7.69	4.79	4.00
LSD 0.05		0.23	0.21	0.14	0.46	0.46	0.064	1.53	1.18	0.87	0.33
Water	Tap water	4.10	2.63	6.449	6.66	0.415	0.690	14.76	16.17	3.53	3.98
quality mean	Tap +river water	3.99	2.61	4.054	4.24	0.422	0.771	12.56	9.83	3.78	4.18
	River water	4.50	2.54	2.947	3.50	0.642	1.015	6.11	7.69	5.15	4.24
LSD 0.05		0.11	ns*	0.073	0.23	0.23	0.017	0.81	0.81	0.7	0.12
Tocopherol mean	0	4.16	4.30	4.30	4.09	0.579	0.982	8.74	5.24	4.47	4.38
	50	4.00	4.43	4.43	4.67	0.513	0.866	10.46	6.49	4.24	4.16
	100	0.33	4.47	4.47	4.99	0.467	0.779	11.36	7.87	4.07	4.08
	150	4.30	4.77	4.77	5.45	0.412	0.676	14.01	11.23	3.84	3.91
LSD 0.05		0.08	0.12	0.08	0.27	0.27	0.037	0.94	0.96	0.45	0.13

 Table (3): Effect of water quality and tocopherol and their interactions on ions concentration of okra leaves.

*ns: No significant

chloride ion in leaves (Dean-Drummond, 1986) or because of the high amounts of Na in leaves inhibited nitrate movement from roots into shoot (Speer & Kaiser, 1991).

The potassium reduction in plants with irrigated with river water may be due to the competitive effect between Na^+ and K^+ on

absorption locations in roots and transport proteins which transport Na⁺ instead of K⁺ (Ashley *et al.*, 2006) because of the big amounts of Na⁺ in river water which caused the reduction in potassium absorption which affected the cell organelles, and the plants have transporters Na⁺ and K⁺ and H⁺ pumps make the essential ion energy inter cells (Zhu, 2003). These results accept with Mbagwu & Adesipe (1987), Unlukara *et al.* (2008), Shahid *et al.* (2011) and Habib *et al.* (2012) who studied on okra plants.

The results indicated that Tocopherol treatment had positively role in reduce the salt stress resulted from river water by increase N and K and decrease Na and Cl % in plants leaves were irrigated with river and develop the K⁺/Na ratio balance. from these results seem that Tocopherol has an important role in an increase the salt tolerance not in reduce an oxidative stress only, but by improving the ion in balance in leaves (Ellouzi et al., 2013). The ion imbalance showed to the role of antioxidants such Tocopherol in membranes selectivity and K⁺/Na⁺ under salt stress. The positive effect may be due to its role in improve the membranes selectivity in addition to increase protein content essential to protect cellular membranes and related enzymes (Farouk, 2011).

Table (4) revealed that studied factors and their interactions affected significantly on total soluble carbohydrates, chlorophyll. Plants irrigated with tap water gave the highest total soluble carbohydrates compared with other water quality which were not differ significantly, however, plants were irrigated with tap and tap water gave the highest total soluble carbohydrates compared with plants were irrigated with river water.

Plants were treated with Tocopherol caused significant effect in carbohydrates and chlorophyll for both seasons and the effect increased with an increment of Tocopherol concentration.

The interaction between both factors caused significant effect in both seasons, plants which irrigated with tap water and sprayed with 150 mg.l⁻¹ tocopherol gave

highest carbohydrates reached to 7.38 and 11.98% in both seasons respectively, compared with the lowest carbohydrates 4.38 and 3.8% for plants irrigated with river water and untreated with Tocopherol in both seasons respectively.

The interaction between both factors caused significant effect in both seasons, plants which irrigated with tap water and sprayed with 150mg.l⁻¹tocopherolgave highest chlorophyll 3.03 and 2.65mg.g⁻¹ fresh weight in both seasons respectively, compared with the lowest chlorophyll concentration which were 1.69 and 2.37 mg.g⁻¹ fresh weight for plants irrigated with river water and untreated with Tocopherol in both seasons respectively.

The results exhibited (Table 3) that water quality had significantly effect in leaves proline content and abscisic acid content (μ g.kg⁻¹ dry matter in both seasons, plants irrigated with river water higher than other treatments while the plants irrigated with tap river water differed significantly compared with plants were irrigated with tap.

Tochopherol reduced the leaves proline content and the reduction in leaves proline content increased with an incensement with Tocopherol concentration.

The interaction caused significant effect in both seasons, plants irrigated with river water and not sprayed with Tocopherol gave the highest in leaves proline content amounted to 275.58 and 279.09 μ g.g⁻¹ dry matter in both seasons, respectively compared with the lowest leaves proline content which were168 and 107.75 μ g.g⁻¹ dry matter for plants irrigated with tap water and treated with Tocopherol at 150 mg.l⁻¹ in both seasons respectively.

The interaction caused significant effect in both seasons, in abscisic acid content (μ g.kg⁻¹

dry matter). Plants which irrigated with river water and not sprayed with Tocopherol gave the highest leaves proline content which amounted to185.35 and 82.59 (μ g.g⁻¹ dry matter) at both seasons respectively, compared with the lowest leaves abssicic acid content which were 80.88 and 54.03 μ g.kg⁻¹ dry matter for plants irrigated with tap water and treated with Tocopherol at 150 mg.l⁻¹ in both seasons respectively.

Table (4) displayed there was reduction in carbohydrates % in plants that irrigated with river water. This reduction maybe due to the increase salt concentration in plant tissues caused an increase the osmotic pressure and decrease water potential which caused stoma closure and in balance in gas exchange finally effect on photosynthesis process (Ashraf & Foolad, 2005) in addition to salt effects on alleviate the enzymes activity such (Rubisco) RUBP carboxylase (Kahrizi et al., 2012), the reduction in this enzyme activity caused reduction in nitrogen assimilation (Matt et al., 2002), as well as the carbohydrates reduction with increase salt levels in water may be due to the salt stress role in leaf area and chlorophyll in plants, so Parida & Das (2005) found that salinity cause reduction in carbohydrates essential in cells growth because of the reduction in photosynthesis rate. These results were in agreement with Balotf & Kavoosi (2011) on Cucurbita pepo plants.

Results also indicated that there was an increase in carbohydrates % with Tocopherol treatment in the second season that may be due to increase chlorophyll concentration and delay leaves senescence (Farouk, 2011) which accumulate additional amounts an of carbohydrates, these results were in agreement with Sadak et al. (2011) on sunflower plants.

An indicator to plant stress tolerance as salt stress, by their role as an osmotic regulators under salt stress and ion leakage reduction and carbon source and free radicals scavengers (Siringam *et al.*, 2011) so the increase of carbohydrates one of the aims for increase salt tolerance.

Table (4) presented there was reduction in chlorophyll in plants were irrigated with river water. This reduction may be due to the salinity harmful effects on chlorophyll pigments (Cha-Um et al., 2010) and proteinpigment-lipid complex with increase in chlorophyllase enzyme activity (Cha-Um & Kirdmanee, 2009) and chloroplast distraction (Ferroni et al., 2007) which caused reduction in chlorophyll concentration however, salinity reduces the chlorophyll synthesis by reduce the glutamate synthesize (Kwinta & Cal, 2005), the started compound to chlorophyll synthesize or may be to the Na and Cl toxic effects in leaf tissues under salt stress caused chlorophyll distraction by instead of Mg in chlorophyll structure as a result of chlorophyll destroyed (Qu et al., 2012) or may be the reason was reactive oxygen species (ROS) which accumulate under salt stress and cause phospholipids and chlorophyll oxidation (Ben-Hassine & Lutts, 2010). These results in the same line agree with Gemes et al. (2008) on tomato plants.

The increase in chlorophyll with Tocopherol spraying may be due to its role as an antioxidant to protect chloroplast from oxidative damage (El-Bassiouny *et al.*, 2005) under salt stress, These results were in agreement with Farouk (2011) who showed that the antioxidants increase and H_2O2 reduction may be the reason in delay leaves senescence.

Water quality	Sprayi ng with Potassi	carbohydrate s%		Chlorophyll mg.g ⁻¹ fresh weight		Proline (µg.g ⁻¹ dry matter)		Abscisic acid (µgg.kg ⁻¹ dry matter			
	um nitrate	2011	2012	2011	2012	2011	2012	2011	2012		
Тар	0	5.34	5.48	2.41	2.36	220.75	116.33	123.88	63.19		
water	50	5.53	9.53	2.86	2.30	215.68	114.58	108.33	62.98		
	100	6.17	11.8 0	3.09	2.83	182.65	111.83	93.00	58.91		
	150	7.43	13.8 0	3.29	2.49	103.98	107.58	87.98	48.62		
Тар	0	3.38	4.31	1.91	2.83	235.65	266.67	158.00	70.76		
+river water	50	3.92	7.24	1.99	2.54	209.98	147.92	149.43	62.64		
	100	5.59	8.58	2.30	2.42	219.10	147.79	145.35	57.75		
	150	7.59	10.7 3	2.42	2.29	127.90	149.42	116.80	54.13		
River water	0	2.94	3.84	1.51	2.72	433.58	275.00	171.55	73.62		
	50	5.62	5.10	1.63	2.64	241.38	269.42	151.60	73.43		
	100	6.24	6.64	1.93	2.58	150.43	267.08	147.75	70.27		
	150	6.23	5.62	2.53	2.30	125.65	265.25	130.40	71.95		
LSD	0.05	0.83	0.65	0.06	0.24	4.66	5.00	5.28	4.96		
Water quality mean	Tap water	6.12	10.1 5	2.91	2.49	180.76	112.58	103.29	58.42		
	Tap +river water	5.12	7.71	2.15	2.52	198.16	177.95	142.39	61.32		
	River water	5.26	5.30	1.90	2.56	237.76	269.19	150.24	72.32		
LSD	0.05	0.44	0.33	0.05	NS	2.52	2.50	2.64	2.87		
Potassiu m nitrate mean	0	3.89	4.54	1.94	2.63	296.66	219.33	151.14	69.19		
	50	5.02	7.29	2.16	2.49	222.34	177.31	136.45	66.35		
	100	6.00	9.01	2.44	2.61	184.06	175.57	128.70	62.31		
	150	7.08	10.0 5	2.75	2.36	119.18	174.08	111.61	58.24		
LSD	0.05	0.51	0.38	0.03	0.14	2.88	2.889	3.63	3.01		

Table (4): Effect of water quality and Potassium nitrate and their interactions on ions percent ratio of okra leaves.

Table (4) revealed there was proline accumulation in leaves under salt stress in plants were irrigated with river water. This may be due to the reduction in proline oxidase which may be the reason in proline accumulation (Girija et al., 2002) or may be due to the reduction in amino acids conversion to proteins (Soliman et al., 1994) or increase in protein hydrolized under salt stress resulted from increase in protease enzyme activity to release amino acids as proline to store or use in osmotic regulation (Azooz, 2004). The proline accumulates in plants under salt stress by increasing synthesized and stop proline distraction (Delauney & Verma, 1993)

The osmotic regulation, cellular organelles protection ,amino acids stored as nitrogen source and free radicals scavenge are an important indicators for accumulate amino acids so proline is one of amino acids has an important role in osmotic regulation and increase salt tolerance and ROS scavengers (Girija *et al.*, 2002)

Table (4) explained there was abscisic accumulation in leaves under salt stress in plants were irrigated with river water. the increase in abscisic acid levels under salt stress may be due to its physiological role included modification in gene expression for protein synthesized, by increase the plant response to stress by increase the plant conditioning ability to abiotic stress, by its role in regulate leaves water content by controlling on guard cells movement and stoma as well as induce gens to synthesize enzymes and proteins to increase salt tolerance and drought and prevent the cells hydrolysis (Zhu, 2003) by induction the transduction single-cell and activate salt stress gens (De Bruxelles et al., 1996) and increase Ca² absorption under salt stress, which regulate cell membrane ability to ion absorption and

transport (Chen *et al.*, 2001), in addition to its role in regulate plant growth under salt stress by occurring changes in cell wall extensibility (Dodd & Davies, 1996; Bacon, 1999) or control on apoplasticpH. Wan *et al.* (2004) recorded that abssisic acid may be induce growth by increase water flow and import of photosynthate assimilators (Munns & Cramer, 1996) and regulate the stomatal conductivity (Wan *et al.*, 2004) and membrane integrity (Chen *et al.*, 2001). These results in agreement with Iqbal & Ashraf (2006) who found that high salinity cause accumulate abscisic acid in leaves (Chen *et al.*, 2001).

Conclusions

The study displayed that we can use EC water more 10 ds.m⁻¹ to irrigate okra plants with Tocopherol foliar application at 150 mg.l⁻¹ for alleviation the effect of salt stress and increase the salt tolerance to water irrigation.

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