Evaluating Jojoba Seedling Growth and Physiological Response to Treated Wastewater Regime

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ABSTRACT

Treated wastewater (TWW) effluent in arid countries is a sustainable source of water and nutrients but has high salinity which can be solved by mixing with freshwater or by selecting appropriate salt tolerant plant. Jojoba is a xerophyte shrub that is economically promising, ecologically posing low risk of link to human food chain, and physiologically surviving high water and salinity stress. The aim of this study was to investigate the impact of TWW on growth and physiology of Jojoba seedlings under greenhouse conditions. Jojoba seedlings growth and physiological parameters were negatively affected by TWW, where seedlings were shorter, leaves smaller, and had lower dry matter by a range between 15-32% compared to FW. Negative physiological response to TWW ranged from 24% for stomata conductance up to 130% for water potential. Mixing FW with TWW gave significant physiological improvement but little growth improvement. It was recommended to use TWW for Jojoba growth only if the economic losses due to salinity are acounted for. Mixing FW with TWW has insignificant practical improvement.

Keywords: Jojoba, Salinity, Chlorine, Wastewater, Seedlings.

INTRODUCTION

It is becoming eminent that arid and semi-arid countries, such as Jordan, will soon become mainly reliant on treated wastewater for agriculture (Lubello et al., 2004; Mohammad and Ayadi, 2004). Although this water source proved to be a good source of essential nutrients for plant growth **(**Stewart and Flinn, 1984; Mohammad and Ayadi, 2004; Alrababah et al., 2008), recent studies have demonstrated that secondary treated wastewater tends to have higher salinity levels than freshwater due to higher Na, Cl, and bicarbonates resembling moderate salinity levels (Feigin et al., 1991; Nirit et al., 2006; Kiziloglu et al., 2008). In Jordan, wastewaters tend to have high salinity levels since water treatment technologies especially waste stabilization ponds (WSP) is subjected to high evaporation rates (Ammary, 2007; Al-Absi et al., 2009; Al-Khashman 2009).

Yield of most vegetable crops grown in the Jordan Valley that relies solely on treated effluent is reduced by up to 50% due to higher salinity levels mainly due to the higher chloride concentration which is considered detrimental to crop plants (Ammary, 2007). Therefore, it is recommended that effluent is mixed with freshwater to reduce their salinity level or find suitable plants that can tolerate the high salinity levels. Since freshwater in arid

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areas is becoming scarcer not even enough to be used to ameliorate the moderately saline effluent, finding suitable plant for such water is needed.

Jojoba (*Simmondsia chinensis* (Link) Schneider (*Simmondiaceae*)) is an evergreen perennial woody shrub that grows naturally in Mexican Sonora desert and in South-West of United States (Hogan, 1978; Benzioni et al., 2006). Jojoba is classified as a true xerophyte with roots penetrating down to 9 m which enables the plant to survive high temperatures and dry conditions (Gentry, 1958; Yermanos, 1974). Renewed interest in commercial cultivation of jojoba stems from various reasons among which is being drought tolerant and of economic and environmental interest. Due to the promising economic potential of the shrub, as 50% of its fruit is composed of high quality liquid wax useful for cosmetics and lubricant industries, and biodiesel (Yermanos and Duncan, 1978; Botti et al., 1998; Cother et al., 2004; Al-Widyan and Al-Muhtaseb 2010), Jojoba is now cultivated more in some arid regions in countries such as Australia, Argentina, India, Mediterranean and African lands (Borlaug et al., 1985; Brown et al., 1996; Lucero et al., 2006; Tsror et al., 2007).

The potential for extending Jojoba cultivation in dry areas, such as Jordan, depends on the response of Jojoba to the use of untraditional water sources such as the treated municipal wastewater with its moderate salinity levels. The available literature on the response of Jojoba to salinity comes from traditional salinity experiments but not from actual salinity from treated wastewater sources. Jojoba is reported as relatively salt tolerant (Benzioni et al., 1992; Roussos et al., 2007) and differences in response among clones have been observed (Rasoolzadegan et al., 1980; Benzioni et al., 1992). The increased level of salinity was reported to have reduced Jojoba shoot and leaf expansion, and the number of leaves and flowers (Rasoolzadegan et al., 1980; Benzioni et al., 1992; Botti et al., 1998; but see Roussos et al., 2007).

The effect of wastewater reuse on Jojoba seedling growth and performance are of prime interest. The aim of the present study was to investigate the impact of treated wastewater on growth and physiology of Jojoba seedlings grown under greenhouse conditions. Our hypothesis was that treated wastewater irrigation can be used for seedling production of Jojoba.

MATERIALS and METHODS

Plant Material

Seeds of jojoba were randomly collected from an open grown plantation located within the campus of the Jordan University of Science & Technology (JUST), Irbid, (32°22` N, 35°49` E with 500 m above sea level). The location is characterized by a semiarid Mediterranean climate of mild-rainy (220 mm annually) winters and hot-dry summers (Department of Meteorology, 2007).

Experimental Design and Irrigation Treatments

The experiment was conducted in a green house at JUST using a Completely Randomized Design (CRD) with three irrigation treatments: (1) freshwater (FW), (2) treated wastewater (TWW), and (3) mixed water (MW) prepared by mixing 1:1 FW:TWW. Each treatment consists of 30 pots was placed in a completely random arrangement in the greenhouse. Fresh water was obtained from well water from JUST while the TWW effluent was obtained from JUST secondary treatment plant (Table 1). Two seeds were planted in each 10-cm-diameter pot in July 2007 in premixed soil (1:1 Peatmos:Perlite) irrigated with fresh water under germination and then leaving only one healthy seedling per pot after emergence. Pots after emergence were irrigated as dictated by weather conditions with an average of three irrigations per week during summer months and one irrigation event per week or every ten days during winter. Irrigation amount was recorded. The experiment was conducted for one year.

Table 1: Wastewater quality of Jordan University of Science and Technology (JUST) treatment plant compared to the maximum allowable national standard value

Test	Unit	JUST Wastewater Quality	Maximum Allowable National Standard Value
pH	SU	7.5	6.0-9.0
TDS	mg/L	750	1500
TSS	mg/L	16.2	159
BOD5	mg/L	6	200
COD	mg/L	34	500
D.O	mg/L	3	>2
NO_3^-	mg/L	32	45
Po_4^{-3}	mg/L	10	30
CL^-	mg/L	194	400
SO_4^{-2}	mg/L	-	500
Pb	mg/L	< 0.01	5
Cd	mg/L	< 0.003	0.01
Zn	mg/L	< 0.01	2
Cu	mg/L	< 0.01	0.2

At the end of the experiment (July 2008), the following growth and physiological parameters were measured; Plant Height (PH), Shoot Dry Weight (SDW), Root Dry Weight (RDW) and Total Dry Weight (TDW), total Leaf Number (LN), average Leaf Area (LA), Transpiration (T), Stomata Conductance (SC) and Water Potential (WP). Physiological parameters (T, SC, and WP) were measured from three or four healthy leaves at mid-day. Transpiration and stomata conductance were measured using steady state porometer (Li-1600), while water potential was measured using pressure chamber. Total number of leaves and leaf area were measured using (leaf area meter). Harvested seedlings were divided into shoot and root to determine dry weight after drying using oven at 70 ^oC for 72 hours.

Statistical Analyses

Data collected were subjected to ANOVA using JMP 5.1.2 software package (SAS, 2004). Irrigation treatments were the main source of variance. Means were separated using Fisher's Least Significant Difference tests (LSD) at 0.05 level of probability.

RESULTS

Analysis of Variance (ANOVA; Table 2) shows that all jojoba parameters under investigation (physiologic and growth) showed a highly significant response to water treatments except for the number of leaves (LN).

Table 2: ANOVA for morphological and physiological parameters of Jojoba plants under different water treatments (treatment df=2, n=90).

Parameter		MS Treatment	MSE	F-ratio	Prob.
Growth	PH	87	6.0	14.5	0.001
	LN	35.4	12.8	2.8	0.078
	LA	4.5	0.43	10.6	0.001
	SDW	1.47	0.111	13.4	0.001
	RDW	0.48	0.026	18.7	0.001
	TDW	3.6	0.17	21.3	0.001
Physiology	WP	1.44	0.008	178	0.001
	T	1942	47.8	40.7	0.001
	SC	1.56	0.023	68.4	0.001

Water Quality Effect on Jojoba Growth

Mean separation of the effect of water quality on Jojoba growth parameters (Table 3) showed that treated wastewater (TWW) negatively affected Jojoba growth parameters compared to the freshwater (FW). The MW showed variable results, where in some parameters, it was intermediate between and significantly different from both TWW and FW such as leaf area (LA), root dry weight (RDW) and total dry weight (TDW). For plant height (PH) and shoot dry weight (SDW), MW was found significantly different from FW but not from TWW, (Table 3).

Plant height growth was significantly higher for seedling under FW (18.8 cm) compared to seedlings under MW (16.0 cm) and TWW (15.7 cm) respectively (Table 3). Leaf area (LA) was found significantly higher for FW (3.7 cm^2) compared to MW (3.1 cm^2) which in turn was found significantly higher than TWW (2.5 cm^2) (Table 3).

The significant effect of water quality on plant height (PH), total leaf number (LN) and leaf area (LA) was reflected on the dry weight of the shoot (SDW), root (RDW) and the whole plant dry weight (TDW). Table 3 shows that shoot dry weight (SDW), root dry weight (RDW) and total plant dry weight (TDW) under FW (1.98, 0.96, and 2.94 respectively) were significantly higher than under TWW (1.55, 0.7 and 2.25 respectively), while MW was intermediate between FW and TWW for root dry weight (0.83) and total plant dry weight (2.52) but not for shoot dry weight (1.69) which was found insignificantly different from TWW but significantly lower than FW (Table 3).

Table 3: Effect of water treatment (Fresh Water, Mixed Water and Treated Waste Water) on the growth parameters of Jojoba plants

Growth Parameters	Fresh Water	Mixed Water	Treated Waste Water
Plant height (cm)	18.8 a	16.0 b	15.7 b
Total number of leaf (leaves)	19.9 a	19.3 a	16.7 a
Leaf area (cm^2)	3.7 a	3.1 b	2.5 c

Growth Parameters	Fresh Water	Mixed Water	Treated Waste Water
Shoot dry weight (g)	1.98 a	1.69 b	1.55 b
Root dry weight (g)	0.96 a	0.83 b	0.70 c
Total dry weight (g)	2.94 a	2.52 b	2.25 c

* Numbers across columns followed by different letters are significantly different at the 0.05 level

Water Quality Effect on Jojoba Physiology

The effect of water quality on the growth parameters was associated with a significant effect on physiological parameters (Table 4). Under TWW, plants expressed significantly higher physiological stress than under MW which in turn expressed significantly higher physiological stress than under FW. Under TWW, water potential (WP), Transpiration rate (T), and Stomata conductance (SC) (-1.15, 76, and 1.0 respectively) were all significantly lower than under MW (-0.62, 90, 1.3 respectively) which in turn was significantly lower than under FW (-0.50, 101, 1.7 respectively) (Table 4).

Table 4: Effect of water treatment (Fresh Water, Mixed Water and Treated Waste Water) on the physiological parameters of Jojoba plants

Physiological Parameters	Fresh Water	Mixed Water	Treated Waste Water
Water Potential (-MPa)	-0.50 a	-0.62 b	-1.15 c
Transpiration (µg cm^{-2} s^{-1})	101 a	90 b	76 c
Stomata conductance (mmol m^{-2} s^{-1})	1.7 a	1.3 b	1.0 c

* Numbers across columns followed by different letters are significantly different at the 0.05 level

Correlation Between Physiological and Growth Parameters

Results of the correlation analysis showed a significant relationship between plant physiological status and growth with varying degree of relationship strength (Table 5). Among the physiological parameters, Stomata conductance (SC) showed the strongest relationship with shoot, root and total dry weight (r=0.73, 0.79, and 0.74 respectively) (Table 5). Leaf area (LA) and plant height (PH) showed a strong relationship to Transpiration (T) (r=0.92, and 0.83 respectively), while the number of leaves (LN) was strongly correlated to SC (r=0.83).

Table 5 Pearson correlation coeeficient between growth and physiological parameters of Jojoba plants.

	WP	T	SC	LA	LN	SDW	RDW	TDW
T	0.48*							
SC	-0.49*	0.20 n						
LA	0.22 n	0.92*	0.56*					
LN	-0.37*	0.21 n	0.83*	0.50*				
SDW	-0.37*	0.40*	0.73*	0.63*	0.92*			

	WP	T	SC	LA	LN	SDW	RDW	TDW
RDW	-0.34*	0.34*	0.79*	0.60*	0.98*	0.97*		
TDW	-0.32*	0.58*	0.74*	0.71*	0.69*	0.86*	0.81*	
PH	0.37*	0.83*	0.49*	0.89*	0.54*	0.63*	0.60*	0.51*

*, n refers to significant and non-significant correlation coefficient at p = 0.05 respectively

DISCUSSION

The serious shortage of fresh water in semiarid areas and in Jordan is a major limiting factor for agriculture production. Jordan's permanent water challenge and exhaustion of water resources (ground and surface water) have lead to the increasing dependence on untraditional uses of water for agriculture. Treated wastewater effluent in Jordan has moderate salinity but severe Cl salinity as the average Cl content of Jordan's wastewater is between 150-450 mg/l with the allowable limit is 400 mg/l (Ammary, 2007; Al-Khashman 2009) as compared to around 6 mg/l reported by Kiziloglu et al., (2008) in Turkey in a location similar to our semiarid Mediterranean conditions. Chlorine salinity was even found high in groundwater in Jordan as a result of higher temperature and due to anthropogenic related factors (Al- Khashman, 2007). This study demonstrated that moderate salinity of the TWW significantly affected Jojoba plants by reducing growth and increasing physiological stress. The resuls also demonstarted that mixing FW with TWW was not efficient to abate negative salinity effect of TWW on growth.

Treated Watsewater Reduce Jojoba Growth Parameters

Although the effect of salinity on jojoba explants was reported positive for modrate levels (Roussos et al., 2006; Roussos et al., 2007), the moderate salinity of TWW in this study showed negative effect on jojoba plants probably due to the specially higher Cl salinity. Mills et al., (2001) reported that both Jojoba salt sensitive and salt tolerant clones showed reduced shoot elongation and biomass under elevated Cl salinity. This study demonstrated that irrigation of Jojoba plants with treated wastewater (TWW) reduced plant height (PH) by 16.5% compared to FW, while MW did not improve PH significantly over TWW and was still reduced by 15% compared to FW. Leaf area (LA) was reduced by 16% and 32% for MW and TWW compared to FW. Reduction in leaf parameters seems to contribute to the reduction of shoot, root and the total plant biomass under TWW by 22, 27 and 23% respectively compared to FW, while MW improved biomass by approximately 7-13% compared to TWW but was still lower than FW by 14%. These findings are supported by other studies where jojoba shoot and leaf expansion, and number of flowers are reduced in response to increased levels of salinity (Rasoolzadegan et al., 1980; Benzioni et al., 1992 as cited in Botti et al., 1998).

It was noted that the differences between treatments only showed significance after 1 year of the experiment. For example, monitoring plant height over the experiment timeline showed insignificant differences between treatments until the tenth month (Figure 1) although a physiological stress was in place from the first day as expected. Hongwei et al., (2008) demonstrated that gene expression responsible for water stress resistance on Jojoba plants was evident after few hours of the stress.

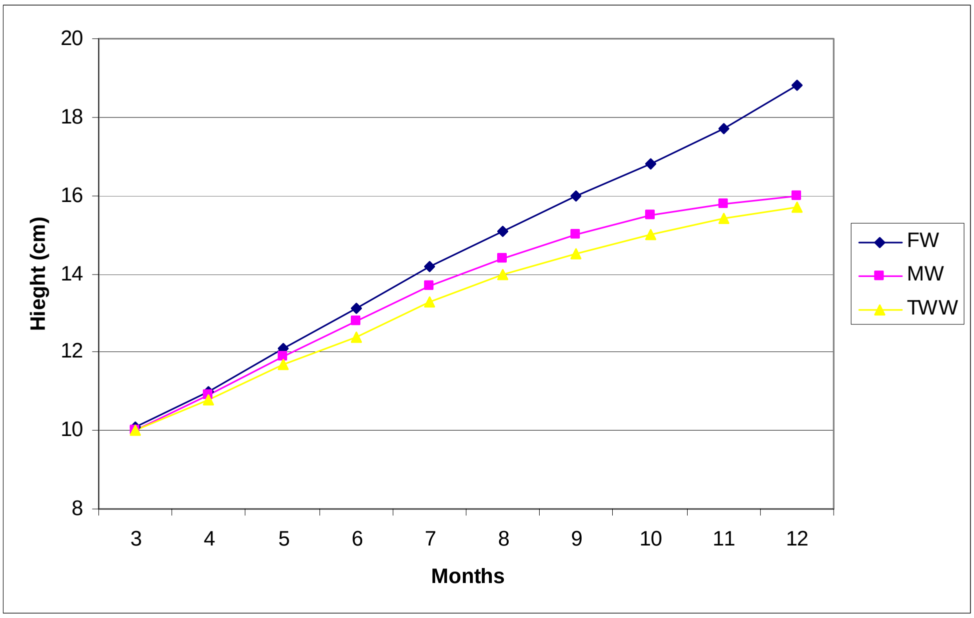

Figure1. Time series of jojoba plant height progress under three water treatments. Significance was only noticed after the tenth month.

Treated Watsewater Effects on Jojoba Physiological Parameters

Physiological response of Jojoba plants was much more pronounced than the grwoth paramteres. Water potential (WP) was reduced by 24% from FW to MW and by 130% from FW to TWW. Transpiration (T) was lower by 11% under MW compared to FW but was lower by 25% for TWW compared to FW. Stomata conductance (SC) was also lower by 24% for MW compared to FW and lower by 42% for TWW compared to FW. For all physiological parameters, MW was intermediate between FW and TWW. It was worth to note that Jojoba water potential (WP) may reach 6.0 MPa in normal field conditions (Nelson and Bartels, 1998) and even 9.5 MPa under extremely dry natural conditions of the southeastern US (Hamerlynck and Huxman, 2009) which is much lower than the 1.15 MPa recorded under TWW in this study. Also, Ceccardi and Ting, (1996) reported that lower water potential, stomata conductance and photosynthetic rate for Jojoba under water stress. The significant negative effect of TWW on Jojoba growth was associated with a significant physiological stress. On individual basis, plants under stronger physiological stress suffered a significant decline in their growth.

Conclusion

Jordan’s TWW salinity levels reduced Jojoba growth by 20-25%. Therefore, it was recommended to use TWW for Jojoba growth only if the economic losses are acounted for. It was also recommended not to mix FW with TWW since adding 50% FW increased seedling biomass by maximum of 13% only. Future study is needed to test the effect of TWW on the production of well established Jojoba plants on the field which may be able to tolerate salinity better than young seedlings. Despite the negative effect of TWW salinity on Jojoba, its use is still of prime interest because other plants may not be able to tolerate salinity levels like Jojoba and the fact that Jojoba wax crop is not linked to human food chain which minimizes the possibility of heavy metal accumulation.

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تقييم النمو والاستجابة الفسيولوجية لبادرات الهوهوبا للري باستخدام المياه العادمة المعالجة

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ملخـص

تمثل المياه العادمة المعالجة في المناطق الجافة مصدرا مستداما للمياه والعناصر الغذائية للنباتات، إلا أنها قد تحتوي على مستويات عالية من الملوحة والتي يمكن تخفيفها بالمزج مع المياه العذبة او يمكن ايجاد النباتات الملائمة للملوحة. يعتبر نبات الهوهوبا نبات متحمل للجفاف والملوحة وهو نبات واعد اقتصاديا ولا يشكل خطرا بيئيا بريه بالمياه العادمة لبعد هذا النبات عن السلسلة الغذائية للانسان. كان هدف هذه الدراسة هو التحقق من مدى تأثير المياه العادمة المعالجة في فسيولوجية ونمو بادرات الهوهوبا تحت ظرف البيت الزجاجي. بينت الدراسة ان نمو وفسيولوجية الهوهوبا تأثرت سلبا بالمياه العادمة المعالجة، حيث نمت البادرات اقصر وباوراق اصغر وقلت الكتلة الحية الجافة بمعدل يتراوح بين 15-32% مقارنة بالري بالمياه العذبة. كما تراوحت التأثيرات السلبية للمياه العادمة المعالجة في فسيولوجية النبات بمعدل يتراوح بين 24% لمعدل ايصال الثغور ولغاية 130% للاجهاد المائي للورقة. ان خلط المياه العادمة المعالجة بالمياه العذبة بنسبة متساوية حسن كثيرا من فسيولوجية النبات لكنه لم ينعكس على نمو النبات في النهاية. توصي الدراسة باستخدام المياه العادمة المعالجة لري الاشتال من الهوهوبا اذا كانت هذه الخسائر الناتجة عن الملوحة محسوبة اقتصاديا. كما توصي الدراسة بعدم فائدة خلط المياه العادمة المعالجة بالمياه العذبة لهذا الغرض.

الكلمات الدالة: الهوهوبا، الملوحة، المياه العادمة.

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