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Effect of irrigation water qualities on *Leucaena leucocephala* germination and early growth stage

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Abstract The evaluation of nonconventional water resources on seed germination and seedling growth performance at early growth stages is still in progress, especially on multipurpose forest trees. This study was designed to test the effect of four water qualities [treated wastewater, industrial, gray and distilled water (control)] on germination and early seedling vigor of Leucaena leucocephala. The results showed that germination was not significantly affected by different water qualities. Seed germination reached a maximum after 17, 14, 14 and 21 days under gray, industrial, treated wastewater and control irrigation, respectively. The highest mean of shoot length was scored under gray water irrigation. Likewise, the highest mean of root length was scored under control, which was not significant from gray water. The means of shoot fresh and dry weight were the highest under treated wastewater. The means of root fresh weight and root dry weight were not significantly different under water treatments. The shoot/root ratios under all water qualities treatments were significantly higher than the control. Growth performance was in progress with no mortality during 21 days of growth. The best nonconventional water quality alternatives based on cleanness, nutrients and toxicity are the gray, treated wastewater and industrial water, respectively.

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Introduction

Water resources are limited all over the world including in both industrial and developing countries. Use of domestic water may contribute considerably to alleviate the pressure of using freshwater resources in irrigation. Several reasons are behind the water shortage such as low rainfall intensity, high evaporation, increasing demands on freshwater and increase in population. As a result, different water qualities are used in agriculture such as wastewater (Lubello et al. 2004; Mohammad and Ayadi 2004). However, using alternative water resources might cause a negative environmental impact that might be overcome through recycling and re-treating such resources (Feigin et al. 1991; Nirit et al. 2006; Kiziloglu et al. 2008) and industrial water (Kisku et al. 2000). Treated wastewater is known to agriculture since the 1980s and is considered a fertile source of essential nutrients necessary for plant growth (Stewart and Flinn 1984; Mohammad and Ayadi 2004). But some researchers have reported that the high level of salinity in treated wastewater has negatively affected crop growth (Botti et al. 1998).

The gray and industrial waters are alternative water resources that could be used in agriculture. Gray water consists of diluted domestic wastewater streams coming from the shower, laundry facilities and/or the washbasins, and kitchen wastewater (Nolde 1999; Jefferson et al. 1999, 2004; Eriksson et al. 2002; Elmitwalli and Otterpohl 2007). Gray water constitutes 50–80% of the total household wastewater (Friedler and Hadari 2006).



Due to the low levels of contaminating pathogens and nitrogen, the reuse and recycling of gray water is receiving more and more attention (Li et al. 2003). The use of gray water for irrigation in countries with low precipitation is an attractive alternative because it produces low salt accumulation in soil compared to other water resources (Abu Ghunmi et al. 2008), while industrial water is produced as a result of fabricating, manufacturing, processing, washing, cooling and mining processes and petroleum refining.

The big challenge in the world, especially in the Middle East, is the serious shortage of freshwater, which is considered as the major limiting factor for agriculture production. The uses of nonconventional water resources such as wastewater, and gray and industrial waters are the best alternative solution.

In agriculture, the water problem is permanent and will lead to exhaustion of ground and surface water resources. Several agricultural crops are sensitive to saline, wastewater, and other nonconventional water resources, while other crops can withstand those resources. One of these species is Leucaena leucocephala, a multipurpose forest tree used for forage production that has been proven to survive in dry regions with poor quality soils because of its nitrogen fixating capability (NAS 2000). It is used as forage source for livestock feed, to improve soil characteristics and in the agroforestry systems and considered a potential species for pasture improvement in the semi-arid region (Kang et al. 1990; Graham and Vance 2003; Arowolo 2007). The lack of literature about the effect of water qualities on seed germination and seedling growth performance for crops in the world motivated us to conduct such research, starting with a potential forage crop candidate L. leucocephala. This study was conducted at the Jordan University of Science and Technology campus, Department of Natural Resources and Environment Laboratory during the growing season 2008-2009, when the seeds of Leucaena were collected. The objective of the present study was to evaluate the effect of different water qualities on the seed germination and early seedling growth performance of L. leucocephala under control conditions.

Materials and methods

An experiment was conducted to study the effect of different water qualities on seed germination and early growth stage in *L. leucocephala*. Seeds of *L. leucocephala* were collected from trees grown locally in the Arboretum at the Jordan University of Science and Technology (JUST), Jordan (latitude 32°34'N, longitude 36°01'E and an altitude 520 m above sea level). Four water qualities were used in this experiment: gray water (GW), industrial water (IW), treated wastewater (TWW) and distilled water (DW). The



 Table 1
 Water quality analysis of the different water qualities used in this experiment was carried out in JUST and NCARE laboratories compared to the Jordan Institution for Standard and Metrology (JISM 2003)

Parameter [Units (SI)]	Treated waste water	Gray water	Industrial waste water	JISM	
рН	7.3	7.9	7.0	6.0–9.0	
EC (dS/m)	1.2	0.7	4.1	1.0-3.0	
BOD (mg/L)	31.0	12.9	NA	200.0	
Ca (mg/L)	50.5	71.9	61.7	400.0	
Mg (mg/L)	29.0	17.3	36.5	60.0	
K (mg/L)	23.1	5.1	39.5	80.0	
Na (mg/L)	151.7	43.5	813.2	230.0	
CL (mg/L)	195.0	85.1	1,010.3	400.0	
Zn (mg/L)	0.4	0.7	0.4	5.0	
Fe (mg/L)	2.0	0.1	1.2	5.0	
Mn (mg/L)	0.0	NA	0.4	5.0	
COD (mg/L)	42.0	29.6	NA	500.0	
B (mg/L)	NA	0.4	NA	2	
PO ₄ (mg/L)	22.4	0.2	NA	30.0	
NO ₃ (mg/L)	47.0	2.0	NA	45.0	
TDS (mg/L)	787.0	429.0	2,600.0	1,500.0	
SAR (%)	4.2	21.9	20.3	9.0	
ESP (%)	4.6	0.5	21.9	NA	
E-coli (Colony/ 1,000)	39.0	408.0	NA	1,000.0	

water quality sources were the following: the treated wastewater was collected from JUST water treatment plant; the industrial water was collected from the textile factory at Prince Al-Ameer Hassan City, north and neighboring part of the university campus; the gray water was collected from the student dorm house on the campus that has a special infrastructure to collect gray water. The gray water was collected in a tank and pumped to another tank through a sand filter to use it for irrigation. In the treatment plants, the wastewater was treated physically, chemically, and biologically. The industrial water was treated physically and chemically only. A complete chemical analysis for the three water qualities is shown in Table 1.

The water samples were analyzed either on campus laboratories or in the water laboratory at the National Center for Agricultural Research and Extension (NCARE), Amman, Jordan.

Germination experiment

Seeds collected were exposed to hot water for 5 min and then soaked in water for 48 h to break the hard seed coat dormancy before being placed on wet filter paper (Albet 150, Albet LabScience, Germany) in glass Petri dishes (11-cm diameter, 2-cm depth) and into incubator for germination. The temperature was set up at 20°C. The Petri dishes were rearranged at randomly every 2 days to ensure no systematic effects due to position within the incubator. All the four treatments consisted of four replicates, each containing 50 seeds.

Visible radicle growth and emergence of hypocotyl and the cotyledons were used to define germination (ISTA 2004). The germination percentage was recorded after 4, 7, 10, 14, 17, and 21 days following the rules of the International Seed Testing Association (ISTA 2004). The final count was recorded after the end of the 21st day of germination. Speed of germination was expressed as the germination rate index (GRI) according to the following formula (AOSA1983):

$$GRI = \frac{\text{No of germinated seed}}{\text{Days of first count}} + \dots + \frac{\text{No of germinated seed}}{\text{Days of final count}}$$

Data collected

The following parameters were measured per plant: seedling (hypocotyl + cotyledons) length (cm), root length (cm), seedling fresh and dry weight (g), and root fresh and dry weight (g). Fresh weight was obtained by weighing on a three-digit balance. Plant materials were dried separately in an oven adjusted at 70°C for 24 h and weighed afterward. The shoot to root length ratio (SL/RL) and shoot to root dry weight ratio (SDW/RDW) were calculated.

Experimental design and statistical analysis

Data collected were subjected to ANOVA using SAS software package 23 (SAS 2002). Water quality treatments were the main source of variance. Means were separated using Fisher's least significant difference tests (LSD) at 0.05 probability level.

Results and discussion

Effect of water qualities on L. leucocephala seed germination

The results presented in this research showed that the overall germination percentage after 21 days was not significantly different between the water quality treatments (Table 2). The highest germination percentage was scored by the TWW (71%) compared to 66, 64, and 64% in GW, IW, and DW, respectively. The germination rate index (GRI) was not significantly different within the treatments. The GRI in the TWW was higher (21) than in the other treatments. The germination percentage on the fourth day was significantly

different within the water treatments where the highest germination percentage was scored by the TWW (52%) compared to 44, 49, 42% in GW, IW, and DW, respectively. On all days of germination, afterward, there were no significant differences (p < 0.05) between treatments. Moreover, there were no significant differences between treatments in the GRI (Table 2). The seeds germinated under TWW had higher GRI (21) compared with the other treatments, with no significant differences (at $p \le 0.05$) and scores of 19, 19, and 18 under GW, IW, and DW, respectively.

Effect of water qualities on L. leucocephala seedling growth

The water qualities showed a significant effect in L. leucocephala morphological parameters in the early growth stages, especially in the hypocotyl and shoot growth parameters. The results presented showed a significant effect of the four water qualities on the shoot and root lengths of Leucaena.

The significant differences (p = 0.05) indicated that the GW scored the highest shoot length per plant (12.64 cm), while there were no significant differences between TWW, IW, and DW (Fig. 1). Root growth showed significant differences between the water quality treatments (p = 0.001). In contrast to the shoot length, root length was the highest in DW (6.61 cm) and was not significantly different from GW (5.43 cm). The root lengths were not significant in the GW, TWW and IW, while TWW and IW were significantly different from DW. The significant effect of water qualities on shoot and root length was reflected on the shoot fresh weight (SFW) and dry weight (SDW) and root fresh and dry weights (RFW, RDW).

Table 2 Germination percentage (%) and germination rate index (GRI) in L. Leucocephala seeds after 4, 7, 10, 14, 17, and 21 days under four water quality treatments

Treatment	Germination percentage (%) day						GRI
	4	7	10	14	17	21	
GW	44	57	61	65	66	66	19
TWW	52	67	69	71	71	71	21
IW	49	59	62	64	64	64	19
DW	42	57	62	63	63	64	18
LSD	7.0	10.3	11.6	11.0	11.3	11.3	2.9
	*	NS	NS	NS	NS	NS	NS

GW Gray water, IW industrial water, TWW treated wastewater, DW distilled water, LSD Fisher's least significant difference to compare treatment means at 0.05 level of probability, NS not significant at p = 0.05

* Significant at p = 0.05





Fig. 1 Shoot length (cotyledons and hypocotyl) and root length (cm) of *L. Leucocephala* as affected by different water quality applications: Gray water (GW), industrial water (IW), treated wastewater (TWW), and distilled water (DW) grown under controlled conditions. Columns with *similar letters* in the same series are not significantly different according to Fisher's least significant difference (LSD = 0.60 and 0.77 for shoot and root length, respectively, at alpha = 0.05)

The shoot and root fresh and dry weights under different water qualities in the early growth stage were studied in this work. The effect of water quality was highly significant (p = 0.001) on the shoot and root fresh weight (Fig. 2). The IW scored the highest shoot fresh weight per plant (0.251 gm) that was not significantly different from GW (0.239 gm) and DW (0.221 gm). The lowest shoot dry weight was scored by TWW (0.208 gm). The root fresh weight was not significantly different for all the water quality treatments (p = 0.05).

The dry weight results showed a variation in the shoot and root per plant. The shoot dry weight did not show any significant differences between the water treatments compared to the root dry weight that showed a significant difference for all the treatments in the dry weight (p = 0.001). The DW treatment scored the highest average root dry weight (0.0028 g) that was significantly different (p =0.001) from GW, TWW, and IW (Fig. 3).

Shoot to root length (SL/RL) was significantly higher for seedling under GW and IW (9.81, and 9.88, respectively), compared to seedlings under TWW and DW (8.52 and 6.98, respectively). Also, shoot to root dry weight (SDW/RDW) was significantly higher for seedlings under IW reaching 2.43 compared to GW (2.36), TWW (2.34). Also, these differences were significantly different from DW (1.88, Fig. 4).

Effects of water quality on germination

The results showed that the different water qualities did not affect the germination percentage, although germination was still in progress till the day 21. The germination and





Fig. 2 Shoot and root fresh weight (gm) of *L. Leucocephala* as affected by different water quality applications: Gray water (GW), industrial water (IW), treated wastewater (TWW), and distilled water (DW) grown under controlled conditions. Columns with *similar letters* in the same series are not significantly different according to Fisher's least significant difference (LSD = 0.03 and 0.007 for shoot and root weights, respectively, at alpha = 0.05)



Fig. 3 Leucaena leucocephala shoot and root dry weight per plant (gm) as affected by different water qualities applications: gray water (GW), industrial water (IW), treated waste water (TWW) and distilled water (DW) and grown under controlled conditions. Columns with *similar letters* in the same series are not significantly different according to Fisher's least significant difference (LSD = 0.0004 for roots weight at alpha = 0.05)

early seedling growth in *L. leucocephala* was in progress and no negative effect or mortality was detected. In contrast, Pang et al. (2009) showed that application of saline water to crops at the early growth stage was not favorable, since most crops were sensitive to salinity in the early stages of growth but developed resistance at later stages, so good quality water should preferably be applied at presowing to improve germination and ensure low salinity at the root zone. This was not the case in *Leucaena*



Fig. 4 Shoot/root ratio based on the length (SL/RL) and dry weight ratio (SDW/RDW) in *L. Leucocephala* as affected by different water quality applications: gray water (GW), industrial water (IW), treated wastewater (TWW) and distilled water (DW) and grown under controlled conditions. Columns with similar letters in the same series are not significantly different according to Fisher's least significant difference (LSD = 0.36 and 1.42 for shoot root length ratios and weight ratio, respectively, at alpha = 0.05)

germination and growth, which was not affected during germination and the early growth stages. There was no difference in germination percentage and GRI between water quality treatments. However, there was variable effect of water qualities depending on chemical composition and salinity (1). It might be an indication of salt tolerance during seed germination, emergence and early growth young in seedling stages in woody plants. The industrial water results showed a positive effect on germination of *L. leucocephala*, which might be due to presence of organic matter (Uzair et al. 2009).

The GW is cleaner and low in salt concentrations and has low levels of contaminating pathogens and nitrogen (Table 1) (Li et al. 2003). Also, GW did not affect germination (66%), but scored almost higher values in root length and shoot to root length ratio compared with other water quality treatments (Abu Ghunmi et al. 2008). The TWW and IW showed a moderate effect on crop growth and germination; this may be due to high levels of minerals and high toxicity and salinity (Na, Cl) (Table 1) (Rai and Rai 2003). These results were in agreement with several reports on IW, where it was a source of pollution and health hazards (McGrath et al. 2000) and adversely affected crop production (Islam et al. 2006), because it had toxic heavy metals that could not be degraded (Smejkalova et al. 2003). Also, TWW had adverse effects on crops growth and production, because it had highly salinity (Mills et al. 2001; Rai and Rai 2003; Nirit et al. 2006; Kiziloglu et al. 2008). Also, using IW and TWW might affect human health when excessively used in irrigation agriculture (Yuan 1993).

Effects of water quality on growth parameters

There were variations in the effects of different water qualities on L. leucocephala growth. There has been no previous work on the effects of these water qualities on seed germination and growth performance, especially in L. leucocephala. Shoot and root length were higher with GW and DW than with IW and TWW; this reduction may be due to increased salinity and minerals (Table 1). These results were in agreement with (Smejkalova et al. 2003) for IW and with (Nirit et al. 2006; Kiziloglu et al. 2008) for TWW. In comparison with the GW, the reduction percentage in shoot length was 7 and 2%, and the root length was 6 and 3%, in TWW and IW, respectively. These parameters directly affect fresh weight, dry weight as well as the length and weight ratios. Shoot fresh and dry weights under IW were higher than under other water quality treatments, which might be due to accumulation of salts in the shoot and leaf (Shannon et al. 1994). Also, there was a reduction in the percentage of shoot fresh weight (13%) and dry weight (6%) under TWW compared to GW, while the reduction in root fresh weight was higher under TWW and IW (6 and 23%, respectively) compared with GW. Similar trends were found for crops, such as soybean and alfalfa (Berstein and Ogata 1966; Kant et al. 1994), grown under saline condition. There is no significant difference in SL/RL ratio for all the treatments compared to the DW. The ratio was higher in SDW/RDW under IW and GW compared with TWW and DW that was reflected mainly by the shoot dry weight. As a result, Leucaena can be irrigated from seed to seedling stages with GW, TWW, and IW.

However, water application management recommends that the direct application of water should be delayed as much as possible till after the early seedling stages to avoid salinity stress (Pang et al. 2009).

Conclusion

The use of different water qualities on *L. leucocephala* production is considered critical especially during the early stages of growth. This research, conducted using three water qualities (GW, TWW, IW) in addition to the control (DW), showed in a primary evaluation that the *L. leucocephala* was not affected by the three water levels, but on the contrary the growth performance was in progress with no mortality during 21 days of growth. Most of the recommendations on water quality use in irrigation mention that crops must not be irrigated at the early growth stages, since it will increase the mortality rate due to the toxicity of heavy metal accumulation and salinity. The use of different water qualities in plant nurseries would be a beneficial



alternative resource to freshwater. On comparing the different water qualities, GW was the best alternative, followed by TWW and then by IW compared to DW, based on cleanness, nutrients, and toxicity. GW had low salinity levels and showed positive effect on germination and seedling growth compared to high salinity TWW and IW. The application of knowledge and extension to such results will need further research to study the effect of different water qualities on the production of well-established *L. leucocephala* plants in the field. Further studies are recommended to illustrate the efficiency of nontraditional water in agricultural production in the field.

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