

# Treated Wastewater Irrigation Promotes the Growth and Nodulation of *Acacia* Species

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## ABSTRACT

The scarcity of irrigation water is one of the vital factors limiting crop production in arid countries. The critical shortage of water necessitates the development of new water sources, and the use of wastewater is considered one of the best solutions for solving this water scarcity. An experiment was conducted to investigate the effect of primary treated wastewater irrigation on growth parameters, nodulation, macro and micronutrients for *Acacia ampliceps* (Maslin.) and *Acacia origena* (Hunde.) seedlings grown in the Dirab Valley, South of Riyadh City. The study included four irrigation treatments: municipal water plus *Rhizobium* inoculum (MWR), primary treated wastewater plus *Rhizobium* inoculum (PTWR), municipal water only (MW), and primary treated wastewater only (PTW). The results revealed that the PTWR and PTW treatments were effective for increasing the growth parameters and nodulation of the *Acacia* seedlings. The combination of primary treated wastewater and inoculation with *Rhizobium* significantly improved the growth parameters, nodulation, and nutrient content of the seedlings. Overall, the combination of treated wastewater and *Rhizobium* can be used as a potential source of nutrients and water for tree legume plantations in the arid areas of Saudi Arabia.

**Keywords:** *Acacia* species; Macro and micronutrients; Nodulation; Primary treated wastewater; Saudi Arabia.

## INTRODUCTION

In many arid and semi-arid regions, water is becoming increasingly limiting resource, and therefore studies need to investigate untraditional ways to meet the increasing demand for water worldwide. Saudi Arabia is an arid country and scarcity of irrigation water is one of the major factors limiting crop production and higher crop yield (Al-Fredan, 2006). In Saudi Arabia, critical shortage of water necessitates the development of new water sources, and the use of wastewater is considered one of the best solutions. (Al-Othman, 2009; Al-Mefarrej, 2013).

The treated wastewater contains organic matter and nutrients that can improve soil and crop productivity. Currently, wastewater is being used for the restoration of degraded land and growth of commercial and environmental crops (Al-Fredan, 2006). After primary

treatment, the wastewater becomes safe for irrigation of non-food crops, such as tree plantations, greenbelts, and forestlands (Hassan et al., 2006; Tabari and Salehi, 2009). Previous studies indicated that using sewage effluent for irrigation improved the soil properties and some growth parameters of forest trees, such as biomass potential, biomass allocations, specific gravity of the wood, fiber length, and volumetric shrinkage (Guang et al., 2010; Ali et al., 2011). Legume trees can absorb wastewater effluent and heavy metals through their root systems, thereby serving as effective biological filters and inhibiting contamination of groundwater sources. Nodulation and nitrogen fixation in the legume-*Rhizobium* relationship is sensitive to water quality, therefore poor water quality can prevent legume growth and reduces crop yield. Both legumes and the nodule initiation process are more sensitive to osmotic stress than *Rhizobium* strains itself (Katerji et al., 2003; Goormachtig et al., 2004; El-Komy, 2005; Gehring et al., 2005; Junior et al., 2005 and Daudin and Sierra, 2008). Poor water quality can result in failure of the infection process and is considered one of the main reasons for unsuccessful symbiosis. In Saudi Arabia, treated municipal wastewater is one of the main sources used for agricultural purposes.

*Acacia ampliceps* (Maslin.) has been introduced to Saudi Arabia in 1980. It is regarded as a fast-growing dense shrub or small tree (3-9 m tall) with a spreading crown. It is adapted at the arid and semi-arid regions of Saudi Arabia. *A. ampliceps* is considered as one of the most salt-tolerant species in Saudi Arabia, *Acacia* species are found on sand plains, flood plains, and along drainage lines (Doran and Turnbull, 1997; Marcar et al., 1999 and Aref et al., 2003). *Acacia origena* (Hunde.) is a medium sized tree (6 m tall) that is native to Ethiopia, West Eritrea, and also across the Red Sea in Yemen and Saudi Arabia.

It is considered as an indigenous tree in southern Saudi Arabia as multipurpose trees, used for, fuel production and reforestation. However, in Saudi Arabia, there is a lack of information on the effect of wastewater irrigation on the growth parameters and nutrient uptake of woody legume trees inoculated with

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*Rhizobium*. Therefore, the aim of the current study was to evaluate the effect of primary treated wastewater irrigation on the growth characteristics of *Acacia* seedlings, inoculated with indigenous *Rhizobium*, under Riyadh region conditions.

## MATERIALS AND METHODS

### 1. Tree growth and soil characteristics

This study was carried out in two successive growing seasonal 2014 and 2015 at the Experimental Station, Faculty of Food and Agricultural Sciences, King Saud University in the Dirab Valley, 60 km south of Riyadh City, Saudi Arabia. The physical and chemical characteristics of the soil used in the experiment are shown in Table (1).

The average temperature during the growing season ranged between 10 °C (winter) and 41 °C (summer), with an annual rainfall of 50 mm. The seeds of *Acacia* species *A. ampliceps* and *A. origena* were obtained from the King Saud University's Range and Forestry Applied Research Unit. Seeds were pretreated with hot water (100 °C) for 15 min, and kept in cool water for 24 h, and then planted in plastic plates containing a mixture of vermiculate and sand (1:2 by volume). Germination started after 7 days, seedlings (two weeks age) were transplanted into plastic pots (20 cm diameter), and each was filled with 5 kg of soil. Fertilizer Ca (H<sub>2</sub>PO<sub>4</sub>), 2CaSO<sub>4</sub> (0.33g/plant) was applied to the pots 30 days after planting.

### 2. Seedling inoculation assay and experimental design

The *Rhizobium* strain (DLR22) used for inoculation was isolated in 2014 from root nodules of *Leucaena leucocephala* (Lam.) seedlings which grown in a Range and Forestry Applied Research Unit Nursery. The isolates were harvested from healthy, unbroken, and pink root nodules collected from the field. Root-nodulating bacteria were isolated from the seedlings, following to the methods described by Vincent (1970). Seedlings were inoculated with the *Rhizobium* strain by applying 10 ml of rhizobia (approximately 1×10<sup>10</sup>) grown in yeast extract mannitol broth, with shaking (200 rpm). After 7 days of incubation period, the pots were organized in a randomized complete design with 5 replications (split plot in CRD, n= 40). The environmental conditions were 24 °C/17 °C (day/night

temperatures), 13 h/11 h (light/dark periods), 75% relative humidity, and a photon flux density was 400–500 μmol m<sup>-2</sup> s<sup>-1</sup>. Four irrigation treatments were used: municipal water plus *Rhizobium* inoculum (MWR), primary treated wastewater plus *Rhizobium* inoculum (PTWR), municipal water only (MW), and primary treated wastewater only (PTW). All water treatments types had the same quantity (650 ml) and irrigated during summer three times and twice every week in winter. The chemical characteristics of the irrigation water used in the study are shown in Table (2). Seedlings were harvested 120 days after planting, and the following measurements were recorded: seedling height and diameter; shoot, root, and total seedling dry matter; number of nodules per seedling; and dry weight of nodules. Seedlings were dried at 70 °C, and the macro and micronutrients were determined. Total nitrogen content was determined using the Kjeldahl method (Kjeldahl, 1883). Potassium (K<sup>+</sup>) was determined using flame photometry (Corning 400, Sherwood Scientific Ltd, Cambridge, UK). Phosphorus (P) was determined using colorimetric determination. However, micronutrients; Cu, Fe, and Mn were measured using atomic absorption spectrophotometry (Perkin Elmer, PinAAcle™ 900F, Massachusetts, USA).

### 3. Statistical analysis

Data were statistically analysed using the analysis of variance procedure for a split plot system in complete randomized design (CRD) using SAS statistical software package program (version 9.10, 2001). The mean values were compared using the F-test, and L.S.D, considering a significance level of p <0.05.

## RESULTS AND DISCUSSION

### 1. Growth and nodulation of *Acacia* species

There were significant differences (p <0.05) between two *Acacia* species for seedling height and nodule dry weight, while seedling diameter, shoot and root dry weight were not significantly in the two seasons. Irrigation treatments significantly affected all the tested parameters, except nodule dry weight. Also, there were significant interaction effects between two *Acacia* species and the four irrigation treatments, except for root and dry weight of total seedling character, particularly in the second season (Table 3).

**Table 1. Physical and chemical characteristics of the soil used in the study**

Particle size distribution (%)			Soil texture	pH	EC <sup>†</sup> (ds m <sup>-1</sup> )	Soluble cations (mg L <sup>-1</sup> )			Soluble anions (mg L <sup>-1</sup> )		Available nutrients (mg kg <sup>-1</sup> )		
sand	silt	clay				Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	SO <sub>4</sub> <sup>-2</sup>	CL <sup>-</sup>	N	P	K
78.7	14.0	7.3	Sandy loam	8.65	1.45	4.45	0.15	6.10	6.0	6.5	13.0	0.15	1.95

<sup>†</sup>EC: electrical *conductivity*

**Table 2. Chemical characteristics of irrigation water types used in the study**

Properties	Types of irrigation Water <sup>†</sup>		Limits <sup>‡</sup>
	MW	PTW	FAO (1992)
pH	7.75	8.25	6.5-8.4
Ec (ds m <sup>-1</sup> )	0.15	1.90	3.0-7.0
TDS (mg L <sup>-1</sup> )	92	1195	1920-44880
Soluble cations (mg L <sup>-1</sup> )			
Ca <sup>2+</sup>	nd <sup>§</sup>	153	-
Mg <sup>2+</sup>	nd	44.5	-
K <sup>+</sup>	0.80	9.36	-
Na <sup>+</sup>	16.0	219.5	-
Soluble anions (mg L <sup>-1</sup> )			
CO <sub>3</sub> <sup>-2</sup>	nd	37.5	-
HCO <sub>3</sub> <sup>-</sup>	31.0	145.0	91.5-518.5
Cl <sup>-</sup>	27.0	313.0	-
SO <sub>4</sub> <sup>-2</sup>	17.5	370	-
Total heavy metals (mg L <sup>-1</sup> )			
Cd	0.004	0.009	0.01
Ni	0.032	0.62	0.20
Pb	0.013	0.209	5.0
Zn	0.490	0.355	2.0

<sup>†</sup>MW: municipal water, PTW: primary treated waste water; <sup>‡</sup>Limits for agricultural reuse of wastewater (FAO, 1992); <sup>§</sup>not detected

The growth of the *Acacia* species was noticeably enhanced by using the PTWR treatment, and, to a lesser extent to PTW treatment. For both seasons, *A. ampliceps* had the highest values of seedling height and diameter followed by the *A. origena* seedlings (Table 4). The incensements in the seedling height were 69% and 13% for *A. ampliceps* and *A. origena*, respectively, while seedling diameter was 13% and 5.5% for *A. ampliceps* and *A. origena*, respectively, in the PTWR treatment than in the MW treatment. Several studies have also showed increasing in the growth parameters of trees and crops after irrigation with treated wastewater (Kiziloglu et al., 2008; Bedbabis et al., 2010; Akponikpèa, 2011; Pandey and Srivastava, 2012, and Selahvarzi and Hosseini, 2012). Moreover, Carvalho et al. (2012) found that the height and nodulation of *Leucaena leucocephala* and *Mimosa caesalpiniaefolia* significantly increased when treated with sewage effluent and inoculated with *Rhizobium*.

The seedling biomass of the two *Acacia* species is presented in Table (5). The shoot and root dry weight in PTWR and MWR were significantly differed with average of 2.7 and 1.31g, respectively, for *A. ampliceps* and 2.73 and 1.30g, respectively, for *A. origena* compared with the other treatments in the first season. In the second season, the shoot dry weight was increased significant only in PTWR with an average of 1.75g, while both the root and total plant dry weight did not show any significant effect. The same trends were noted in root and dry weight of total seedling in both

PTWR and MWR treatments, which gave high mean values. MWR treatment in *A. origena* gave the highest root and dry weight of total seedling values compared with *A. ampliceps* in the second season (Table 5). These results are in accordance with Shetta et al. (2009), who found that the total dry weights and the fresh and dry weights of stems and branches of *Casuarina* trees treated with liquid sludge were higher than those dewatered sludge treated trees and control treatments. Also, Kaur et al., (2013) demonstrated the significant influence of rhizobia isolates on growth and biomass in *Albizia lebbek* seedlings.

Inoculation with appropriate *Rhizobium* isolates ideally compatible with the species and environment in terms of nitrogen fixation and assimilation enhanced the productivity of the forests and plantations. Most of leguminous plants are able to increase nitrogen fixation and remove heavy metals from the soil at the same time (Ike et al., 2007). The nodulation values for the *Acacia* species under the different irrigation treatments are presented in Table (6). Statistically, both MWR and PTWR are not different. For the two *Acacia* species, the MWR treatment induced had the highest mean value of nodulation and nodule dry weight/ seedling, followed by the PTWR treatment. In the first season, high mean nodules seedling<sup>-1</sup> and nodule dry weight seedling<sup>-1</sup> were noted for *Acacia origena* (14.2 nodules seedling<sup>-1</sup> and 0.034g nodule dry weight, respectively) than for *Acacia ampliceps* (10.4 nodules seedling<sup>-1</sup> and 0.032g nodule dry weight, respectively).

**Table 3. Analysis of variance of the seedlings growth parameters for two *Acacia* species subjected to four irrigation treatments during two growing seasons**

Source of Variation	df	First season (2014)										Second season (2015)					
		H <sup>†</sup>	D <sup>‡</sup>	SD <sup>§</sup>	RD <sup>¶</sup>	TPD <sup>**</sup>	ND <sup>††</sup>	Nod <sup>§§</sup>	H	D	SD	RD	TPD	ND	Nod		
Replicate	4	50.7	0.07	0.23	0.39*	0.44	0.00021	6.4	3.91	0.14	0.12	0.09	0.15	0.0001	0.31		
Species (Sp.)	1	705.6**	0.21	0.01	0.01	0.0012**	3.03	324.9**	0.05	0.24	0.08	0.05	0.0002**	0.40			
Treatment (Trt.)	3	599.3**	0.33*	2.94**	0.31	2.25**	0.0001	278.6**	765.7**	0.04	0.82**	0.30	1.49*	0.0001	388.1**		
Sp. × Trt.	3	293.1**	0.46**	1.39**	0.43*	3.34**	0.00011*	13.8*	163.1**	0.08*	0.19*	0.26	0.87	0.0002*	21.5**		

\*Significant at the 0.05 probability level; \*\*Significant at the 0.01 probability level; †seedling height; ‡seedling diameter; §shoot dry weight; ¶root dry weight; \*\*total dry weight; ††nodules dry weight; §§number of nodules seedling<sup>-1</sup>.

**Table 4. Mean values of the seedlings growth characteristics for two *Acacia* species subjected to four irrigation treatments during two growing seasons**

Species	Treatment	First season (2014)			Second season (2015)		
		H (cm) <sup>††</sup>	D (mm) <sup>‡‡</sup>	Nod <sup>§§</sup>	H (cm)	D (mm)	Nod
<i>Acacia ampliceps</i>	MW <sup>†</sup>	41.6 ± 2.5e	4.33 ± 0.1ab	6.4	70.0 ± 3.5b	5.38 ± 0.1ab	0.31
	MWR <sup>‡</sup>	55.2 ± 5.8d	4.40 ± 0.17ab	3.03	74.4 ± 3.1b	5.24 ± 0.29b	0.40
	PTW <sup>§</sup>	62.6 ± 7.6cd	4.42 ± 0.5ab	278.6**	72.4 ± 2.8b	5.53 ± 0.15a	388.1**
	PTWR <sup>#</sup>	70.6 ± 8.5ab	4.51 ± 0.15a	0.00011*	83.3 ± 5.3a	5.5 ± 0.13ab	21.5**
<i>Acacia origena</i>	MW	60.4 ± 5.6cd	2.83 ± 0.28c	13.8*	53.5 ± 8.5c	5.40 ± 0.31ab	0.31
	MWR	71.8 ± 5.8a	4.05 ± 0.5bc	163.1**	67.2 ± 5.7b	5.45 ± 0.14ab	0.40
	PTW	63.0 ± 7.1bcd	4.59 ± 0.12a	0.08*	73.8 ± 4.1b	5.48 ± 0.17a	0.31
	PTWR	68.4 ± 4.8abc	4.6 ± 0.15a	0.19*	82.8 ± 6.3a	5.6 ± 0.32a	0.40

†municipal water; ††municipal water with *Rhizobium*; ‡primary treated wastewater; ‡‡primary treated wastewater with *Rhizobium*; §seedling height; †seedling diameter; Within columns, values with the same letters are not significantly different at a 0.05 level of probability (i=5; means ± SD).

**Table 5. Mean values of seedlings biomass for two *Acacia* species subjected to four irrigation treatments during two growing seasons**

Species	Treatment	First season (2014)				Second season (2015)			
		Seedling dry weight (g seedling <sup>-1</sup> )		Seedling dry weight (g seedling <sup>-1</sup> )		Seedling dry weight (g seedling <sup>-1</sup> )		Seedling dry weight (g seedling <sup>-1</sup> )	
		DS <sup>††</sup>	DR <sup>‡‡</sup>	TPD <sup>§§</sup>	DS	DR	TPD	TPD	
<i>Acacia ampliceps</i>	MW <sup>†</sup>	0.95 ± 0.4c	0.61 ± 0.3b	1.56 ± 0.2c	0.84 ± 0.30c	0.52 ± 0.28a	1.37 ± 0.49a		
	MWR <sup>†</sup>	2.5 ± 0.2ab	0.95 ± 0.05ab	3.45 ± 0.06ab	1.58 ± 0.20ab	0.99 ± 0.77a	2.57 ± 1.4a		
	PTW <sup>§</sup>	1.87 ± 0.4b	1.05 ± 0.3ab	2.92 ± 0.3b	1.24 ± 0.24bc	0.82 ± 0.24a	2.06 ± 0.33a		
	PTWR <sup>#</sup>	2.7 ± 0.2a	1.31 ± 0.2a	4.01 ± 0.2a	1.75 ± 0.71a	0.98 ± 0.68a	2.73 ± 0.48a		
<i>Acacia origena</i>	MW	1.54 ± 0.1b	0.92 ± 0.5ab	2.46 ± 0.9b	0.98 ± 0.39c	0.59 ± 0.25a	1.6 ± 0.79a		
	MWR	2.73 ± 0.8a	1.3 ± 0.6a	4.03 ± 0.9a	1.46 ± 0.41ab	1.2 ± 0.45a	2.66 ± 0.88a		
	PTW	1.95 ± 0.8b	0.70 ± 0.4b	2.65 ± 0.4b	1.22 ± 0.35bc	0.82 ± 0.49a	2.04 ± 0.44a		
	PTWR	1.92 ± 0.1b	0.94 ± 0.5ab	2.86 ± 0.5b	1.13 ± 0.10bc	1.1 ± 0.78a	2.23 ± 0.41a		

municipal water; <sup>†</sup>municipal water with *Rhizobium*; <sup>‡</sup>primary treated wastewater; <sup>#</sup>primary treated wastewater with *Rhizobium*; <sup>§</sup>shoot dry weight; <sup>††</sup>root dry weight; <sup>‡‡</sup>total dry weight; <sup>§§</sup>total dry weight. Within columns, values with the same letters are not significantly different at a 0.05 level of probability (p=5; means ± SD).

**Table 6. Mean values of nodulation for two *Acacia* species seedlings subjected to four irrigation treatments during two growing seasons**

Species	Treatment	First season (2014)		Second season (2015)	
		ND <sup>††</sup> (g seedling <sup>-1</sup> )	Nod <sup>‡‡</sup>	ND (g/plant)	Nod seedling <sup>-1</sup>
<i>Acacia ampliceps</i>	MW <sup>†</sup>	0.001 ± 0.001c	3.0 ± 1.2d	0.001 ± 0.001c	2.0 ± 1.1e
	MWR <sup>†</sup>	0.032 ± 0.01a	10.4 ± 1.5b	0.044 ± 0.012a	15.4 ± 3.6a
	PTW <sup>§</sup>	0.022 ± 0.007b	7.4 ± 3.1c	0.017 ± 0.007b	6.6 ± 3.7d
	PTWR <sup>#</sup>	0.030 ± 0.004a	10.2 ± 1.8b	0.030 ± 0.004a	10.4 ± 1.1bc
<i>Acacia origena</i>	MW	0.001 ± 0.001c	3.5 ± 1.1d	0.002 ± 0.002c	4.3 ± 1.0e
	MWR	0.034 ± 0.010a	14.2 ± 2.9a	0.034 ± 0.022a	12.0 ± 1.4b
	PTW	0.016 ± 0.005b	6.8 ± 3.1c	0.016 ± 0.005b	8.6 ± 2.3cd
	PTWR	0.022 ± 0.003b	9.2 ± 1.9bc	0.031 ± 0.009a	11.0 ± 1.5bc

municipal water; <sup>†</sup>municipal water with *Rhizobium*; <sup>‡</sup>primary treated wastewater; <sup>#</sup>primary treated wastewater with *Rhizobium*; <sup>§</sup>number of nodules seedling<sup>-1</sup>. Within columns, values with the same letters are not significantly different at a 0.05 level of probability (p=5; means ± SD).

In the second season, higher mean nodules seedling<sup>-1</sup> and nodule dry weight seedling<sup>-1</sup> were noted for *Acacia ampliceps* (15.4 nodules seedling<sup>-1</sup> and 0.044g nodule dry weight, respectively) than for *Acacia origina* (Table 6).

In the *Rhizobium*–legume symbiotic relationship, the plant is the more sensitive to metal toxicity compared with bacteria. The nodulation and nitrogen fixation of legumes has been suggested as an indicator of soil pollution, based on the sensitivity of the host legumes and *Rhizobium* strains to heavy metals and industrial wastes. In polluted environments, nodules can help plants survive, because the bacteroids in nodules counter the metal stress (thiol inactivation), and this finding further supports the fact that symbiosis is mutually beneficial to legumes and rhizobia (Abd-Alla et al., 2012). In both *Acacia* species, the MWR and PTWR water treatments affected on nodulation, they significantly increasing the number and dry weight of nodules. Similar results have been reported in other studies (Stamford and Silva 2000; Al-Fredan, 2006; Carvalho et al., 2012). Abd-Alla et al. (2012, 1999) indicated that moderate application rates of sewage sludge increased the nodulation and nitrogen fixation in some legume crops. Overall, the nodulation results detected in this study are in agreement with the previous results of many studies (e.g., Michalak, 2006; Sharma et al., 2007; Selahvarzi and Hosseini, 2012 and Abd-Alla et al., 2014).

## 2. Macronutrients accumulation in *Acacia* species

For both seasons, the concentration of N, P, and K in *Acacia* seedlings significantly ( $p < 0.05$ ) affected by species, irrigation application, and their interactions. Concentration of Nitrogen was insignificant in *Acacia* species (Table 7). In the first season, the mean value of N concentration was highest in the MW treatment (12.2mg seedling<sup>-1</sup>), while in the second season, the PTWR treatment resulted in the highest N concentration (12.01 mg seedling<sup>-1</sup>; Figure 1A). This result is in accordance with the finding of Abd-Alla et al. (1999), who found an increase in the N concentration in some legume plants. Also, phosphate (P) concentration gave higher value in seedlings of *Acacia ampliceps* treated with the MWR treatment (1.68 mg seedling<sup>-1</sup>; Figure 1B). Available P and N were highest in the PTWR and MWR treatments than that of the MW treatment, which is similar to the findings reported by Ali et al. (2011). The potassium (K<sup>+</sup>) concentration in the seedlings of *Acacia origina* showed an incensement at PTWR treatment with an average 127.6 mg seedling<sup>-1</sup>, in the first season and 206.8 mg seedling<sup>-1</sup>, in the second season as compared with *Acacia ampliceps* under the

same treatment. The maximum concentration of K<sup>+</sup> was 131.8 and 202.4 mg seedling<sup>-1</sup>, respectively, in the two seasons (Figure 1C). Generally, the K<sup>+</sup> concentration in the *Acacia* species was higher in the second season than that of the first season. The present results were consistent with other studies, which indicated that the concentration of N, P, and K<sup>+</sup> increased in plants when treated with wastewater (El-Sayed, 2005; Singh and Bhati, 2005 and Selahvarzi and Hosseini 2012). These increases in the mineral concentration might be attributed to increases in the occupancy root zone. In addition, Falkiner and Smith (1997) found that irrigating *Pinus* and *Eucalyptus* spp. with sewage effluent increased the macronutrient concentrations in the forest soil.

## 3. Micronutrients accumulation in *Acacia* specie

In this study, trace elements concentration in the seedlings tissues was varied between species and tissues. During the two growing seasons, the Cu, Fe, and Mn concentrations were differed between *Acacia* species. In the first season, Cu, Fe, and Mn were highest in *Acacia origina* seedlings, while in the second season, *Acacia ampliceps* had the highest Cu, Fe, and Mn values (Figure 2). In the first season, Cu concentration was highest in *Acacia origina* seedlings treated with MWR, followed by seedlings treated with PTWR. In the second season, the highest Cu concentration was noted in the PTWR treatment (6.4 µg seedling<sup>-1</sup>; Figure 2A). In the first and second seasons, Fe concentration was highest in *Acacia origina* seedlings treated with PTW (193.3 µg seedling<sup>-1</sup>) and PTWR (209.8 µg seedling<sup>-1</sup>), respectively (Figure 2B). For Mn, the highest concentration in the first season was found for *Acacia ampliceps* seedlings treated with PTWR (182.1 µg seedling<sup>-1</sup>) while, in the second season, *Acacia origina* had the highest concentration when treated with PTWR (181.2 µg seedling<sup>-1</sup>; Figure 2C). The wastewater may have contained large amounts of Cu, Fe, and Mn, which improved nodulation of the *Acacia* species. These results were in agreement with those obtained by Sharma et al. (2007) and Selahvarzi and Hosseini (2012). In contrast, Ike et al. (2007) found decreased in concentrations of Cu, Fe and Zn with long-term periods of soil irrigation. Accumulation of these metals in plant tissues can cause harmful effects on bacterial enzymes, and these metals cannot be chemically or biologically degraded (Abd-Alla et al., 2014). The results also demonstrated that inoculation *Acacia* species with *Rhizobium* combined with the application of the primary treated wastewater leads to significant improvements in seedlings growth parameters and macro and micro- nutrients contents.

**Table 7. Analysis of variance of macro and micronutrients accumulation for two *Acacia* species seedlings subjected to four irrigation treatments during two growing seasons**

Source of Variation	df	First season										Second season				
		N	P	K	Cu	Fe	Mn	N	P	K	Cu	Fe	Mn			
Replicate	4	1.68	0.05	755.6	0.61	397.4	234.1	2.7	0.24	650.6	0.23	521.4	175.6			
Species (Sp.)	1	5.45	1.44*	22132.3**	14.21**	21465.6**	19549.2*	2.98	1.04	18020.1**	9.10**	147.11046.3**	6343.5**			
Treatment (Trt.)	3	19.58**	1.36**	7367.6**	0.66*	8818.9**	8215.6 <sup>†</sup>	30.16**	1.84**	12410.4**	5.49**	8683.6**	12691.4**			
Sp. × Trt.	3	9.17**	0.99**	6889.4**	0.44	4791.4**	12557.7**	10.46**	0.61	9565.2**	4.04**	4930.5**	6188.1**			

\*Significant at the 0.05 probability level; \*\*Significant at the 0.01 probability level.

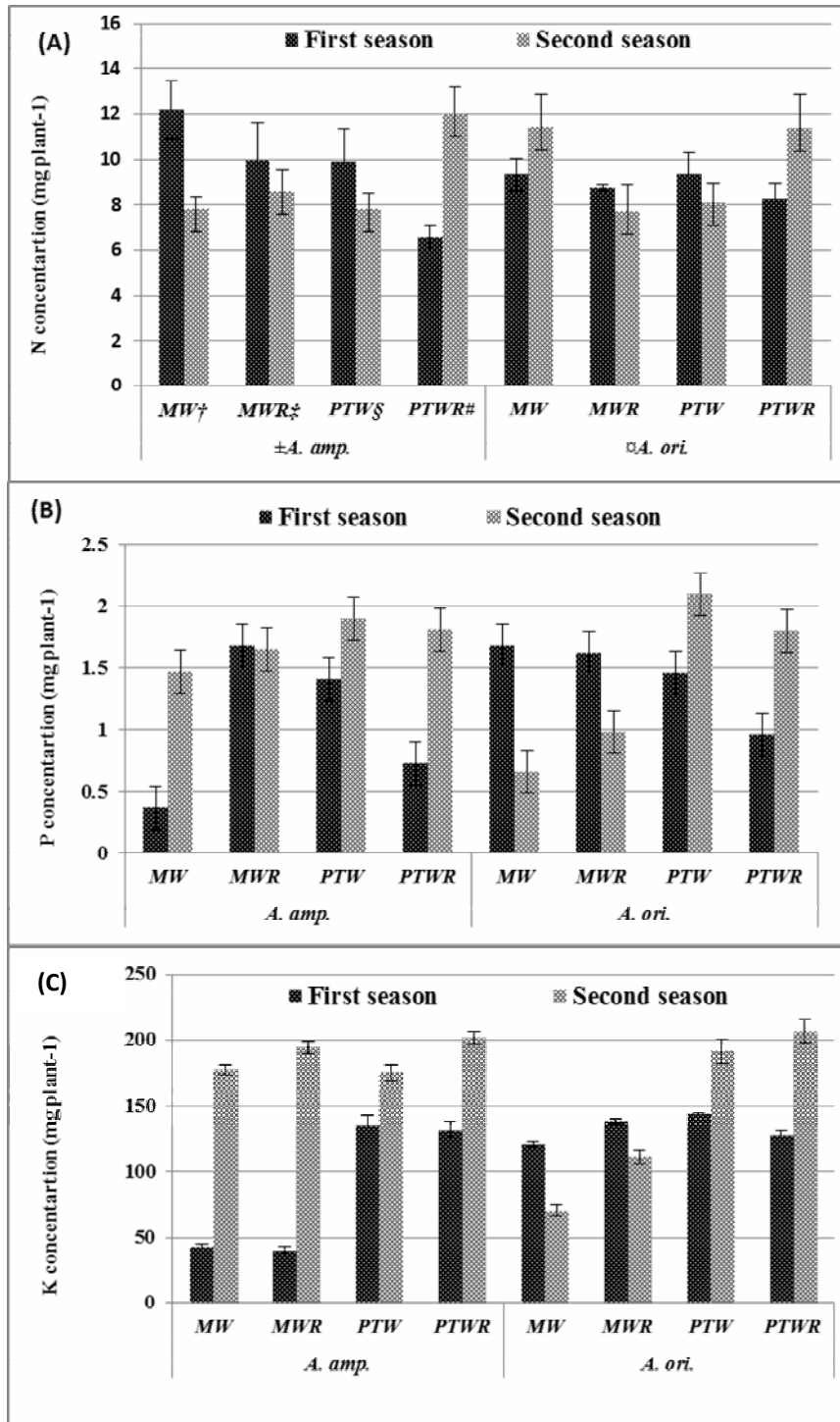


Figure 1(A, B and C). Effect of irrigation treatments on N (A), P (B), and K<sup>+</sup> (C) concentration in *Acacia* species during two seasons. <sup>†</sup>municipal water; <sup>‡</sup>municipal water with *Rhizobium*; <sup>§</sup>primary treated wastewater; <sup>#</sup>primary treated wastewater with *Rhizobium*; <sup>±</sup>*Acacia ampliceps*; <sup>□</sup>*Acacia origena* (n=5; means ± SD)



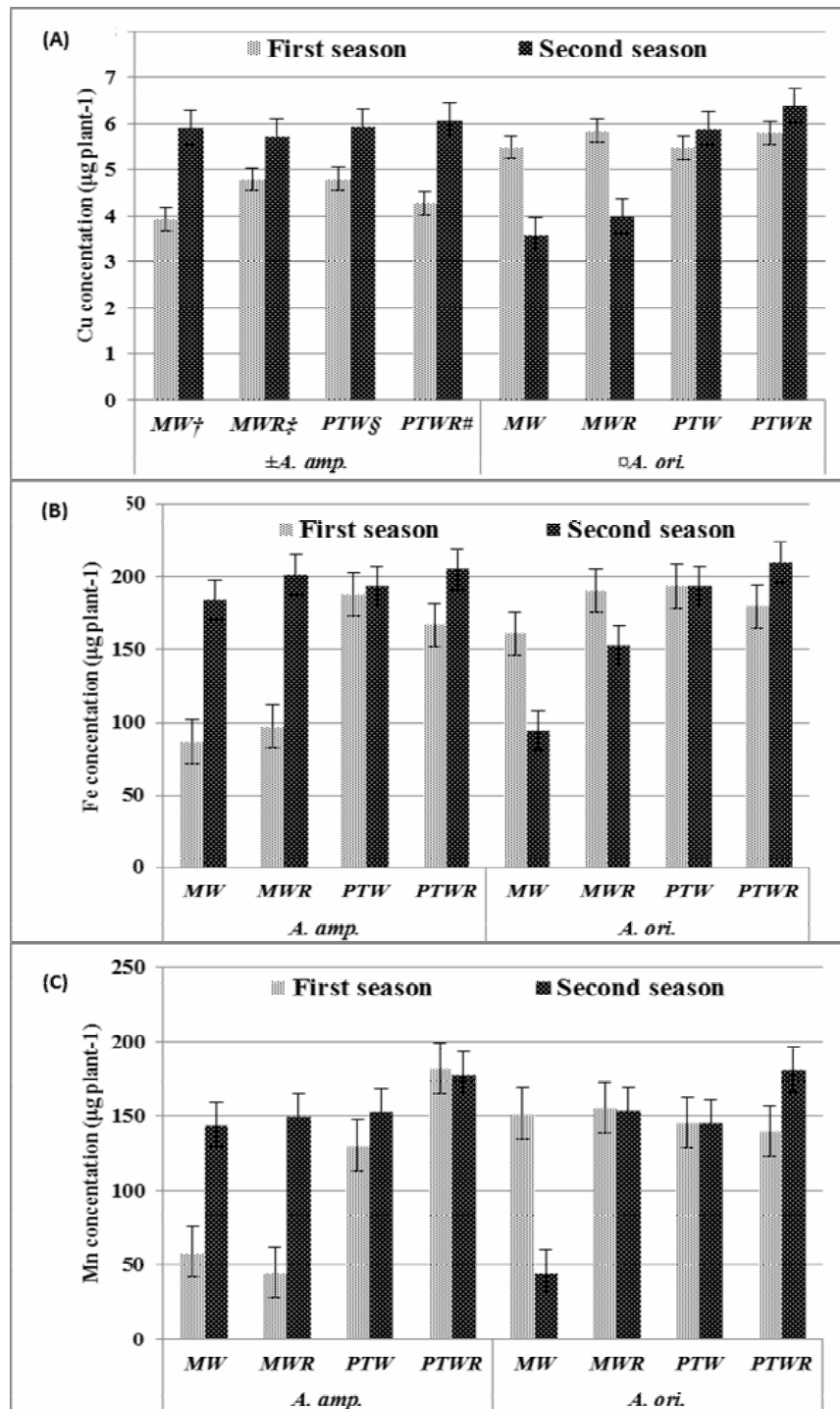


Figure 2 (A, B and C). Effect of irrigation treatments on Cu (A), Fe (B), and Mn (C) concentration in *Acacia* species during two seasons. <sup>†</sup>municipal water; <sup>‡</sup>municipal water with *Rhizobium*; <sup>§</sup>primary treated wastewater; <sup>#</sup>primary treated wastewater with *Rhizobium*; <sup>±</sup>*Acacia ampliceps*; <sup>□</sup>*Acacia origina* (n=5; means ± SD)

## CONCLUSION

Overall, primary treated wastewater was effective in increasing seedlings growth and can be used as an alternative resource in leguminous trees plantations. The results also demonstrated that, when combined with the application of the primary treated wastewater, inoculation with *Rhizobium* significantly improved the growth parameters, and macro and micronutrient content. In conclusion, this study showed that treated wastewater could be used as a potential source of nutrients and water for tree legumes. However, we suggest continued observations on the wastewater used in the agricultural purposes in order to ensure that the water is a suitable quality before it is readily used as an irrigation resource in arid or desert lands.

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*(Acacia origena)*

*(Acacia ampliceps)*

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