

# Responses of *Acacia gerrardii* and *Vachellia origena* Seedlings to Mineral Fertilization and Salinity Stress in Saudi Arabia

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## ABSTRACT

This study has investigated, over two seasons, salinity stress, and two mineral nitrogen fertilizer effects on *Acacia gerrardii* (Benth.) and *Vachellia origena* (Hunde.) growth. Photosynthesis and growth characteristics both responded positively to fertilization. *Vachellia origena* growth improved and become more tolerant to salinity stress by aid of fertilization. Calcium nitrate fertilization has induced the highest seedling height, diameter, and dry weight. The salt concentrations negatively affected growth, particularly 5000 ppm. Calcium nitrate or ammonium sulfate can improve the growth and the biomass of the tree seedlings and increase tolerance to high salinities either in nursery or in the field.

**Key Words:** *Acacia* and *Vachellia* species, Nitrogen fertilizers, Salinity stress, Saudi Arabia.

## INTRODUCTION

Fertilizers have different effects on agrochemical soil properties and most soils do not have enough nitrogen to ensure high yields. This means that the application of nitrogen fertilizers is an important area of research because nitrogen is one of the basic elements required by plants. However, ammonium sulfate can impair the agrochemical properties of soils, which become damaged if it is used over a long period<sup>1</sup>. Many studies have shown that following the application of nitrogen fertilizer to the soil, plants make better use of nitrogen in the soil. This finding suggests that applying nitrogen fertilizers will ensure a large supply of soil nitrogen to plants even when the distribution of the fertilizer in the soil is not uniform (Korenkov, 1976).

Salinity negatively affects all plant developmental stages and induces premature leaf senescence, which reduces plant yield (Flowers et al., 2010). Salinity occurs through natural or human-induced processes and these can lead to salt accumulating in soil water at concentrations that inhibit plant growth (Ghassemi, et al., 1995 and Munns, 2002). Furthermore, salt can move laterally into water, which also leads to increase salinity. Salts in the soil water can inhibit plants in two ways: the salt in the soil solution could reduce the ability of the plant to take up water, or excessive amounts of salt enter the plant in the transportation stream and cause injury to the cells found in transpiring

leaves. Both effects reduce plant growth rates (Greenway and Munnus, 1980). Salt-stressed plants face at least three major constraints: water deficit, ion toxicity, and ion imbalance. Reduced water availability due to the decrease in soil osmotic potential affects sensitive species that are unable to regulate their water potential with respect to the soil, which then results in the loss of cell turgor. Additionally, excess anion levels, especially Na<sup>+</sup> and Cl<sup>-</sup>, are extremely toxic to most crop plants, because they compromise plant metabolism and growth, negatively affect enzyme activity and membrane stability, and enhance reactive oxygen species production (Cabot et al., 2014). The most important strategies employed over recent years to reduce the effects of salt stress on legume production have focused on the selection of host genotypes that are tolerant to high salt conditions. However, accurate selection requires an understanding of the mechanisms that enhance the productivity of plants adversely affected by salt stress (Kenenil, et al., 2010 and Farissi et al., 2010).

The *Acacia* spp. are important vegetation types in Saudi Arabia, which has very little vegetation cover and large expanses of desert. In Saudi Arabia, the acacia communities represent the climax stage of xerophytic vegetation, which generally has a high cover rate, but low species diversity (Shaltout and Mady, 1996 and Aref, and El-Atta, 2010), 10. Fifteen indigenous *Acacia* species are widely distributed throughout Saudi Arabia in the arid and semiarid regions. Most of these provide wood for fuel and timber and are a good source of gum, tannins, and forage. Additionally, the acacia trees form a good habitat for honeybees, which produce high quality honey (Chaudhary, 1983 and Aref, et al., 2003).

*Acacia gerrardii* is found in Iraq, Jordan, Sinai Peninsula and the Arabian Peninsula. *Acacia gerrardii* was grown in many areas especially at South, Southwestern of Saudi Arabia and classified as one the most promising species cause of wide genetic variation within the species and its wide distribution are likely to be a good basis for selection for desirable traits especially in central region of Saudi Arabia. The trees are considered multipurpose trees, and are used for fuel

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Received July 24, 2016, Accepted September 18, 2016

production and reforestation (Aref, and El-Juhany, 2001).

*Vachellia origena* (Hunde.) (Synonyms; *Acacia origena*) is a medium sized tree (6 m tall) that is native to Ethiopia, west Eritrea, and across the Red Sea in Yemen and Saudi Arabia; it is considered an indigenous tree in southern Saudi Arabia. The most popular acacia species in Saudi Arabia include *Acacia ehrenbergiana* and *Vachellia origena* as (*Acacia origena* Hunde), *Acacia tortilis* and *Acacia gerrardii* (Benth.). Only a few acacia species are used for firewood and charcoal production, such as *A. ehrenbergiana*, *A. tortilis*, and *A. gerrardii*, whereas the others are regarded as good source of forage, pole timber, gums, and tannins (Nasser and Aref, 2014).

The weather in the Riyadh region is dry and very hot. It reaches about 50°C during May to October, but it is dry and cold in winter when the temperature may drop at night to freezing point. Under these conditions, evaporation from the land increases, and this leads to a rise in salinity, which reduces plant growth and may lead to death. There have been attempts over the last three decades to solve this problem in Saudi Arabia. These have included selecting species that are more tolerant to salinity, adopting new irrigation techniques and new fertilizer application regimes, and using new fertilizers or other nitrogen sources. *A. gerrardii* and *Vachellia origena* are considered the most popular local trees in Saudi Arabia, but to date, there have been no studies on their ability to endure salinity stress, particularly at the seedling stage. The aim of this study was to investigate the effect of two nitrogen fertilizers on the growth of two economically important trees: *A. gerrardii* and *Vachellia origena*, under salinity stress and under the prevailing climatic conditions in the Riyadh region.

## MATERIALS AND METHODS

### Site and the woody tree species used in the study

The experiment was conducted at the Forest Physiology Laboratory and in the shade house facilities at the Range and Forestry Applied Research Unit nursery, Food and Agricultural Sciences experimental station, Dirab Valley, South of Riyadh City, Saudi

Arabia (N.24° 24' 33", E.46° 39' 40"), over two growing seasons (2014 and 2015).

The physical and chemical characteristics of the soil used in the study are shown in Table (1).

### Tree growth and experimental design

*Acacia gerrardii* and *Vachellia origena* seeds were obtained from the Range and Forestry Applied Research Unit nursery and planted outdoor during the first weeks of October, 2013. Seeds were immersed in hot water (100 °C) for 15 min and in cool water for 24h to break seed coat dormancy, thereafter sown in plastic tray troughs containing a mixture of vermiculate and sand (1:1). After two weeks, seedlings were transplanted into (25 cm diameter) plastic pots filled with 10 kg of sandy loam soil. The seedlings remained in the pots until the third week of September 2014 and 2015. Each pot contained two seedlings and the average heights and diameters of the seedlings in each pot were measured. The nitrogen fertilizers used in this study were ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (4.9 g per each pot (N= 200kg/hectare) and calcium nitrate Ca (NO<sub>3</sub>)<sub>2</sub> (6.5 g per pot (N= 200kg/hectare). Sodium chloride (NaCl) was applied at concentrations of 1000, 2000, 3000, 4000, and 5000 ppm, and was prepared by adding 1, 2, 3, 4, and 5 g/L, respectively, to the irrigation water. Six months after planting, each pot received one of the fertilizer treatments two times per season and the pots were irrigated with one of the salt solutions twice a week until the end of the experiment. The control treatment was irrigated with tap water three times per week in the summer and twice every week in the winter. The pots were arranged in randomized complete block design with three replicates of each treatment out door. The following vegetative growth parameters were recorded: shoot height (cm), stem diameter (mm), branch number/plant, stem, branch, and root dry weights (g), and chlorophyll *a*, *b*, and *a+b* (µmol/L).

### Chlorophyll extraction

The chlorophyll was extracted from the leaves of the *Acacia* trees by *N, N*- dimethyl formamide (DMF) using the method described by Porra *et al.* (1989). Chlorophylls *a* and *b*, and Chlorophyll *a+b* contents were then calculated in µmol/L from the equations used by Porra *et al.*, (1989).

**Table 1. Physical and chemical characteristics of the soil of the Dirab Valley**

Particle size distribution (%)			Soil texture	pH	EC (m mhos/cm)	Soluble cations (meq/L)			Soluble anions (meq/L)		Mineral elements (ppm)		OM %
sand	silt	clay				Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>++</sup>	SO <sub>4</sub> <sup>-</sup>	CL <sup>-</sup>	N	P	
78.7	14.0	7.3	Sandy loam	8.2	1.01	1.04	0.52	0.25	7.2	2.2	29.2	7.7	1.0

### Statistical Analysis

The analytical work was completed at the mid of 2016. The growth parameters and chlorophyll content data were statistically analyzed using analysis of variance (ANOVA) at the  $p \leq 0.05$  level, as described by Gomez and Gomez (1984). Completely Randomized Block Design (RCBD) with three replications was used. The treatment means were compared using the least significant difference (L.S.D) test at  $p \leq 0.05$  (Snedecor and Cochran, 1980).

## RESULTS

### Effect of nitrogen fertilizers on tree growth characteristics under salt stress

There was no significant difference in the growth of the tree species between fertilizer treatments as the two species were grown under salt stress over the two seasons. There was also no significant difference between the fertilizers or in the interaction between seedling height and diameter. *Acacia gerrardii* had a higher average of seedling height (51.39 and 62.26 cm

in the first and second season, respectively) than *Vachellia origena* (49.43 and 61.87 cm, respectively). The averages of diameter and number of branches per plant were varied in *A. gerrardii* and *V. origena* over the two seasons (Figure 1), but these differences were not significant between each other. The data indicated that *V. origena* had significantly higher foliage and plant dry weights than *A. gerrardii* in both seasons, but there were no significant differences between stem and root dry weights (Figure 2). The results suggested that *V. origena* dry weight was more sensitive positively to the two nitrogen fertilizer treatments, whereas the fertilizer treatments produced a greater increase in *A. gerrardii* height than *V. origena* under field conditions.

Statistically, no differences were found in chlorophyll content between the tree species within each season. However, the mean values of chlorophyll content in *V. origena* contained more chlorophyll *a*, *b*, and *a+b* than *A. gerrardii* in the two seasons (Figure 3).

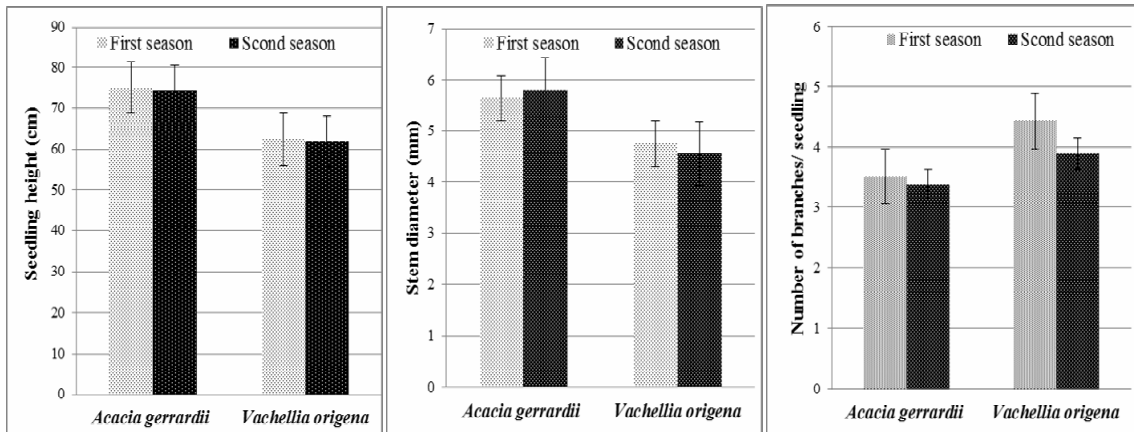


Figure 1. The mean effects of tree species on the heights, diameters and number of branches over the two seasons (n=36 mean  $\pm$ SD)

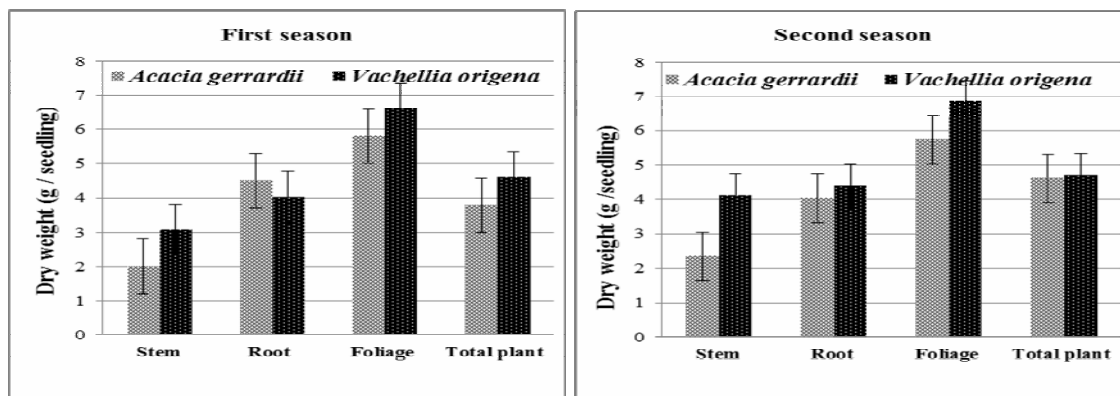
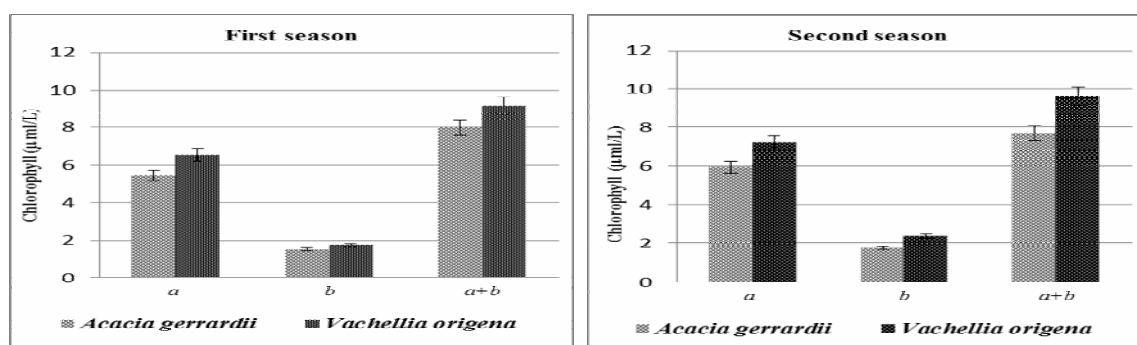


Figure 2. The mean effects of tree species on dry weight (g) of stem, root, foliage and total plant over the two seasons (n=36 mean  $\pm$ SD)



**Figure 3. The mean effect of tree species on chlorophyll content over the two seasons (n=36 mean ±SD)**

The effect of nitrogen fertilizer type is shown in Table (2). The results indicated that none of growth characteristics are statistically significant except for  $(\text{NH}_4)_2\text{SO}_4$  foliage dry weight in the second season. The mean values for the growth characteristics varied between the two fertilizer types. The calcium nitrate  $\text{Ca}(\text{NO}_3)_2$  increased the seedling height and diameter values more than ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4$  in the first season, whereas in the second season, the  $\text{Ca}(\text{NO}_3)_2$  averages were lower than  $(\text{NH}_4)_2\text{SO}_4$  in its impact. The ammonium sulfate fertilizer increased seedlings dry weight in the second season than that the calcium nitrate did (Table 2).

Chlorophyll content showed similar trends. The two fertilizer treatments had significantly different effects on chlorophyll content. The  $\text{Ca}(\text{NO}_3)_2$  fertilizer treatment led to increase chl *a*, chl *b*, and chl *a+b* contents in both *Acacia* and *Vachellia* species over the both growth seasons than that induced by  $(\text{NH}_4)_2\text{SO}_4$ , except for chl *a+b* in the second season (Table 3).

**Effect of salinity concentration on the growth characteristics of the tree species**

The effects of the salt concentration treatments applied to the seedlings under the two nitrogen

fertilization regimes are shown in Table (4). The results indicated that the salt treatments had significantly different effects on seedling height and diameter, and the number of branches per plant over the two seasons. The 5000-ppm salt treatment had the highest negative effect on seedling height, stem diameter and the number of branches per plant in both seasons. The reductions in height for the first and second seasons was accounted for 53.6% and 31.8%, respectively, while the reduction in seedling diameter was 43.6% and 36.3%, respectively. The same trend was found for the number of branches, where the reduction in the number of branches was 60% and 42.1% for the two growth seasons, respectively. The effects of the other salt concentrations on seedling height and diameter, and number of branches per plant varied and showed no specific trend (Table 4).

Significant differences were found between the salt concentration treatments for stem, root, and foliage dry weights, but there were no significant differences found for plant dry weights and the interaction between tree species and salt concentration in the first season, while no significant differences between stem, root and foliage dry weights in the second season were noticed.

**Table 2. The mean effects of fertilizer type on growth characteristics over the two seasons**

Growth characteristic	Fertilizer Type			
	First season		Second season	
	$(\text{NH}_4)_2\text{SO}_4$	$\text{Ca}(\text{NO}_3)_2$	$(\text{NH}_4)_2\text{SO}_4$	$\text{Ca}(\text{NO}_3)_2$
Height (cm)	60.24a	72.63a	61.08a	72.05a
Stem diameter (mm)	5.55a	5.95a	6.18a	6.24a
Stem dry weight (g)	2.81a	3.23a	4.28a	4.19a
Root dry weight (g)	4.56a	4.87a	5.11a	5.31a
Foliage dry weight (g)	4.13a	4.26a	11.57b	13.03a
Total Plant dry weight (g)	9.50a	10.08a	18.96a	20.53a
Number of branches	7.66a	7.60a	4.36a	5.94a

Lowercase letters show significant differences between species (in row within the same season); values with the same letter are statistically similar and those with different letters are significantly different at  $P > 0.05$ . Each value is an average of 36 seedlings.  $(\text{NH}_4)_2\text{SO}_4$ : Ammonium sulfate;  $\text{Ca}(\text{NO}_3)_2$ : Calcium nitrate.

**Table 3. The mean effect of fertilizer type on chlorophyll content over the two seasons**

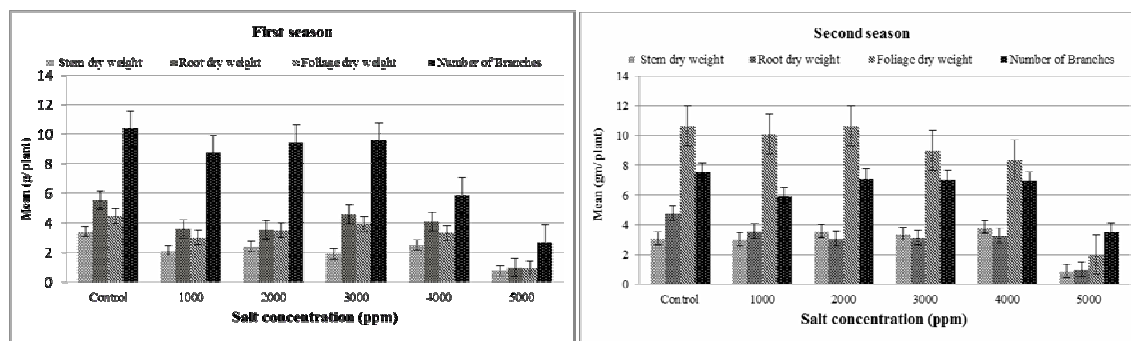
Chlorophyll content ( $\mu\text{mol/L}$ )	Fertilizer			
	First Season		Second Season	
	$(\text{NH}_4)_2\text{SO}_4$	$\text{Ca}(\text{NO}_3)_2$	$(\text{NH}_4)_2\text{SO}_4$	$\text{Ca}(\text{NO}_3)_2$
Chlorophyll <i>a</i>	4.10b	6.7a	6.51b	6.67a
Chlorophyll <i>b</i>	3.20b	3.5a	3.2a	2.45 b
Chlorophyll <i>a+b</i>	7.19a	8.21a	8.70a	8.12b

Lowercase letters show significant differences between species (in row within the same season); values with the same letter are statistically similar and those with different letters are significantly different at  $P > 0.05$ . Each value is an average of 36 seedlings.  $(\text{NH}_4)_2\text{SO}_4$ : Ammonium sulfate;  $\text{Ca}(\text{NO}_3)_2$ : Calcium nitrate.

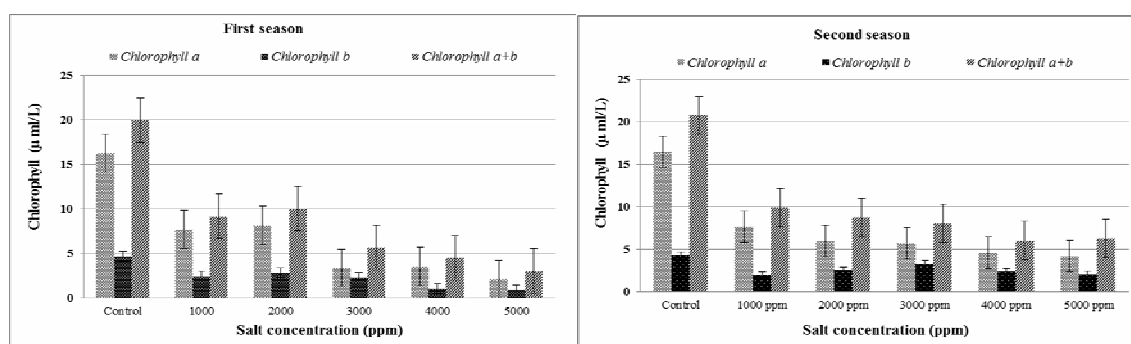
The 5000-ppm salt treatment had the greatest effect on the plant dry weights of the tree species in both seasons, and the reduction in the stem, root, foliage, and total plant dry weights for the first season was 68%, 82.1%, 79%, and 78.3%, respectively. The data trend for the second season was similar to that obtained in the first one, but the reduction in such parameters was less than those in the first season (Figure 4).

The chlorophyll content was significantly related to the salt treatments over the two seasons. The data showed an opposite relationship between the salt

concentration and chlorophyll content (Figure 5). The chlorophyll content was declined as the salt concentration increased in the two seasons. The reduction in the chlorophyll content for the 5000-ppm salt treatment in the first season was 87.2%, 80.7%, and 85% for chlorophyll *a*, *b*, and *a+b*; respectively (Figure 5). The trend in the second season was similar to that obtained in the first one, and the reductions in the chlorophyll *a*, *b*, and *a+b*, contents were 74.3%, 51.5% and 69.6%, respectively (Figure 5).



**Figure 4. The mean effects of salt concentration on stem, root and foliage dry weight and number of branches over the two seasons (n=12 mean  $\pm$ SD)**



**Figure 5. The mean effects of salt concentration on chlorophyll content over the two seasons (n=12 mean  $\pm$ SD)**

**Table 4. The mean effects of salt concentrations on seedlings growth over the two seasons**

Growth characteristic	Salt concentration (ppm)											
	First season					Second season						
	control	1000	2000	3000	4000	5000	control	1000	2000	3000	4000	5000
Height (cm)	66.7a	50.8b	57.7ab	60.1ab	55.2b	30.9c	69.8a	55.4b	63.5ab	63.4ab	62.8ab	47.6b
Stem diameter (mm)	6.5a	5.5ab	5.1ab	5.2ab	4.75b	3.66c	5.9a	5.6ab	5.5ab	5.7a	5.4a	3.76b
Number of Branches	7.5a	4.7b	5.1b	4.86b	4.98b	3.0c	7.6a	5.9b	6.3ab	6.4ab	6.9ab	4.4c

Lowercase letters show significant differences between species (in row within the same season), values with the same letter are statistically similar and those with different letters are significantly different at  $P > 0.05$ . Each value is an average of 12 seedlings.  $(\text{NH}_4)_2\text{SO}_4$ : Ammonium sulfate; Ca  $(\text{NO}_3)_2$ : Calcium nitrate.

## DISCUSSIONS

Plants require large amounts of nitrogen as a mineral element, which means that nitrogen deficiency rapidly inhibits plant growth. NPK treatments applied to soils have been shown to significantly increase leaf amino acid content, mineral composition, and total carbohydrates (Hu, and Schmidhalter, 2005 and Osman, 2010). Results of the present study demonstrated that the growth and photosynthesis characteristics of the two tree species (*A. gerrardii* and *V. origena*), and these results agreed with those reported by Bargali and Singh (1993) and El-Kohen and Mousseau (1994). Nitrogen fertilizer applications are particularly important because nitrogen is one of the basic elements required by plants and most soils do not have enough nitrogen to ensure high yields. Fertilizers have various effects on different agrochemical soil properties, but they generally improve the growth of tree seedlings (Berger and Glatzel, 2001; Hu and Schmidhalter, 2005; Gbadamosi, 2006; Oskarsson et al., 2006; Oskarsson, and Brynleyfsdottir, 2009; and Dianda, et al., 2009). Both fertilizer types used in this study, to somewhat, had the same effect on tree seedling growth and photosynthesis, but the calcium nitrate improved the growth characteristics and photosynthesis of the two tree species in the field more than ammonium sulfate. The results were consistent with the findings of Oliet et al., (2004); Oskarsson and Brynleyfsdottir, (2006); Osman et al. (2010); and Aref and Shetta (2013).

The chlorophyll content was also affected by the nitrogen fertilizer applications. It was higher in seedlings treated with calcium nitrate under salt stress than in seedlings treated with ammonium sulfate, and these results were in agreement with the findings reported by Chartzoulakis, (2005) and Tabatabaei, (2006). In contrast, the results disagreed with Kao et al., (2001) and Gebauer et al., (2004). Furthermore, increased salinity significantly reduced chlorophyll *a*; *b*, and *a+b* contents. This was consistent with the findings reported by Abdul Qados (2011); Aref and Shetta, (2013) and Latrach et al., (2014).

Wang and Nil, (2000); Chartzoulakis and Klapaki, (2000) and Parida and Das (2005) reported that the immediate response to increasing salt stress is confined in a reduction in the rate of leaf surface expansion, which ultimately ceases shoot expansion. Salt stress also results in considerable decreases in leaf, stem, and root fresh and dry weights. In this study, seedling growth was more affected by increased salinity than root and stem dry weights. Previous studies have reported that salinity reduces shoot and root weights in several legumes (Alshammarya, et al., 2004 and Ltaief,

et al., 2007). The results of this study show that salt stress caused a significant reduction in seedling height, and stem and root dry weights in both tree species, and it seemed to reduce the availability of the nutrients required for the growth and development of the trees. Growth limitation at high salinity may be due to a reduction in the energy needed for growth. Cordovilla et al., (1995); Tejera et al., (2004); Bouhmouch et al. (2005) and Imada et al., (2009). reported that under salt stress, chlorine ions limit the absorption of  $\text{NO}_3^-$ . An antagonistic effect between  $\text{Cl}^-/\text{NO}_3^-$  is better known in glycophytes than halophytes, because the latter are able to efficiently absorb  $\text{NO}_3^-$  even under highly saline conditions. Salinity, expressed as excessive a mixture of NaCl and  $\text{CaCl}_2$ , has been shown to reduce *Kentucky bluegrass* growth by 50% at a salt concentration of 7500 ppm (about 11 dS/m) (Horst and Taylor, 1983), which is considered as higher than the value obtained in this study. Qian et al. (2001), also reported that a 2 dS/m difference in salinity caused 50% shoot growth reduction in two *Kentucky bluegrass* cultivars. It has also been found that under salt stress, increasing the N concentration to 200 mg/L in salt-sensitive cultivars may have a beneficial effect, whereas in salt-tolerant cultivars, increasing N fertilization can restore the decreased growth caused by high salinity (Chartzoulakis, 2005 and Tabatabaei, 2006).

## CONCLUSION

The results show that, *Vachellia origena* was the most responsive to nitrogen fertilizer and was more salt tolerant than *Acacia gerrardii* under the field conditions around Riyadh. The results also indicated that, to somewhat, there were no differences between the impact of the two fertilizer types on growth of the two tree species. The calcium nitrate fertilizer produced higher mean values for seedling height, stem diameter, and plant dry weight than those induced by ammonium sulfate fertilizer. Therefore, this study suggests using calcium nitrate to obtain good quality seedlings in the nursery. The results indicated that a salt concentration of 5000 ppm had the harmful effect on the seedling growth of both tree species. The seedlings were not tolerant to 5000 ppm NaCl, but the tolerance to the other salt concentrations were varied and had different effects on the growth characteristics of the tree species. Therefore, this study suggests using calcium nitrate ( $\text{CaNO}_3$ ) as the nitrogen source as it can improve the growth and biomass of the *A. gerrardii* and *V. origena* seedlings in the nursery by improving seedling tolerance to salt stress in the fields and soil under environmental conditions prevailing Riyadh region.

## ACKNOWLEDGEMENTS

The authors thank the College of Food and Agriculture Sciences and the Research Center and the Deanship of Scientific Research, King Saud University, Saudi Arabia for supporting this work.

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(*Vachellia origena*)

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