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THE INFLUENCE OF AL-MADINAH AL-MUNAWWARA TREATED AND UNTREATED DOMESTIC WASTEWATER ON GROWTH AND PHYSIOLOGY OF THREE TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)GENOTYPES

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Abstract

The impact of irrigation with Al-Madinah Al-Munawwara domestic wastewater on three tomato genotypes (AL, P and VF) was investigated. Five treatments including Tap water, untreated (TN), primary (T1), secondary (T2) and tertiary (T3) treated wastewaters were used for irrigation. The physico-chemical characteristics of wastewater were determined. Leaves were analysed for N, P, K and heavy metals (Copper, Cadmium, Lead and Nickel). The growth parameters assessed were % germination, plant height, shoot and root dry weights, and total leaf dry weight. Some physiological parameters such as photosynthetic light response curve, maximum gross photosynthesis (A_{max}), dark respiration (*DR*), chlorophyll fluorescence parameters (*Fo*, *Fm* and *Fv / Fm*), chlorophyll content index and stomatal conductance were detected. % germination was decreased in both A1 and P genotype, with no effect on VF genotype. Most growth parameters were increased in genotype A1, followed by VF then P genotype which had a sensitive leaf dry weight to T2 and T3. Photosynthesis was mainly increased in A1 genotype with a decrease in VF genotype. *DR* was negatively affected in VF genotype with no response of A1 genotype. Chlorophyll fluorescence showed an increase in *Fo* in VF genotype but a decrease in *Fv / Fm* in both A1 and VF genotypes. Chlorophyll content index was decreased but only in A1 and VF genotypes under TN. Treatment with TN and / or T1 decreased stomatal conductance in all genotypes. The levels of heavy metals in wastewaters used were lower than the standard limits; however, plant chemical analysis showed that the leaves of the three tomato genotypes accumulated heavy metals but differently with higher levels at TN and lower levels at T3.

Key words: Domestic wastewater; Tomato; Growth; Photosynthesis; Chlorophyll fluorescence.

Introduction

Water is very important for the survival of plants and plays a very important role in the protection of our environment. The availability of clean fresh water is a major concern in many parts of our planet. The re-use of wastewater in agriculture in the last few decades has become an acceptable agricultural practice in modern agriculture (Gatta *et al*., 2015). For example, Iqbal *et al*. (2017) reported that wastewater is a very good source of some essential and beneficial elements, such as N, P, K, Mg, S, Ni and Na. Due to continuously increasing world's population, there is an increasing demand of water for domestic uses, as well as energy, agricultural and industrial productions. As a result of the rapid increase in human population, the yearly per capita water supply on the globe has been reduced between the years 1850-1993 from 33,300 to 8,500 cubic meters (Swain, 1997). More and more regions are joining the list of water shortage including the Middle East and North African regions. According to FAO AQUASTAT (Anon., 2005), the average renewable water supplies per capita per year for 2005 were about 20,000, 11,000, 4,000 and 1,500 cubic meters for North America, Europe, Sub-Saharan Africa and MENA regions (the Middle East and North Africa), respectively. However, more efficient use of water resources is required in both urban and rural environments. For example, the re-use of treated wastewater is a very important source that can increase water use efficiency.

According to many studies, wastewater can enhance the soil biological, chemical and physical characteristics (Kiran *et al*., 2012; Yang *et al*., 2015; Kaur & Najam, 2016). Due to the fact that wastewater is very rich in nutrients and can increase crop yield and soil fertility, it was suggested by Erfani *et al*. (2002) to use wastewater as an alternative to chemical fertilizers. Such water has a potential to be used to irrigate agricultural crops (Toze, 2004). It has been reported by many investigators, that wastewater can enhance the growth and productivity of various crops; wheat (Akhtar *et al*., 2012), turnip (*Brassica rapa*) (Parveen *et al*., 2013) and Chili (Iqbal *et al*., 2015). Irrigation with wastewater increased significantly many growth and physiological parameters including plant height, root length, fresh and dry weights, leaf area, rate of photosynthesis, chlorophyll content, stomatal conductance, water use efficiency and yield of chili plants (Iqbal *et al*., 2015). The re-use of treated wastewater has many advantages (Toze, 2004): 1) it is a continuous reliable water supply; 2) reduces wastewater discharge into the environment; 3) reduces the use of water from natural sources (Gregory, 2000).

The assessment of the impact of the re-use of wastewater on plant growth and physiology has been investigated in a number of studies. For example, Türkmen *et al*. (2001) reported that when wastewater was used to irrigate cucumber plants, plant height was increased progressively with increasing wastewater concentration. Similarly, when treated and untreated wastewaters were used to irrigate *Delonix elata* plants, plant height was stimulated, especially in untreated and partially-treated wastewater compared to fully-treated sewage and tap waters (Al-Zahrani & Nahari, 2006). Similarly, when root system length was measured under irrigation with wastewater, Saravanamoorthy & Kumari (2007) observed an increase in root length of peanut plants. In contrast, Huma *et al*. (2012) observed that plumule and radicle length of *Brassica juncea* L., *Brassica napus* L.and *Coriandrum sativum* L. was decreased in response to irrigation with wastewater. In chickpeas, treated and untreated wastewaters decreased root system length with the effect of untreated wastewater being more pronounced (Garg & Kaushik, 2006). In the case of plant dry weights, wastewater had a negative effect on cucumber (Türkmen *et al*., 2001), maize (Galavi *et al*., 2009) and *Delonix elata* (Al-Zahrani & Nahari, 2006) plant roots. Furthermore, dry weight of the stems (Garg & Kaushik, 2006) and leaves (Galavi *et al*., 2009) followed that of the roots in chickpeas and maize plants, respectively. The improvement of crops irrigated with wastewater can be ascribed to the regular supply of mineral nutrients to the rhizosphere (Tak *et al*., 2013). The increase in the density of soil microorganisms due to irrigation with wastewater, makes nutrients more available to plants (Mekki *et al*., 2006; Tak *et al*., 2012b). The effects of wastewater on leaf area was also investigated by many researchers, who observed that wastewater had a positive effect on leaf area (Türkmen *et al*., 2001; Saravanamoorthy & Kumari, 2007); however, others observed the opposite effects (Al-Zahrani & Nahari, 2006). The increase in leaf area due to wastewater was explained by an increase in the number of leaves per plant (Türkmen *et al*., 2001). It has been reported that 100% of wastewater of chili in plants was more effective in increasing growth, photosynthesis and yield, followed by 50% wastewater in terms of productivity, compared to that of fresh water (Iqbal *et al*., 2017). The use of treated municipal water (TMW) could be a valuable alternative to the use of fresh water (*FW*), by enhancing the nutritional condition of olive trees and enhancing oil production and quality (Bourazanis *et al*., 2016).

The contradictory effects of wastewaters on plant growth parameters observed by the researchers were due to the chemical composition of the wastewater used to irrigate the plants; the negative effects were mostly caused by high levels of heavy metals present in wastewater (Yim & Tam, 1999; Singh & Agarwal, 2007). The effects of wastewater on plant growth could be due to the effects of the chemical composition on the physiology of the plants. For example, it was observed that treated wastewater caused an increase in chlorophyll content in broad bean plants (Zeid & Abou El Ghate, 2007), peanut plants (Saravanamoorthy & Kumari , 2007) and *Delonix elata* plants in early stages of growth (Al-Zahrani & Nahari, 2006). Moradi *et al*. (2016) also reported chlorophyll content increases with increasing wastewater concentration. The rate of photosynthesis (Antolín, 2010) and the maximum quantum yield of the photosystem II (Singh & Agarwal, 2007) were also declined in plants irrigated with wastewater. It was also reported that when plants were irrigated with secondary treated or untreated wastewater, an increase in protein and carbohydrates contents (Zeid & Abou El Ghate, 2007; Galavi *et al*., 2009), and proline (Singh & Agarwal, 2007), were observed.

One of the disadvantages of the wastewater re-utilization in agriculture is the worries and questions, about the effect of the wastewater quality on crops and consumers of such crops. Such waters may be highly concentrated in nutrients, salts, heavy metals, pathogens, and pharmaceutical drugs (Toze, 2004). The accumulation of certain heavy metals in plant organs has been studied by many authors, who concluded that the level of heavy metals in plant organs was within the permitted limits; this was observed in broad bean plants (Zeid & Abou El Ghate, 2007), cucumber plants (Türkmen *et al*., 2001) and citrus trees (Pedrero & Alarcon, 2009). It was also reported that plant roots accumulate generally more heavy metals than the shoots (Singh & Agarwal, 2007). For example, in maize plants, leaves were found to accumulate more nitrogen than the other plant parts (Fonseca *et al*., 2005). Furthermore, Al-Zahrani & Nahari (2006) reported that macronutrients were accumulated more in the shoots than the roots in plants irrigated with untreated or partially treated wastewater.

The present study investigated the effects, on three tomato genotypes, using all three levels of wastewater treatments practiced in the region of Al-Madinah Al-Munawwara, Kingdom of Saudi Arabia. The assessment included the effects of treated and untreated wastewater on plant growth, physiology and the accumulation of some heavy metals in leaves.

Materials and Methods

Plant material: Seeds of three tomato (*Lycopersicon esculentum* Mill.) genotypes used in the present study, were provided by MODESTO SEED CO., INC. (MODESTO, CALIFORNIA 95357, U.S.A.). The genotypes were: *Pearson* A1(A1), *Pakmor* (P) and *Marmandi VF* (VF). The experiments were conducted in controlled environment plant growth chambers situated at the Department of Biology, Faculty of Sciences, Taibah University, Al-Madinah Al-Munawarh, Kingdom of Saudi Arabia.

Growth conditions: Seeds were washed and then soaked in distilled water for 2 hours prior to germination, placed on distilled water saturated Whatman No 2 filter paper in Petri dishes and then incubated in the dark at 25 ± 0.2°C. Seedlings of uniform size were planted in 12 cm x 20 cm plastic pots filled with compost and sand mixture (3:1). The pots were put in a controlled growth chamber set a temperature 25 ± 0.2°C. Illumination was provided by fluorescent tubes lamps giving 150 µmol quanta / m^2s at plant level amid 14 hours photoperiod. The relative humidity within the cabinet was set at around 60 ± 0.5%.

Wastewater: Wastewater was collected from Alkhalil wastewater treatment station in Almadinah Almunawwara, Kingdom of Saudi Arabia. Four different wastewaters were used, untreated TN, primary treated T1 (physical treatment), secondary treated T2 (biological treatment) and tertiary treated T3 (chemical treatment) wastewaters.

Wastewater treatments: Soon after planting, plants were watered either with one of the four wastewaters (untreated, primary treated, secondary treated or tertiary treated) or the control (tap water). Pots contained three plants each receiving 200 ml every other day; each treatment comprised of three pots in a replicate.

Growth parameters: The determination of Leaf Area was carried out using a portable LI-3000C Leaf Area Meter provided by LICOR Inc. (Lincoln, NE, USA).

Dry weights of leaves, stems and roots were determined by harvesting and oven drying fresh materials at 80 °C for 48 hours.

Photosynthesis measurements: Photosynthetic rate was measured as described by Akhkha *et al*. (2011) on the fully expanded fifth intact tomato leaves of five weeks old plants, using an LI-6400 XT Infra-Red Gas Analyser (IRGA), which was supplied by LICOR Inc. (Lincoln, NE, USA). Light intensities used were 0, 50, 150, 500, 750, 1000 and 1500 µmol quanta $m^{-2}s^{-1}$.

Chlorophyll fluorescence measurements: Chlorophyll fluorescence parameters (*Fo*, *Fm*, *Fv/Fm*) were determined during the daylight at the 5^{th} fully expanded youngest leaves of five weeks old tomato plants with a *Hansatech* Chlorophyll Fluorometer (*Hansatech* Instruments, Narborough Road, Norfolk, PE32 1JL, United Kingdom); the protocol followed that of Akhkha & Boutraa (2010), involving adaptation of leaves to darkness for 15 minutes before measurements were taken.

Chlorophyll content index determination: Chlorophyll content index was determined in the fifth leaves at daylight, using a portable Chlorophyll Content Meter (Apogee Instruments Inc., 721W 1800N, Logan, UT 84321, USA).

Sulfuric acid digestion: The method of digestion followed that of Jones and Case (1990). Each sample of 0.5g dry leaves material was digested for 30 min at room temperature by adding 3.5-ml of concentrated Sulfuric acid (H_2SO^4). Then, a 3.5-ml of H_2O_2 (30%) was added and heated up for 30 min at 250°C. This was followed after cooling down by adding again a 1-ml of H_2O_2 (30%). Therefore, the sample was filtered using Whatman No. 42 and <0.45 µm Millipore filter papers and the volume was made up to 25-ml by adding distilled water.

Heavy metal analysis: The determination of the concentrations of heavy metals such as Cu, Cd, Pb and Ni in plant samples was carried out using an Atomic Absorption Spectrometer (AAS) (Hitachi Z-8100, Japan).

NPK analysis: A 0.5 g of dried plant samples were powdered, digested then analysed for N, P and K. "Kjeldahl method" was used to determine N concentration after mineralization with Sulphuric acid (Bremner & Mulvaney, 1982), K was determined by flame emission, and P was determined using a colorimetric method (Tran & Simard, 1993).

Water analysis: The standard method for examination of water and wastewater (Anon., 1998) was performed for wastewater analysis.

Statistical analyses: The arrangement of treated pots in the plant growth chamber followed the randomized complete block design. Excel 2016 to calculate the means, standard deviations and standard errors. Analysis of variance (ANOVA) was performed using General Linear Model GLM (Minitab 17). One-Way ANOVA Multiple Comparison followed that of Tukey's at 5%. All treatments were replicated three times.

Results

Chemical analyses

Wastewater analysis: Wastewater analysis in Table 1 showed that untreated wastewater (TN) had the highest levels of all determined elements. However, as wastewater is passed through the different treatments, the level of the elements decreased gradually, with T3 containing the lowest levels of the chemical parameters determined. Wastewater had very low levels of heavy metals such as Lead, Arsenic and Cadmium. COD and BOD were very high in TN (873.8 and 481.3, respectively), and very low in T3 (30 and 7.41, respectively).

Table 1. Caption missing (Please check it)

	Drinking water	TN	T1	T2	T3	Saudi water standards for irrigation*
TDS	120	579	520	472	460	2500
pH	7.2	7.89	7.6	7.53	7.44	6-8.4
NH_3-N	4	37.92	35.5	32.67	19.38	5
NO_3-N	3	5.5	5	4.65	4.08	10
Chloride	40	209	201	176	180	100**
Fe	Nd	1.743	0.573	0.428	0.116	5
Cd	Nd	LOW	LOW	LOW	LOW	0.01
Chrome	Nd	0.0144	0.0075	0.0034	0.0018	0.1
Copper	Nd	0.032	0.0105	0.0083	0.0081	0.4
Lead	Nd	LOW	LOW	LOW	LOW	0.1
Nickel	Nd	0.0163	0.0147	0.012	0.011	0.2
Zinc	Nd	0.058	0.055	0.032	0.038	4
Arsenic	Nd	LOW	LOW	LOW	LOW	0.1
Manganese	Nd	0.043	0.0404	0.0386	0.0359	0.2
COD	-	873.8	106	26	30	50**
BOD	-	481.3	163.6	140.6	4.71	40

***** 2006-MWE maximum allowable contaminant levels in restricted irrigation waters set by the Saudi Ministry of Water and Electricity (Anon., 2006)

** COD and Chloride set by 2003-MMRA (Saudi Ministry of Municipal and Rural Affairs) (Anon., 2003)

Plant tissue analysis

N, P and K Levels: Results in Table 2A show the different levels of the major elements N, P and K in leaves of the three tomato genotypes after treatments with treated and untreated wastewater. In general, plants treated with TN had the highest levels of the major elements with a gradual decrease toward T3. The control plants had the lowest levels compared to all the plants treated with the different wastewaters. T3 plants had less NPK compared to all other treatments including the controls. Genotypes had A1 and VF had relatively higher levels of N and K than P genotype.

Heavy metals levels: Results in Table 2B showed that the three genotypes differed in the accumulation of heavy metals in their leaves. All genotypes accumulated Cd and Ni with the exception of A1 and VF genotypes which accumulated no Cd when plants were irrigated with T3.

Lead (Pb) was absent in all genotypes and all the treatments with the exception of P genotype showing some level of Pb accumulation when plants were irrigated with untreated TN and primary treated T1 wastewaters.

In the case of Copper (Cu), VF genotype accumulated some levels except when irrigated with T3. In Contrast,A1and P showed no accumulation of Cu except when plants were irrigated with the untreated wastewater TN.

The accumulated Cu and Pb levels were in general lower than the safe limits set by WHO / FAO (2007). In Contrast, Cd was higher than safe limits in all genotypes showing accumulation of such heavy metal. The safe limit of Ni is unknown.

Table 2A. Effects of wastewater treatments on the percentage of Nitrogen, Phosphorus and Potassium levels in the leaves of the three Tomato genotypes.

Genotypes	Treatments	% N	% P	% K
A1	Control	4.71	1.10	1.01
	Untreated	5.73	2.50	3.16
	Primary treated	5.53	2.10	2.78
	Secondary treated	5.32	2.00	2.72
	Tertiary treated	4.91	2.10	2.66
P	Control	2.76	1.60	0.48
	Untreated	5.94	2.80	1.97
	Primary treated	5.12	2.50	1.88
	Secondary treated	4.91	2.50	1.56
	Tertiary treated	4.40	1.90	1.39
VF	Control	5.12	1.45	2.06
	Untreated	5.73	2.15	3.74
	Primary treated	5.63	1.95	2.82
	Secondary treated	5.43	1.90	2.80
	Tertiary treated	5.43	1.90	2.19

Table 2B. Effects of wastewater treatments on the percentage of Nitrogen, Phosphorus and Potassium levels in the leaves of the three Tomato genotypes.

Genotypes	Treatments	Cu (mg/Kg)	Cd (mg/Kg)	Pb (mg/Kg)	Ni (mg/Kg)
A1	Control	Nd	0.46	Nd	0.05
	Untreated	6.31	6.84	Nd	45.94
	Primary treated	Nd	2.99	Nd	39.88
	Secondary treated	Nd	1.00	Nd	17.50
	Tertiary treated	Nd	Nd	Nd	14.86
P	Control	Nd	1.35	Nd	11.00
	Untreated	0.98	6.84	3.36	26.29
	Primary treated	Nd	4.50	0.51	24.61
	Secondary treated	Nd	3.60	Nd	23.46
	Tertiary treated	Nd	2.61	Nd	17.29
VF	Control	5.73	Nd	Nd	11.125
	Untreated	9.775	4.90	Nd	28.85
	Primary treated	4.20	2.48	Nd	26.30
	Secondary treated	0.85	2.29	Nd	26.51
	Tertiary treated	Nd	Nd	Nd	22.36
WHO/FAO (2007) Safe limits for heavy metals in plants (mg/kg)		40	0.2	5.0	10*
European Commission regulation (ECR, 2006)		-	0.2	0.3	-

* WHO (1996)

Plant growth

Seed germination: The effect of different wastewater treatments on % germination was investigated; results were summarized in Table 3. ANOVA analysis showed that in genotype A1, all wastewater treatments inhibited significantly ($p<0.05$) the % germination, compared to the control. However, such inhibition did not last and the % germination reached that of the control by day 3 onward. In the case of P genotype, showed similar pattern with the exception of TN that had no significant ($p>0.05$) effect. In contrast, VF genotype did not respond significantly ($p>0.05$) to any of the wastewater treatments.

Growth parameters: The results of plant height as affected by different irrigation wastewater treatments (Table 4), showed that VF tomato genotype was the most responding to the treatments as plant height was significantly increased ($p<0.05$) when plants were irrigated with all different wastewater treatments. P genotype also responded with a significant increase in plant height to the untreated wastewater (TN), while other treatments had no significant effect ($p>0.05$). In contrast, A1 genotype responded negatively but only to T2 and T3.

Shoot dry weight increased significantly ($p<0.05$) in TN and T1 in A1 genotype, and in T2 and T3 in VF genotype; while, P genotype responded to T1, T2 and T3 with a significant increase ($p<0.05$) in shoot dry weight in comparison to the control plants.

Total leaf dry weight followed that of shoot dry weight with an effect of all treatments except T1 in both P and VF genotypes.

The results of root dry weight (Table 4) showed that the three tomato genotypes did not behave differently in response to wastewater treatments with T1 causing a significant increase ($p<0.05$) in root dry weight in all genotypes. This parameter was also increased significantly when TN or T2 were used to irrigate plants of A1 and VF genotypes respectively.

Gas exchanges

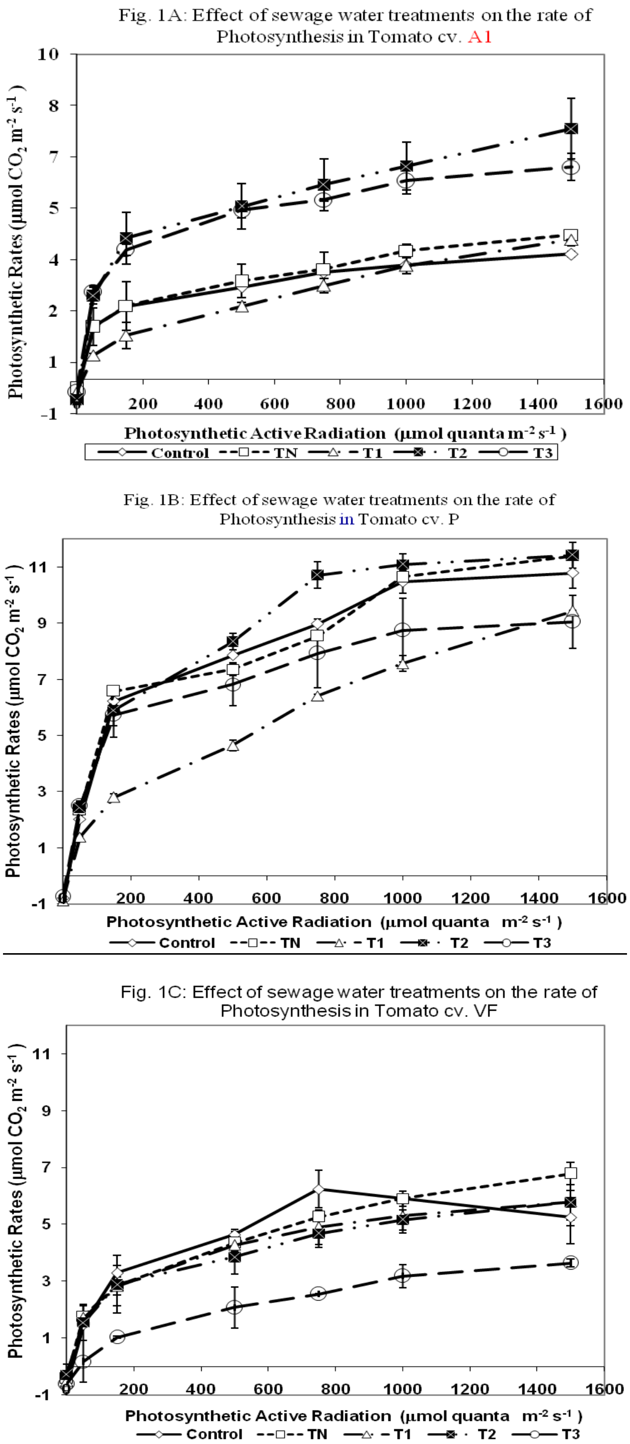
Light response curve photosynthetic rates: Gas exchange results (Figs. 1A, 1B, 1C) showed that the rate of photosynthesis presented as a light response curve was affected differently depending on the genotype and the type of wastewater treatment received. The genotype A1 responded to the wastewater treatment by a significant increase ($p<0.05$) in the rate of photosynthesis when plants are irrigated with T2 and T3. The rate of photosynthesis was increased also in P genotype when plants are irrigated with T2; however, treatments T1 and T3 reduced the rate of photosynthesis in the genotype P and genotype VF when the latter was used to irrigate the plants.

Table 3. Effects of wastewater treatments on % Germination of three Tomato genotypes (n = 4, Mean ± S.E.).

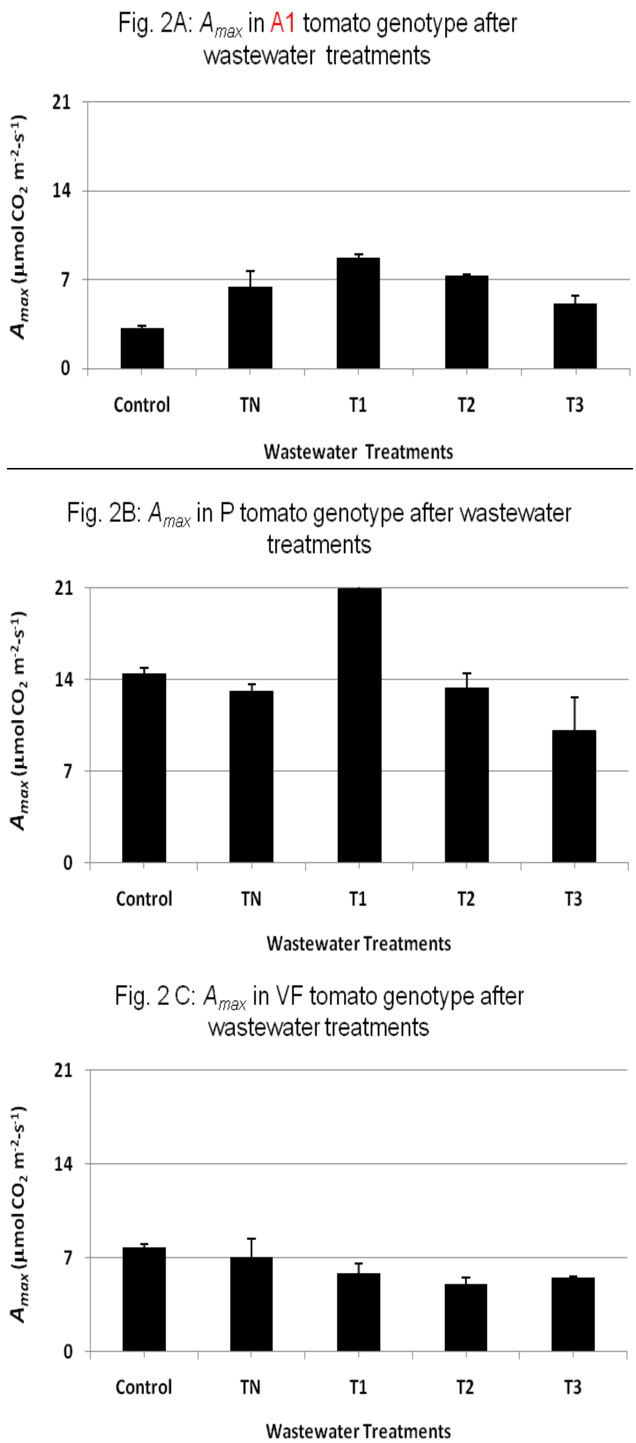
Genotypes	Treatment	Days after germination						
		1	2	3	4	5	6	7
A1	Control	0.00 ± 0.00	25.0 ± 8.70	52.5 ± 4.80	62.5 ± 4.8	67.5 ± 6.23	67.5 ± 6.30	67.5 ± 6.30
	TN	0.0 ± 0.00	2.5 ± 0.25	47.5 ± 7.50	52.5 ± 6.30	65.0 ± 8.70	67.5 ± 7.50	67.5 ± 4.80
	T1	5.0 ± 0.29	7.5 ± 2.50	52.5 ± 4.80	60.0 ± 7.70	72.5 ± 4.80	72.5 ± 4.80	72.5 ± 4.80
	T2	5.0 ± 0.50	7.5 ± 0.48	60 ± 5.77	67.5 ± 2.50	67.5 ± 2.50	75.0 ± 2.90	75.0 ± 2.90
	T3	2.5 ± 0.48	7.5 ± 0.48	57.5 ± 10.3	67.5 ± 10.3	72.5 ± 8.50	82.5 ± 8.50	82.5 ± 8.50
P	Control	2.5 ± 0.25	17.5 ± 1.30	50.0 ± 4.10	55.0 ± 6.50	67.5 ± 10.3	72.5 ± 7.50	77.5 ± 7.50
	TN	2.5 ± 0.25	15 ± 0.65	55.0 ± 6.50	67.5 ± 4.80	70.0 ± 7.10	77.5 ± 7.50	77.5 ± 7.50
	T1	5.0 ± 0.29	10.0 ± 0.41	62.5 ± 9.50	65.0 ± 6.50	75.0 ± 6.50	80 ± 4.10	82.5 ± 2.50
	T2	2.5 ± 0.25	5 ± 0.50	52.5 ± 4.80	67.5 ± 8.50	72.5 ± 7.50	75.0 ± 6.50	77.5 ± 4.80
	T3	0.0 ± 0.00	5 ± 0.29	62.5 ± 8.50	75.0 ± 2.90	82.5 ± 4.8	90.0 ± 4.10	90.0 ± 4.10
VF	Control	0.0 ± 0.00	5.0 ± 0.29	45.0 ± 2.89	67.5 ± 2.50	80.0 ± 4.10	85.0 ± 2.90	85.0 ± 2.90
	TN	0.0 ± 0.00	0.0 ± 0.00	45.0 ± 6.50	65.0 ± 6.50	70.0 ± 8.20	70.0 ± 8.20	70.0 ± 8.20
	T1	2.5 ± 0.25	7.5 ± 0.48	60.0 ± 7.50	77.5 ± 7.50	82.5 ± 4.80	85.0 ± 5.00	85.0 ± 5.00
	T2	0.0 ± 0.00	0.0 ± 0.00	37.5 ± 6.3	85.0 ± 6.50	87.5 ± 6.30	95.0 ± 2.90	97.5 ± 2.50
	T3	2.5 ± 0.25	2.5 ± 0.25	35.0 ± 5.00	82.5 ± 7.50	82.5 ± 4.80	85.0 ± 6.50	85.0 ± 6.50

Table 4. Effects of wastewater treatments on growth parameters of three Tomato genotypes. (n = 4, Mean ± S.E.).

Genotypes	Plant height (cm)					Shoot dry weight (g)				
	C	TN	T1	T2	T3	C	TN	T1	T2	T3
A1	31.0±2.0	29.0±1.5	29.0±0.7	23.0±0.5	25.0±1.3	0.5±0.08	0.8±0.22	0.9±0.17	0.6±0.06	0.4±0.01
P	22.0±0.5	26.0±0.5	24.0±0.9	24.0±1.1	22.0±0.3	1.4±0.1	1.5±0.1	1.2±0.4	1.1±0.2	0.9±0.1
VF	25.0±0.8	31.0±2.1	30.0±0.4	28.0±0.6	30.0±0.1	0.5±0.01	0.6±0.01	0.5±0.07	0.7±0.10	0.8±0.07
Genotypes	**Total leaf dry weight (g)**					**Root dry weight (g)**				
	C	**TN**	**T1**	**T2**	**T3**	**C**	**TN**	**T1**	**T2**	**T3**
A1	0.18±0.05	0.47±0.18	0.45±0.15	0.30±0.05	0.12±0.01	0.17±0.001	0.21±0.003	0.26±0.002	0.16±0.002	0.17±0.001
P	0.74±0.10	0.79±0.12	0.78±0.34	0.53±0.05	0.24±0.03	0.70±0.001	0.72±0.014	0.92±0.011	0.71±0.006	0.70±0.001
VF	0.20±0.01	0.29±0.01	0.20±0.03	0.33±0.10	0.45±0.07	0.19±0.001	0.18±0.003	0.25±0.002	0.29±0.002	0.19±0.001



Figs. 1. Effects of wastewater treatments on the light response curve Photosynthesis rate of three Tomato genotypes A1 (A), P (B) and VF (C). (n = 4, Mean ± S.E.).

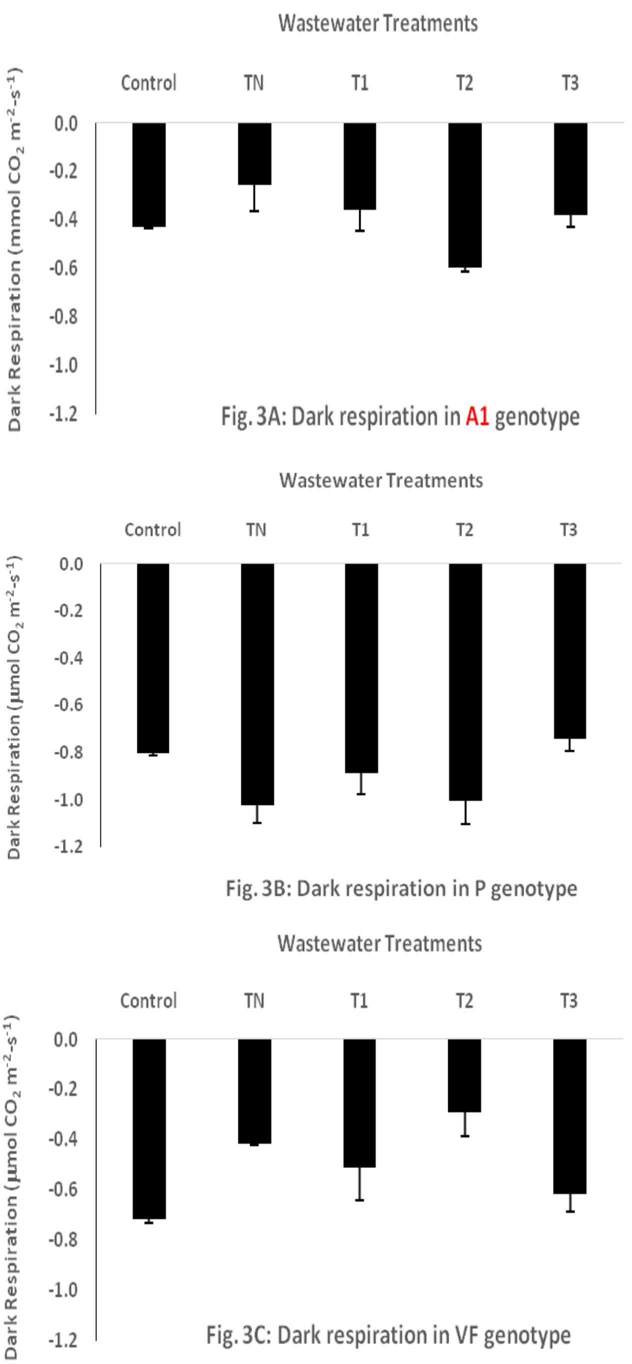


Figs. 2. Effects of wastewater treatments on the Gross Maximum Photosynthesis (A_{max}) of three Tomato genotypes A1 (A), P (B) and VF (C). (n = 4, Mean ± S.E.).

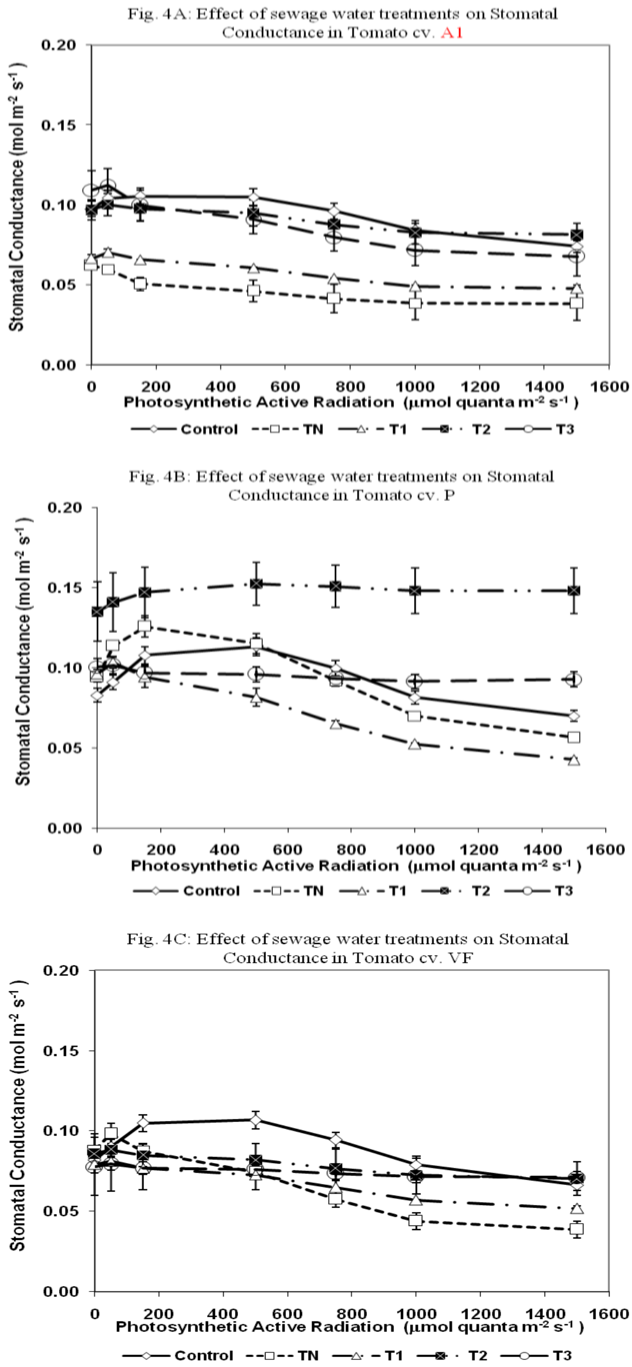
Gross maximum photosynthesis: The maximum gross photosynthesis (A_{max}) was also estimated using Light Response Curve fitted to the non-linear model of Marshal and Biscoe (Marshal & Biscoe, 1980; Akhkha *et al.*, 2001; Akhkha, 2010).

Results in Figs. 2A-C showed that A_{max} increased significantly ($p<0.05$) in plants irrigated with T1 and T2 in A1 genotype, while T1 was the only treatment affecting P genotype with an increasing A_{max}. In contrast, VF genotype showed a decrease in the A_{max} when irrigated with T2 and T3 treatments.

Dark respiration: Dark respiration (*DR*) in Figs. 3A-C, showed a significant increase ($p<0.05$) in A1 genotype plants treated with T2, while other treatments had no significant effect ($p>0.05$). In genotype P, there was an increase in dark respiration under TN, T1 and T2; however, ANOVA analysis showed no significance ($p>0.05$). In contrast, VF genotype showed a significant decrease in the rate of *DR* under treatments with TN, T1 and T3 compared to the control plants.



Figs. 3. Effects of wastewater treatments on Dark Respiration (*DR*) of three Tomato genotypes A1 (A), P (B) and VF (C). (n = 4, Mean ± S.E.).



Figs. 4. Effects of wastewater treatments on Stomatal Conductance of three Tomato genotypes A1 (A), P (B) and VF (C). (n = 4, Mean ± S.E.).

Chlorophyll fluorescence: Chlorophyll fluorescence parameters were measured to see the effect of different wastewater treatments in order to detect any change in the flow of electrons in photosystem II, which may indicate signs of stress (Percival, 2005). The results summarised in Table 5 showed that *Fo* was significantly ($p<0.05$) increased in VF genotype when plants were irrigated with TN, T2 and T3; while the other two genotypes were not affected by any of the treatments. In the case of *Fm*, no significant ($p>0.05$) changes were detected under any treatment in any of the three genotypes.

Results showed that *Fv/Fm* was significantly decreased ($p<0.05$) when plants were irrigated with the untreated wastewater (TN) in both A1 and VF genotypes; while P genotype did not show any significant changes in *Fv/Fm*. Statistical analysis also indicated that *Fv/Fm* values under TN treatments were significantly lower ($p<0.05$) than those of T1, T2 and T3 in A1 and VF genotypes.

Stomatal conductance: Stomatal conductance was shown in Figs. 4A-C to be decreased significantly ($p<0.05$) in both A1 and VF genotypes when TN and T1 were used to irrigate the plants. In P genotype, stomatal conductance was reduced significantly ($p<0.05$) under T1 treatment but increased significantly ($p<0.05$) under T2 treatment.

Table 5. Effects of wastewater treatments on chlorophyll fluorescence parameters: *Fo*, *Fm* and *Fv/Fm*, of three Tomato cultivars. (n = 4, Mean ± S.E.).

Genotypes	Wastewater treatments	Fluorescence parameters		
		Fo	***Fm***	***Fv / Fm***
A1	C	327 ± 7.0	1874 ± 37.7	0.817 ± 0.01
	TN	344 ± 23.0	1733 ± 74.2	0.757 ± 0.02
	T1	322 ± 3.5	1874 ± 37.3	0.822 ± 0.01
	T2	332 ± 15.5	1861 ± 56.4	0.821 ± 0.02
	T3	317 ± 22.4	1824 ± 18.6	0.826 ± 0.01
P	C	280 ± 2.5	1774 ± 37.6	0.842 ± 0.01
	TN	305 ± 11.3	1780 ± 9.8	0.832 ± 0.01
	T1	292 ± 13.1	1927 ± 43.9	0.835 ± 0.01
	T2	277 ± 7.6	1716 ± 48.6	0.839 ± 0.00
	T3	310 ± 2.6	1815 ± 48.9	0.829 ± 0.01
VF	C	286 ± 9.9	1850 ± 49.7	0.839 ± 0.00
	TN	369 ± 17.0	1827 ± 58.8	0.778 ± 0.02
	T1	345 ± 16.7	1770 ± 67.6	0.825 ± 0.01
	T2	388 ± 21.1	1842 ± 19.6	0.816 ± 0.01
	T3	315 ± 6.3	1866 ± 24.5	0.830 ± 0.01

Table 6. Effects wastewater treatments on Chlorophyll Content Index of three Tomato cultivars. (n = 4, Mean ± S.E.).

Genotypes	Chlorophyll content index (Arbitrary)				
	Control	**TN**	**T1**	**T2**	**T3**
A1	12.73 ± 1.03	9.05 ± 0.72	8.20 ± 0.74	11.80 ± 0.23	8.40 ± 0.41
P	21.40 ± 1.01	14.17 ± 0.85	13.00 ± 0.9	15.00 ± 1.03	12.87 ± 0.51
VF	11.17 ± 0.84	9.70 ± 0.06	10.35 ± 1.59	9.63 ± 2.48	12.37 ± 0.38

Chlorophyll content index: Results in Table 6 showed that treatments with wastewater decreased chlorophyll content index in A1 and P genotypes but not in VF; however, ANOVA showed that only T1 and T3 were significant ($p<0.05$).

Discussion

The current study investigated the effects of four wastewater treatments regimes, untreated (TN), primary (T1), secondary (T2), and tertiary (T3) treatments, on growth and physiology of three tomato (*Lycopersicon esculentum*) genotypes.

Irrigation of plants with different wastewater treatments reduced the % germination mainly in the genotype A1 followed by the P genotype; however, such effect did not last for long and VF genotype showed no response to any of the treatments. Other studies involving wastewaters reported an inhibitory effect of seed germination; for example, a number of metal-tolerant plants including mustard greens (*Brassica juncea* L.), rapeseed (*Brassica napus* L.), coriander (*Coriandrum sativum* L.), fennel flower (*Nigella sativa* L.), fenugreek (*Trigonella foenum-graecum* L.) and barley (*Hordeum vulgare* L.) had their seed germination inhibited (Huma *et al*., 2012). Untreated wastewater was also found to be highly toxic with an inhibitory effect on germination of seeds and seedling growth of oats (*Avena sativa* L.) (Fendri *et al*., 2013). In contrast, the study carried out by Ravindran *et al.* (2016) showed that treated wastewater was beneficial to the germination of four commercial crops including, tomato (*Lycopersicon esculentum*), radish (*Raphanus sativus*), carrot (*Daucus carota*) and onion (*Allium cepa*). Similarly, with rice, Gassama *et al.* (2015) reported stimulatory effect at low wastewater concentrations (<25%) and inhibitory effect at high concentrations (>50%). It was suggested that the inhibitory effect of wastewater at high concentrations on seed germination was due to reduced levels of lipase and amylase enzymes (Fendri *et al*., 2013). In contrast, such activities may be increased at low wastewater concentrations as suggested by Zeid and Abou El Ghate (2007) who reported that the increase in amylase, invertase and protease activity could be due to the mineral ions present in wastewater, acting as enzymes activators.

Irrigation with treated or untreated wastewater was also found to impair plant growth. For example, in a study carried out on tomato plants, wastewater was found to cause an increase in plant height, fresh and dry biomass (Khan *et al*., 2011). Aiello *et al*. (2007), also reported a high marketable tomato yield when three genotype tomato plants were irrigated with wastewater from *Sicily* (*Italy*) wastewater treatments plants. Castro *et al.* (2013) investigated the effect of treated wastewater on some growth parameters and observed that wastewater increased plant height, dry weight, fresh weight and diameter of lettuce (*Lactuca sativa* L.). This was in accordance with the present study which demonstrated that when growth parameters are affected in response to treated or untreated wastewater, they were increased with the exception of plant height in genotype A1 under T2 and T3, and total leaf dry weight under T3 in A1 genotype and T2 and T3 in P genotype. The decrease in some growth parameters under some treatments in some genotypes was in line with a study carried out by Alghobar and Suresha (2016) who reported that growth and yield characters of rice crop were not improved as a result of irrigation with untreated and treated wastewater; the high concentration of trace metals in wastewater decreased the number of grains/panicle, weight of 1000 seeds and yield/plant, when plants were irrigated with untreated and treated wastewater as compared to ground water control. The authors attributed the effects to higher accumulation of micronutrients and macronutrients in soil and plant tissues. Similarly, Belhaj *et al*. (2016) reported that considerable decreases in leaf area, shoot and root dry weights were detected in three vegetable crop plants (tomato, radish and lettuce) when irrigated with untreated (T1) and untreated diluted (T2) wastewater. The study attributed such negative effect to the toxic levels of heavy metals in wastewater.

The present study confirmed that the effect of wastewater on the rate of photosynthesis depended on the type of wastewater treatment and also on the genotype used. The rate of photosynthesis increased in A1 genotype but decreased in genotype VF, while P genotype showed an increase and a decrease in the rate of photosynthesis depending on the type of treatment. The increase in photosynthetic rate in A1 genotype was reflected on the growth parameters such as shoot, root and total leaf dry weights. The increase in A_{max} in P genotype was also reflected in an increase of shoot and root dry weight. In contrast, A1 genotype showed no correlation between the rate of photosynthesis and growth parameters.

The response of A1 genotype of the present study was in line with the study carried out by Tak *et al*. (2010) who reported that wastewater irrigation of chickpea plants increased the rate of photosynthesis and such increase was reflected in the growth parameters. The authors concluded that wastewaters proved to be effective as a source of water and nutrients enhancing growth, photosynthetic rate and plant yield. Singh and Agrawal (2010) also reported an increase in the rate of photosynthesis when plants were irrigated with wastewater compared to ground water irrigated ones. Such increase was attributed to the concentrations of toxic heavy metals that were not high enough to impair the photosynthetic machinery. In the present study, genotypes P and VF showed a decrease instead in photosynthesis when plants were irrigated with T1 or T3 and T2 or T3 respectively. Such decrease in the rate of photosynthesis was also reported in many studies involving irrigation with wastewater. For example, Greenhouse experiments investigating the effect of irrigation with wastewater on some crop plants (tomato, lettuce and radish) showed that the untreated wastewater had negative effects on the rate of photosynthesis and antioxidant enzymes content. However, such adverse effect was substantially reduced when a 50 % dilution was used (Belhaj *et al*., 2016). Da Silva *et al*. (2014) also observed that when Eucalyptus plants were irrigated with wastewater, the photosynthetic rates were adversely affected. The above studies attributed such negative effect of wastewater to the high levels of heavy metals, which are well known to be toxic to plants disturbing photosynthetic capacity, pigment synthesis, protein metabolism, and the integrity of the membranes (Yang *et al*., 2008).

Stomatal conductance was examined in the present study in order to determine the impact of stomatal closure on photosynthetic rate in response to treatments with wastewater. Changes in stomatal conductance did not correlate with changes in photosynthesis rate in response to different wastewater treatments; our findings showed that wastewater irrigation decreased stomatal conductance in all genotypes when plants were irrigated with TN and T1. However, T1 and T2 when used to irrigate genotype P plants caused a decrease and an increase of stomatal conductance respectively; such changes correlated well with changes in photosynthesis which suggests that the effect of wastewater on photosynthesis may occur partly due to effects on stomatal conductance. Such increase of stomatal conductance in P genotype was in line with the findings of Singh and Agrawal (2010) who observed an increase in stomatal conductance, as well as the rate of photosynthesis; such positive response was explained by the presence of low levels of heavy metals in the used wastewater. The decline in stomatal conductance observed in our studies especially inA1and VF genotypes was reported also by Tak *et al*. (2012a) using chickpea plants and explained by phosphorus limitation in the wastewater used.

Chlorophyll content measured as chlorophyll content index is another limiting factor of photosynthesis; the present study found that this parameter was reduced under T1 and T3 treatments in both A1 and P genotypes with no changes in VF genotype. This was in line with the study carried out by Khaleel *et al*. (2013) who observed that chlorophyll content was decreased when plants were irrigated with 100% raw wastewater, while enhanced at diluted wastewater due to high nutrients uptake. Chlorophyll content and the photosynthetic rate were also enhanced in plants grown under wastewater in an investigation involving bean (*Phaseolus vulgaris*), which indicated the possible involvement of Mg^{2+} in addition to other nutrients (Zeid & Abou El Ghate, 2007). Gassama *et al*. (2015) reported also that treated and untreated domestic wastewaters inhibited chlorophyll content of rice leaves at > 50% concentration, while promoting effects were observed at lower concentrations (< 25%). Similarly, in a study carried out by Manisha and Angoorbala (2013) who observed a maximum decrease in chlorophyll *a*, chlorophyll *b* and total chlorophyll contents at wastewater dilutions of 0% and 50% respectively.

The determination of chlorophyll fluorescence was to investigate the status of Photosystem II in plants irrigated with wastewater treatments, as this parameter was reported to be a good measure of *stress* in plant leaves (Murchie & Lawson, 2013) that might cause damage to the Photosystem II in response to wastewater irrigation. The present study, showed that *Fo* has been increased, but only in the genotype VF, while *Fm* was not affected in any genotype. In contrast, *Fv/Fm* was decreased in bothA1and VF genotypes in leaves of plants irrigated with the untreated wastewater (TN). Therefore, the PS II reaction center and functions in leaves of tomato plants were inhibited under untreated wastewater; this might weaken the light energy utilization and transformation capability, causing dissipation of absorbed light energy through heat energy mostly (ZhiGang *et al*., 2009). Such decrease in *Fv/Fm* did not reflect in the photosynthesis rate, as no changes were recorded under TN treatment. In contrast, Singh and Agrawal (2010) observed no significant changes in *Fv/Fm* ratio of plants treated with wastewater or ground water, suggesting an un-stressful condition of the photosynthetic apparatus.

Dark respiration (*DR*) is another important parameter that was not extensively looked at in plants irrigated with wastewater; the present work concluded that dark respiration increased in A1 genotype treated with T2; however, while expecting a decrease in the rate of photosynthesis the opposite was recorded. Such increase in the rate of *DR* due to irrigation with wastewater, was also observed by Paudel *et al*. (2016), but in Citrus root systems and not in leaves. In contrast, VF genotype showed a decline in the rate of *DR* in response to TN, T1 and T2 treatments; such decline was not reflected in the rate of photosynthesis which decreased under T2 treatment.

Many authors reported that the negative effect of wastewater on plant growth could be due to high levels of heavy metals present in wastewater (Mangabeira *et al*., 2001; Jomova & Morovi, 2009). In the present study, the levels of heavy metals in different treated and untreated wastewaters were lower than the limits set by the Saudi Ministry of Water and Electricity (Anon., 2006) and the Ministry of the Saudi Municipal and Rural Affairs (Anon., 2003) for irrigation water; hence any negative effect on growth or physiology of the plants could not be due to such levels of heavy metals. However, the plants were irrigated extensively with different wastewaters for about 6 to 7 weeks, which is enough to accumulate gradually high levels of heavy metals in the soil and consequently in plant tissues. This could explain the negative effect on some growth and physiological parameters such as % germination, plant height, total leaf dry weight, rate of photosynthesis, *Fv/Fm*, Chlorophyll Content Index and stomatal conductance. Plant chemical analysis showed that the leaves of the three tomato genotypes accumulated heavy metals but differently. A1 and P genotypes accumulated mainly Cd and Ni, while VF genotype accumulated Cu in addition to Cd and Ni. All genotypes showed higher levels of the heavy metals when irrigated with TN, then the levels decease gradually toward irrigation with T3. However, Cu and Pb levels were lower than the WHO/FAO standards. Cd levels in the contrary were higher than the safe limit, which suggests a potential health hazard if such crop when consumed. As mentioned before, such accumulation was due to the accumulation of the heavy metals in the soil. Belhaj *et al*. (2016) concluded that tomato, lettuce, and radish plant growth was negatively affected due to accumulation of heavy metals in leaves and roots exceeding the permissible levels. High accumulation of heavy metals was reported to have a negative impact on the quality of edible parts of some vegetables making them unsafe for human consumption (Perveen *et al*., 2012; Ahmad *et al*., 2014; Khan *et al*., 2016).

Such negative effect could also be due to high level of salinity (Tuna *et al*., 2007). Although TDS in our used wastewaters was lower than the 2006-MWE standards; Al-Jasser (2011) stated that such standard is too high and a TDS of 1000 mg/l or less should be used to allow for good drainage. The extensive irrigation with wastewaters for 6-7 weeks in our case would increase soil salinity and this may be at least partly responsible for the decline of some growth and physiological parameters. Hussain *et al*. (2010), reported that soil salinity increases in proportion to the salinity of irrigation water, which would increase the osmotic potential of soil solution and consequently reducing plant growth. However, it was suggested that using good drainageand blending of fresh and saline waters would help in overcoming salt toxicity of crops (Hussain *et al*., 2010). The COD reported in our study exceeded that of 2003-MMRA standards in TN and T1, while the BOD exceeded that of 2006-MWE in TN, T1 and T2.

The present study also showed an increase in the concentrations of N, P and K in tomato leaves as a response to irrigation with the different wastewaters. This is possibly due to the accumulation of high concentrations of N, P and K in the soil due to extensive irrigation. These results are in line with the studies conducted in rice (Alghobar & Suresha, 2016), foxtail millet (Aghtape *et al*., 2011) and corn forage (Tavassoli *et al*., 2010), where they showed that irrigation with wastewater significantly increased the levels of N, P and K. This increase could be related to the amount of sufficient nutrients elements present in wastewater (Alghobar & Suresha, 2016). Such increase was not reflected in all growth parameters, wastewater treatments and genotypes; this may be due to the high levels of heavy metals mentioned before that might impair growth; this was more prominent in P genotype. Alghobar and Suresha (2016) also reported that wastewaters did not improve growth of rice plants due to heavy metals, despite high levels of N, P and K in wastewaters and soil.

Conclusion

Irrigation with wastewater led to the accumulation of heavy metals in different proportions in the leaves of the three tomato genotypes due to accumulation in soil. The difference in heavy metals accumulation in the three genotypes reflects different uptake capabilities. However, only Cd levels were above the safe limits regardless of the wastewater treatment used. VF was the most genotype to accumulate Cu. The negative effect of wastewater treatments on some growth and physiological parameters was probably due partly to heavy metals and partly to TDS. Some of growth and physiological parameters showed an increase in response to different wastewater treatments, such increase could be due to high level of nutrients in such waters.

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