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Impact of Irrigation with Treated Domestic Wastewater on Squash (*Cucurbita pepo* L.) Fruit and Seed under Semi-Arid Conditions

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Abstract: The present study investigated the effect of using municipal treated wastewater in irrigation on plant growth and seed quality of squash as compared to fresh water. The physico-chemical properties of both water sources were investigated. Soil, fruits and seeds were tested for heavy metals presence and accumulation. A number of seed composition parameters were also measured. Growth parameters (fruit length, diameter and oven-dried weight) were increased in response to irrigation with treated wastewater as compared to control. All tested heavy metals concentrations were below the toxic limit of the Jordanian standards. Crude protein content was highest (41.28%) in naked seeds under treated wastewater treatment, whereas the lowest content (33.57%) was under freshwater treatment of the whole seeds.

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1. Introduction

Water availability and scarcity is a major issue worldwide [1]. It has been projected that by 2025, half of the world's population will live in water scarce areas [2]. This water scarcity would be attributed to non-uniform distribution of water between countries accompanied with variations in rainfall, population growth, water pollution, periodic drought spells and climate changes [1,3–6]. Moreover, increasing the water portion of household and industrial sectors at the expense of the agricultural sector has aggravated water shortage and scarcity problems [1,7].

In order to solve this problem, non-conventional water sources have been suggested, including but not limited to treated wastewater [8–11]. Treated wastewater (TWW) is always available in large quantities. Therefore, it can be used as a non-conventional agricultural water resource for irrigation to conserve fresh water [1,5,6]. Using industrial and municipal treated wastewaters to irrigate agricultural fields has become more common in industrialized and developing countries [12,13], particularly in the Mediterranean climate countries, to alleviate the pressure on freshwater sources during drought periods of summer, while the significant rainfall amount during winter is capable of leaching the accumulated salts in soil as a result of using TWW for irrigation [11,14].

Moreover, using wastewater for irrigation has several advantages, including the low-cost water, increasing soil organic matter content which improve soil physical and chemical properties, and reducing the chemical fertilizer used due to the significant nutrient contents in the TWW such as nitrogen (N), phosphorous (P) and potassium (K), which can improve crop production and enhancing soil fertility [3,6,11,15–17]. However, in accompaniment to these beneficial nutrients, wastewater contains other various materials that could be toxic and negatively affect soil, plants and the environment, depending on their source [1,8,18]. These various sources of wastewater (i.e., municipal, industrial, and hospital wastewater)

are all unique in their composition [19]. For example, industrial wastewater contains heavy metals that are beneficial for plant growth at low concentrations [20]. However, accumulation of their concentration in soil leads to an adverse effect on soil and plants, which can result in soil quality degradation and reduction in plant production and quality, which poses a threat to human and animal health [13,21].

Vegetables are a group of crops consumed by all categories of people and can be eaten raw or processed. Therefore, their consumption can cause serious health problems if contaminated [19]. Using wastewater may affect human health as well as the environment because of its content of salts, heavy metals, bacteria and viruses, and contaminants of emerging concern (CECs), depending on the source of wastewater, its composition, and treatment technique [6]. Therefore, many laboratory studies investigated the potential uptake, translocation and accumulation of CECs in plants even at high concentrations that do not exist actually in nature [4,22–29]. Similarly, Naser et al. [30] also reported an accumulation of some heavy metals in leafy vegetables irrigated by municipal and industrial untreated wastewater. Moreover, Brar et al. [31] concluded that potato leaves and tubers accumulated some heavy metals concentrations after irrigation with contaminated wastewater. Fazeli et al. [32] investigated the effect of untreated effluent from a paper mill on rice. They found the concentration of heavy metals in the seeds is significantly lower than that existing in the effluent. Also, Al-Ansari [33] reported an increase in the yield of eggplant after using TWW. Although heavy metal concentrations remained below the permissible limits, the chemical composition of wastewater has to be monitored regularly because of the use of different sources of TWW that comes from different plants which use different treatments (primary, secondary, and tertiary Treatment). Therefore, the objectives of this study were to determine the growth of squash plants irrigated by treated municipal wastewater and to evaluate seed quality under semi-arid climate conditions.

2. Materials and Methods

2.1. Experimental Site

The experiment was carried out in an open field of the Faculty of Agriculture (32°28′01.6″ N 35°58′31.1″ E) Jordan University of Science and Technology (JUST), Jordan during the year 2014. Eight growing beds per each season were prepared (7.7 m long, 0.7 m width and 0.45 m height) and filled with topsoil collected from same location, containing on average 48% clay, 37% silt, and 15% sand. The soil has an electrical conductivity (EC) of 2.10 dS m^{−1} and pH of 7.95.

Certified squash seeds (*Cucurbita pepo* L.) cv. Shorouq (*Syngenta*) were planted directly in the soil; two seeds were planted every 40 cm close to the emitters of the irrigation pipeline (16 plants per bed). Then, soil was covered with a black plastic sheet to reduce the evaporation in order to reduce water consumption and prevent the growth of weeds. Immediately after sowing, all seeds were irrigated with fresh water until seedlings emerged and developed the first true leaves, after which seedlings were thinned out into one plant. Two types of water quality have been used in the experiment, freshwater (FW) and treated wastewater (TWW) produced from secondary treatment (Table 1). Experimental design was Randomized Complete Block Design (RCBD) with four replicates.

2.2. Fruit Growth

Fruit diameter was measured daily using a digital caliper at the swollen zone of fruit, whereas fruit length was measured from the pedicle zone to the blossom end. Samples were selected randomly from replicates and marked for daily diameter measurement until they reached maturity, by changing color with hard leathery rind and withering of the mother plant. At the end of season, all matured fruit were collected and sliced to measure the fresh and dry weight.

Table 1. Mean values of selected properties of the used TWW and FW.

Parameter	Unit	TWW	FW
pH	-	7.8	7.4
EC	dS m ⁻¹	1.5	0.6
Na	mg L ⁻¹	161.3	22.9
NO ₃ ⁻		38.3	20.1
PO ₄ ²⁻		4.0	udl
K		37.7	3.5
Ca		70.1	25.1
Mg		41.3	30.8
Cl		255.8	51.3
DOM		70	nd
TSS		30	nd
TDS		1050	500
BOD		15	nd
COD		57	nd
Cd		<0.0003	<0.0003
Zn		0.017	0.020
Cu		0.018	0.010
Pb		0.004	<0.0003
Cr		udl	Udl

nd: not determined; udl: under detection limit; EC: electrical conductivity; DOM: dissolved organic matter; TSS: total suspended solid; TDS: total dissolved solid; BOD: biological oxygen demand; COD: chemical oxygen demand.

2.3. Heavy Metal Accumulation in Soil, Fruit and Seed

A subset of fruit, seed and soil was selected randomly from both water sources, control (FW) and wastewater treatments, for analysis of major heavy metals (Zn, Mn, Fe, Cu, Cr, As, Ni, Cd) using an atomic absorption instrument following Abbruzzini et al. [34]. For soil sample preparation, 2 gm was taken from each sample, then dissolved in 5 mL HNO₃, 0.5 mL HF, and 0.5 mL HCl in a Teflon vessel. Then, 0.5 gm was taken from each fruit and seed samples, which had been oven-dried beforehand. Samples were dissolved in 6 mL 69% HNO₃ and 3 mL HCl in a Teflon vessel in a Microwave Digestion System. The digested samples were then transferred into a Teflon beaker and the volume was completed to 50 mL with de-ionized water. The digested solution was filtered by using 0.45 µm syringe filter and stored in 50 mL polypropylene tubes to be analyzed.

2.4. Chemical Analysis of the Seeds

The seed proximate composition was analyzed following the standard official methods of the Association of Official Analytical Chemists [35].

Crude protein was calculated from the nitrogen content by Kjeldahl method using factor 6.25 (method 979.09). Ash content was evaluated by incinerating a few grams from each sample at 550 °C in a muffle furnace for 6 h (method 923.03). Moisture was determined gravimetrically to a constant weight in an oven at 100 °C (method 925.10). Crude fat of the sample was determined according to the Soxhlet extraction method by mixing of the fine powder of the sample with ether extract solution (method 960.39). The fat content was determined gravimetrically after the extract was dried to a constant weight.

2.5. Mineral Composition of the Seeds

To determine the mineral content of squash seeds, 3 g from each sample was incinerated in a furnace at 500 °C and the residues dissolved in 50 mL of 2.5% HNO₃ solution. The concentrations of Na, Ca, K, Li and Ba was determined using a flame photometer following [36]. Phosphorus was determined using the ammonium molybdate/ammonium vanadate method of Chapman and Pratt [37].

2.6. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) by One-way ANOVA using SPSS (Statistical Package for the Social Sciences). Mean comparison was performed using the Fishers Least Significant Difference (LSD) at $p \leq 0.05$.

3. Results and Discussion

3.1. Fruit Growth

Table 2 shows the effect of irrigation water treatment on fruit length, diameter and oven-dried weight. The results show that TWW as irrigation water significantly increased the length and diameter of the squash fruit. The average length was 25 and 20.7 cm for the TWW and FW treatments, respectively. Similarly, average diameter was 10.2 and 8.8 cm for the TWW and FW treatments, respectively. These measurements were reflected on the oven-dried weight for each treatment; the average oven-dried weight was 41.9 and 27.5 g cm for the TWW and FW treatments, respectively. This increase could be explained by the high nutrient content of TWW which plays a crucial role in plant growth improvement [38–40]. These results are in agreement with Al-Lahham et al. [41], who reported an increase in the diameter of tomato after using TWW in irrigation compared to FW. Similarly, Kumar et al. [40] reported a significant increase in the growth parameters of cauliflower irrigated by TWW compared to well water. They attributed this difference to the nutrients content in the TWW which make the farmers attracted to the use of TWW.

Table 2. Mean values of fruit length, diameter and oven-dried weight of the used TWW and FW.

	FW (SE ²)	TWW (SE)	<i>p</i> -Value
Fruit mean ¹ length (cm)	20.7 (1.602)	25.0 (1.954)	0.151
Fruit mean ¹ diameter (cm)	8.8 (0.708)	10.2 (0.835)	0.253
Oven-dried mean ¹ weight (g)	27.5 (0.143)	41.9 (0.128)	<0.001 *

¹ mean is the average of 5 fruit ² standard error. * statistically different.

3.2. Heavy Metal Accumulation in Soil, Fruit and Seed

The effect of using TWW on heavy metals accumulation in soil, fruits and seeds is tabulated in Table 3. The results show that there were no significant differences in the accumulation of heavy metals in soil, fruits and seeds between the TWW and FW. Moreover, all the concentrations reported in Table 3 are below the toxic limit of the Jordanian standards (Jordanian standard for reclaimed wastewater, Ministry of Water and Irrigation, 893/2006). These results could be explained by the fact that the TWW used in the experiment is municipal, not industrial, wastewater. Therefore, it contains very low concentrations of heavy metals (Table 1). On the other hand, Naz et al. [42], reported that industrial wastewater contains heavy metals in concentrations considered toxic for plants. These results are in line with the results obtained by Zavadil [43], who reported no significant effect of using TWW on accumulation of heavy metals in lettuce, radish, carrot and potatoes. Similarly, Hussain et al. [44] showed that using TWW to irrigate radish, spinach and carrot plants showed lower levels of heavy metals (i.e., Cd, Co, Cu, Cr, Mn, Ni, Pb and Zn), below toxic limits. Moreover, Kiziloglu et al. [45] reported that TWW can be used for irrigating crops for long terms and no accumulation of heavy metals occurred in cauliflower and red cabbage. A similar conclusion was drawn by Kim et al. [46], that using treated domestic wastewater in agriculture is safe for human consumption.

Table 3. Effect of using TWW and FW on heavy metal accumulation in soil, fruits and seeds.

Heavy Metal	Unit	Soil		Fruits		Seeds	
		FW	TWW	FW	TWW	FW	TWW
Ni	ppm	0.028	0.032	0.003	0.003	0.003	0.002
Zn		0.081	0.079	0.044	0.052	0.114	0.116
Cu		0.022	0.021	0.026	0.039	0.038	0.042
Mn		0.303	0.329	0.000	0.000	0.026	0.040
Fe		42.339	42.632	0.150	0.183	0.153	0.437
Cd	ppb	7.032	7.893	0.770	1.232	0.158	0.295
Cr		205.205	204.376	0.913	1.304	0.254	1.859
As		653.236	625.004	2.056	3.782	1.509	2.292

3.3. Seed Proximate Analysis

The total seed number of plants irrigated using FW was 484 seeds, compared to 774 seeds of same number of plants irrigated by TWW. This means that the usage of TWW resulted in a significant increase in the number of seed production. This result is comparable with Day et al. [47], who reported a significant increase in the seed yield as a result of using wastewater in irrigating plants.

Table 4 shows the effect of using TWW and FW on seed dry matter, crude fat and crude protein contents. The seeds of plants irrigated by TWW had higher dry matter percentage (90.23%) than that irrigated by FW (87.96%). Similarly, dry matter of the naked seeds was higher in the TWW treatment (68.18%) compared to the FW plants (66.43%). Several studies reported an increase in the dry matter as a result of using TWW in irrigation plants because of its content of the nutritional elements required by plants to grow [48–50].

Table 4. Effect of using TWW and FW on seed proximate analysis.

		F.W		T.W.W		p-Value
		Mean	SE ⁴	Mean	SE	
Whole	Actual DM ¹	87.96	3.719	90.23	4.807	0.730
	CP% ²	33.57	1.574	35.85	0.525	0.254
	CF% ³	28.45	1.628	29.84	1.165	0.549
Naked	Actual DM	66.43	1.569	68.18	6.325	0.743
	CP%	39.44	1.349	41.28	0.837	0.309
	CF%	36.89	0.444	34.57	1.775	0.246

¹ dry matter; ² crude protein; ³ crude fat and ⁴ Standard Error.

According to Table 4, the highest crude protein (CP) content in the squash seeds was found in naked seeds of the plants that were irrigated by TWW (41.28%), whereas the lowest content was found under FW treatment of the whole seeds (33.57%). This increase in crude protein percentage could be linked to the availability of various amounts of elements in the TWW such as nitrogen, potassium and phosphorous.

These results are in agreement with Aghtape et al. [51] who studied the effects of irrigation using wastewater on some forage characteristics of foxtail millet (*Setaria italic*). They found that crude protein percentage was the highest under TWW treatment for all growing stages. Similarly, Ghanbari et al. [52] reported a higher percentage of crude protein using TWW compared to FW in wheat.

It is a well-known fact that seeds contain storage food in their cotyledons such as lipids, proteins and mainly carbohydrates. Crude fat content of squash seeds is tabulated in Table 4. The highest crude fat content was found under FW treatment of the naked seeds (36.89%) whereas the lowest content was found under FW treatment of the whole seeds (28.45%). However, these differences are not statistically significant. These results are in

agreement with the results of Kiziloglu et al. [53], who found that TWW had no significant difference on fat content.

3.4. Mineral Composition of the Seeds

Table 5 shows the effect of using TWW and FW on seed mineral composition (Na, K, Ca and P). The highest concentration of sodium (Na) was found in the plants irrigated by TWW in the whole and naked seeds, at 730.49 and 628.57 mg/L, respectively (Table 5). Similarly, the highest concentration of potassium (K) was found in the plants irrigated by TWW in the whole and naked seeds, at 5957.4 and 4350.3 mg/L, respectively. Also, the highest concentration of calcium (Ca) was found in the plants irrigated by TWW in the naked seeds (149.73 mg/L) and lowest concentration in the plants irrigated by TWW in the whole seeds (114.21 mg/L). Moreover, phosphorous (P) concentration in the plants irrigated by TWW was higher compared to plants irrigated by FW, at 3.64 and 1.29 mg/L, respectively.

Table 5. Effect of using TWW and FW on mineral composition of the squash seeds.

		Na	K	Ca	p
		mg/L			
Whole	TWW	730.49	5957.4	114.21	3.64
	FW	537.5	3545.5	136.36	2.03
Naked	TWW	628.57	4350.3	149.73	3.58
	FW	571.79	3876.7	114.66	1.29

Khan et al. [54], who studied the nutrient content of sunflowers (*Helianthus Annuus* L.) irrigated by TWW, found that using TWW in irrigation caused a significant increase in the concentration of Na and Ca. Similarly, Omeir et al. [55] found a higher phosphorus content in the seeds of plants irrigated by TWW compared to FW.

3.5. Effect of Treated Wastewater on Fruit and Seed of Mycotoxin Availability

The effect of irrigating squash plants by municipal TWW on mycotoxin content in seed and fruit is tabulated in Table 6. There was no significant difference in the aflatoxins content (B1, B2, G1, G2 and M1) for both water sources (FW and TWW) where both values were much below the acceptable level (4–15 µg/kg) set by the European Union [56].

Table 6. Effect of water source on mycotoxin in seed.

Aflatoxin	FW		TWW		p-Value
	Mean	SE	Mean	SE	
B1	0.357	0.0049	0.361	0.0102	0.723
B2	BDL	BDL	BDL	-	-
G1	0.365	0.0041	0.359	0.0032	0.260
G2	BDL	BDL	BDL	-	-
M1	0.097	0.0635	BDL	-	0.170

SE: standard error; BDL: Below detection limit.

These results could be explained by the fact that the origin of the used wastewater was a municipal source, and it was treated by a secondary treatment plant.

4. Conclusions

The TWW, depending on its composition to original source (municipal or industrial), can supply plants with the required nutrients for growth. High concentrations of these nutrients probably stimulate plant growth in the TWW treatment compared to the FW treatment. According to the results, plant growth was satisfactory in irrigation with TWW

treatment. Therefore, municipal TWW could be noticeable as a source of irrigation in agriculture, especially under arid and semi-arid climates where water is scarce.

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