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Published version:

DOI:10.1016/j.agwat.2016.11.026

(Article begins on next page)

Effects of zeolite and water stress on growth, yield and chemical compositions of *Aloe vera* L.

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Abstract
A.vera is one of the most economically important medicinal plants in many countries which is widely used in food, cosmetics and pharmaceutical industries. Water stress is the primary environmental factor that limits crop production. Therefore, in order to study the effects of water stress (20, 40, 60 and 80% of the filed capacity (FC) and zeolite (0, 4 and 8 g kg^{-1} soil) on growth, yield and chemical compositions of *A. vera* an experiment was conducted in 2013 and 2014. The plants were harvested 90, 180 and 270 days after imposing the treatments. The greatest number of new leaves and pup were produced by the plants irrigated 20 and 40% FC with 8 g zeolite, respectively. Generally, the highest leaf fresh weight and gel fresh weight were observed 270 days after imposing the treatments when plants were irrigated after depleting 40% of the FC and treated with 8 g zeolite. Water use efficiency of *A. vera* increased with less water and more zeolite availability. In addition, the results indicated that the maximum aloin and proline accumulation were obtained 90 days after imposing the treatments when the plants were irrigated after depleting 80% and 60% of the FC where no zeolite was applied, respectively. Irrigation after 80% depletion of the FC without zeolite application resulted in highest fructose and glucose content. In general, zeolite application could alleviate water stress adverse effects, and improved plant growth and yield. Severe water stress decreased leaf yield and plant growth while caused an increase in phytochemical and biochemical compounds.

Keywords: *Aloe vera*, Aloin, Growth, Water deficit, Yield

1. Introduction

Aloe vera L. (syn. *Aloe barbadensis* Miller) is a fleshy perennial plant originated from Africa which is cultivated widely in warm and dry regions of the world. The plant belongs to Xanthorrhoeaceae family and includes more than 548 species (Cousins and Witkowski, 2012; Ray and Gupta, 2013) used in pharmaceutical and food industries. Extracted gel and sap are also used commercially in cosmetics and alternative medicine industries (Sahu et al., 2013; Murillo-Amador et al., 2014; Radha and Laxmipriya, 2015).
Water is one of the most limiting factors in crop production worldwide (Sankar et al., 2007; Al-Busaidi et al., 2011). It is also one of the main factors affecting plant growth and development as well as morphological and physiological adaptation to environmental conditions. It has been reported that crop yield, especially in arid and semi-arid regions, strongly correlates water availability of and seasonal changes (Cousins and Witkowski, 2012). Although *A. vera* is a drought tolerant species, its water requirement depends on soil water holding capacity (Delatorre-herrera et al., 2010; Cousins and Witkowski, 2012). It has been stated that *A. vera* growth and yield would decrease with reducing soil moisture content (Rodríguez-García et al., 2007). According to the previous findings, water stress limits *A. vera* growth and production, however due to crassulacean acid metabolism (CAM) this plants shows a high water use efficiency (Nobel and Zutta, 2007; Delatorre-herrera et al., 2010). Higher water use efficiency in CAM plants as an important eco-physiological index, assists this plant to use available water more efficiently in arid regions. The water-use efficiency of CAM plants can be 5-fold higher than that of C3 plants and 3-fold higher than for C4 plants (Lüttge, 2004; Geerts and Raes, 2009). The higher water use efficiency in CAM plants is due to the fact that the stomata can open at night for CO_2 fixation (Lüttge, 2004; Winter et al., 2005). Another factor which increases drought tolerance in these plants is stomata density and osmotic biosynthesis (Delatorre-herrera et al., 2010). Considering the fact that the major components in *A. vera* are made of polysaccharides and aloin (Hamman, 2008; Ray et al., 2013a), gel production and phytochemicals are highly affected by environmental factors and growth stages (Lucini et al., 2013; Ray et al., 2013a). Several studies have reported that water deficit stress increases total sugar content, soluble sugars, proline accumulation and secondary metabolites in *A. vera* (Joyce et al., 1992; Moreira and Filho, 2008; Delatorre-herrera et al., 2010). Increased synthesis of these compounds, increases water use efficiency in *A. vera* grown under water deficit stress conditions (Delatorre-herrera et al., 2010). In addition, increased aloin content has been reported in many studies in which *A. vera* plants were

subjected to salinity (Rahimi-Dehgolan et al., 2012), high light densities (Lucini et al., 2013) and sodium (Rahi et al., 2013).

Several studies have been conducted focusing on *A.vera* adaptation to water deficit stress; however not enough information is available about water stress reducing factors. Application of soil amendments in order to improve soil physical properties is one of the most important approaches to overcome with water deficit stress (Polat et al., 2004). Zeolites are microporous, aluminosilicate minerals commonly used as commercial adsorbents and catalysts. This mineral is found almost in all regions of Iran (Sepaskhah and Barzegar, 2010). The most famous and abundant type of zeolites called clinoptilolite which has been discovered in 1890. Zeolite application into the soil leads to increase water retention capacity. In addition zeolite acts as a chemical sieve allowing some ions to pass through while blocking others (Gholamhoseini et al., 2012). Zeolite application in sandy soils subjected to drought stress can improve final yield via increase of soil water capacity. Absorption and controlled release of moisture by zeolite improves plant growth and yield under drought conditions (Sepaskhah and Barzegar, 2010; Al-Busaidi et al., 2011). Reduced adverse effect of water deficit stress by zeolite has been reported by Gholizadeh et al. (2010) and Najafinezhad et al. (2014). Considering the importance of *A. vera* plants in industry and since limited information is available about the response of *A. vera* under water deficiency with application zeolitethe current experiment was carried out and aimed to study the effects of water stress with zeolite application on growth, yield and chemical compositions of *A. vera*.

2. Material and methods

2.1. Experimental design, treatments and growth conditions

A factorial in time experiment was carried out in a randomized complete block design with four replications. Sampling time was considered as sub factor. The factorial combination of three zeolite rates (0, 4, and 8 g kg^{-1} soil) and four irrigation regimes (irrigation after depleting 20, 40, 60 and 80% of the filed capacity) were considered as main factors. The plants were grown in a research greenhouse of Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran in 2013 and 2014. The 18-20 cm pups (small plants growing from the sides of the mother plant) were planted in plastic pots and placed in greenhouse for two months, irrigated equally. After this time, pups were transplanted into new pots filled with 18 kg similar soil but amended with the abovementioned amounts of zeolite and then water deficit treatments were imposed. The irrigation began in June 2013 which the total number of irrigation during the experimental period were: FC 20 (%)+Zeolite 0 (74), FC 20 (%)+Zeolite 4 (74), FC 20 (%)+Zeolite 8 (71); FC 40 (%)+Zeolite 0 (56), FC 40 (%)+Zeolite 4 (56), FC 40 (%)+Zeolite 8 (54); FC 60 (%)+Zeolite 0 (33), FC 60 (%)+Zeolite 4 (33), FC 60 (%)+Zeolite 8 (31); FC 80 (%)+Zeolite 0 (13), FC 80 (%)+Zeolite 4 (13); FC 80 (%)+Zeolite 8 (12). The total volume of water in each irrigation event were 140, 105, 75 and 40 L plant^{-1} for 20, 40, 60 and 80 of FC, respectively. The physical and chemical soil and zeolite properties are shown in Tables 1 and 2. The first, second and third samplings were performed on 22nd September 2013, 20th January and 21st March 2014, respectively, at 90, 180 and 270 days after imposing the treatments. Four plants from each treatment and replicate were selected and leaf and pup number were registered. Harvested plants were transferred to laboratory and after removing upper and lower parenchyma; gel fresh weight was recorded.

2.2. Soil moisture content

Soil moisture content was monitored daily using TDR (Time Domain Reflectometer) device (TRIM-FM 10776, Germany). A 20 cm three pointed rods probe was used to measure volumetric moisture. The data were confirmed using gravimetric moisture. The following equation was used for this purpose.

$$\Theta_v \,\% = \Theta_G \times Ps/Pw \quad (1)$$

Where Θ_G is the gravimetric water content; Θ_V is the volumetric soil moisture; Pw is the water density; Ps is the density of soil.

The water was replaced in the pot each time the soil water content indicated a water loss of 10%. After water application, In order to reduce evaporation, soil surface pots were covered by aluminum foil and drained water was collected and measured. The percolated water was collected and measured.

Soil moisture at filed capacity point and wilting point were determined (Table 1). Furthermore, pressure plate apparatus was used to determined soil pF and then soil moisture curve was plotted.

2.3. Water use efficiency

The water use efficiency (WUE) was calculated 270 days after imposing the treatments as the quotient of leaf biomass production or gel production and the total water applied per treatment, expressed in grams per liter (Silva et al., 2010) according to the following formula.

$$WUE = \text{yield (g)} / \text{Total consumed water (L)} \quad (2)$$

WUE was also calculated based on fresh weight.

2.4. Proline accumulation

Proline content was determined according to Bates et al. (1973) method. Samples were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3 % w/v), and then centrifuged at 18,000 g for 15 min. Two millilitres of the supernatant was then added to a test tube, to which 2 ml glacial acetic acid and 2 ml freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml glacial acetic acid and 20 ml 6 M orthophosphoric acid) were added. The test tubes were incubated in a water bath for 1 h at 100° C and then cooled at room temperature. Four millilitres of toluene was then added to the tubes and then mixed on a vortex mixer for 20 s. The test tubes were allowed to stand for at least 10 min, to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was calculated from a standard curve, and was expressed as mmol g^{-1} fresh weight.

2.5. Soluble sugars content

Content of sugars (sucrose, glucose, fructose, maltose and xylose) was determined according to Sturm et al. (2003) method with some minor modification using high performance liquid chromatography (HPLC) (Agilent Technologies 1200 series) equipped with refractive index detector RID and Zorbax Carbohydrate 5 Micron column (4.6 × 250 mm). Test conditions: mobile phase mixture of acetonitrile with deionized water in ratio 65: 35 (v/v); flow rate 0.8 ml min^{-1}; temperature of column and detector 30̊ C. Dried gel samples were prepared by adding 2 ml of deionized water and mixing for 1 min. Samples were centrifuged at 6000 rpm for 18 min., and supernatant was isolated, and finally filtered through membrane filter (0.45 µm). Each sample (20 µl) was injected to HPLC and analyzed in triplicate. Chromatograph software calculated content of sucrose, glucose, and fructose, maltose and xylose comparing chromatograms of gel samples with standard curves of respective sugar.

2.6. Aloin content

Aloin content was measured according to Waller et al. (2004) method. After cutting the leaf, yellow syrup that leaked from wound sites was collected carefully and stored in liquid nitrogen until being analysed. Samples were then freeze-dried (Labogene ScanVac Cool Safe Freeze Dryer System (CS55-4, Lynge, Denmark)) for 24 h. Aloin was determined using high-performance liquid chromatography (Waters, USA; 4.6 × 250 mm, dp 10 µm column, µBondapack C18). Aloin standard was purchased from Sigma-Aldrich, USA. Stock solution was prepared by dissolving aloin into water methanol solvent (1:1 v: v) and used to make standard solutions. Aloin concentration was calculated by using external standard and aloin standard curves.

2.7. Statistical analysis

Main and interaction effects of experimental factors were determined from analysis of variance (ANOVA) using the general linear model (GLM) procedure in Statistical Analysis System (SAS) software Institute Inc. (2002). The PROC UNIVARIATE within SAS was used to test the assumptions of ANOVA, and residuals were normally distributed. Least significant difference (LSD) test at the 0.05 probability level was used to check significant differences between means.

3. Results

3.1. Growth and yield

3.1.1. Leaf and pup number

As Table 3 shows, water deficit stress and zeolite had significant effect on leaf and pup number during different growth stages. The maximum and minimum leaf number was observed when plants were irrigated after depleting 20% FC with 8 g zeolite and 80% of the filed capacity and no zeolite was applied, respectively. In non stressed plants, leaf number increased on account of 8 g zeolite. Moreover, zeolite application increased leaf number compared with the control treatment. When plants were irrigated after depleting 20% of the filed capacity, leaf number increased to 1.88, 1.5 and 1.56 leaves per month from each plant during first, second and third sampling, respectively. The maximum leaf number was observed 270 days after imposing the treatments when plants were irrigated after depleting 20% of the filed capacity and 8 g zeolite was applied (24.25 leaves by plant). The results demonstrated that pup number was affected by water deficit stress and zeolite application. In all stages, the maximum pup number was observed in plants which were irrigated after depleting 40% of the filed capacity and treated with 8 g zeolite (Table 3). Furthermore, different stages showed a significant effect on pup number. The maximum pup number was observed 90 days after imposing the treatments (6.75 pups by plant) (Table 3).

3.1.2. Leaf and gel fresh weight

Leaf and gel fresh weight were affected by water deficit stress and zeolite application. Leaf and gel fresh weight increased with increasing water availability and zeolite application (Table 3). The maximum value was observed 90 and 180 days in plots where plants were irrigated after depleting 20% of the filed capacity and 8 g zeolite was applied. Whereas, 270 days after imposing the treatments, the highest leaf and gel fresh weight was obtained when plants were irrigated after depleting 40% of the filed capacity and 8 g zeolite was applied, that increased by 55 and 61% compared with irrigation after depleting 80% of the field capacity leaf and gel fresh weight, respectively. In addition, under mild and severe stress conditions, zeolite application could increase leaf and gel fresh weight compared with control treatment (Table 3).

3.2. Water use efficiency

According to Fig. 1 and 2, water use efficiency affected by water deficit stress and zeolite application. The highest water use efficiency leaf and gel were obtained from plants which were irrigated after depleting 60% of the field capacity and treated with 8 g zeolite (20.81 and 13.13 g L^{-1}), respectively. Conversely, the lowest value was recorded from plants which were irrigated after depleting 20% of the filed capacity without zeolite. Generally, water use efficiency increased with increasing water deficit stress intensity and zeolite applied. In other words, water use efficiency in all stress treatments was more than control treatment. In this study, zeolite increased water use efficiency so that the highest value was obtained when 8 g zeolite was applied.

3.3. Chemical analyses

3.3.1. *Soluble sugars*

Following the injection of standard solutions, glucose and fructose were the only detected sugars in dried gel. It has been reported that glucose and fructose are the main sugars in *Aloe* species (Paez et al., 2000; Christopher and Holtum, 1996). As can be seen from Table 4, there was more fructose than glucose in samples. Results were indicating that the amount of fructose was much higher in *A. vera* plants compared to glucose. Also results indicated that increasing water stress severity positively correlates with the accumulated sugars (glucose and fructose) in *A. vera* plants. The highest fructose and glucose content was obtained when plants were irrigated after depleting 80% of the filed capacity and no zeolite was applied at 270 days after imposing the treatments, respectively (249.87 and 87.30 mg g DW^{-1}). Zeolite application prevented the increase of soluble sugars under water deficit stress conditions. Application of zeolite increased moisture retention in the soil with improving the water supply, and that makes the plant more water available for metabolic processes and probably does not require osmotic adjustments, which lowers the concentration of sugars.

3.3.2. *Proline*

Proline accumulation increased with increasing water stress intensity (Table 4). In all stages, the highest proline content was obtained when plants were irrigated after depleting 60% of the filed capacity and no zeolite was applied. The highest and lowest proline accumulation was observed at 270 and 180 days after imposing the treatments, respectively.

3.3.3. *Aloin content*

Water deficit stress and zeolite application during different growth stages had significant effect on aloin content of *A. vera*. Aloin content increased with increasing water stress intensity. The highest aloin content was found 90 days after imposing the treatments in plants which were irrigated after depleting 80% of the field capacity and no zeolite was applied (234.8 mg g DW^{-1}). Conversely, the lowest aloin content was observed 180 days after imposing the treatments in plants which were irrigated after depleting 20% of the field capacity and 4 g zeolite was applied. There was a negative correlation between yield and aloin content. Zeolite application diminished water deficit stress effects and aloin content (Fig. 3).

4. Discussion

Leaf number is one of the most important factors affecting *A. vera* growth and yield. As it was found water availability affect the number of leaves produced. Similar results have been reported by Silva et al. (2010) who studied the effect of evapotranspiration on *A. vera* growth. In the current study, the increasing leaf number was observed 90 days after imposing the treatments in summer. Similar to previous findings (Hernandez et al., 2002) the leaf growth rate was registered as 1.8 leaves per month. To maintain production over time, the number of leaves harvested should be equivalent to the number of new produced during the year and for the conditions of the experimental site (Silva et al., 2010). The main propagation method for *A. vera* is the vegetative pups (Hazrati-Yadekori and Tahmasebi-Sarvestani, 2012). It has been confirmed that environmental factors, such as soil moisture, temperature and light intensity affect pups production. For example, it has reported that pup number decreased with decreasing soil water content (Silva et al., 2010). Comparing harvests dates, the highest rate of new pup production occurred in summer, which were the months with highest light intensity in this period. Increased pup number on account of light has been reported by Paez et al. (2000**).** In addition, regardless of dates, zeolite increased pup production**.** The results showed that leaf and gel yield affected by severe water stress (Table 3). This result is in agreement with findings of other researchers (Hernandez et al., 2002; Rodríguez-García et al., 2007). The yield loss in third harvest was more than the first and second harvest (Table 3). Similar results have been reported by Delatorre-herrera et al. (2010) who studied the effect of soil moisture content on *A. vera* leaf yield. *A. vera* keeps its stomata open under water deficit stress conditions and continues CO_2 fixation (Lüttge, 2004; Cousins and Witkowski, 2012). Our results showed that zeolite application could decrease water deficit stress adverse effects. This is because of its properties in holding water and nutrients (Gholamhoseini et al., 2013). The results of the current study revealed that water use efficiency is high in *A. vera* plants. Thus our results demonstrate that 80% of FC is not the appropriate irrigation for the plants. The WUE of *A. vera* plants increased with water restrictions (Figs. 1 and 2). Our results also show that 20% of FC decreased the fresh weight of the leaves and gel production compared to control plants and with plants irrigated with 60% of FC. These results are in agreement with those reported in literature for *A. vera* (Delatorre-herrera et al., 2010; Silva et al., 2010; Silva et al., 2014). *A. vera* plants under water restriction increased the levels of soluble sugars. Polysaccharides improve water stress resistance and increase water use efficiency (Delatorre-herrera et al., 2010). Silva et al. (2014) have shown that there is correlation between water use efficiency and drought stress resistance in *A. vera*. Zeolite application increases plant growth, yield and water use efficiency through water uptake. Similar results have been reported by Sepaskhah and Barzegar (2010) and Gholamhoseini et al. (2013). A higher WUE always was related to a lower water use under drought condition. The most efficient plants were 40% FC with 8g zeolite; the least efficient were the 80% FC without zeolite aplication. The quality and quantity of gel components changes due to water deficit stress. Soluble sugars are the most important compounds in *A. vera* gels (Hamman, 2008). Soluble sugars play a critical role in cell division and leaf growth. Previous findings indicate that soluble sugars would increase in warm seasons compared with other seasons (Zapata et al., 2013). In CAM plants, sucrose is the final product in Calvin cycle which helps plant to overcome with drought stress through osmotic adjustment (Delatorre-Herrera et al., 2010). On the other hand, proline as an important amino acid plays a pivotal role in plants grown under drought stress conditions (Joyce et al., 1992; Balibrea et al., 1999). Increase in proline accumulation in water stressed plants has been reported by Delatorre-herrera et al. (2010). In the current study, *A. vera* plants under water restriction increased their levels of proline.

Aloin is one of the most important compounds found in *A. vera* (Groom and Reynolds, 1987). It has been reported that aloin content would increase in warm seasons of the year (Zapata et al., 2013). This might be due to more light in summer and its effect on secondary metabolites synthesis (Beppu et al., 2004). In most cases, environmental stresses increase aloin content (Rahimi-Dehgolan et al., 2012; Lucini et al., 2013). According to the previous results, aloin content in young leaves is more than old ones; in addition, aloin synthesis in summer is more than other seasons (Lucini et al., 2013; Ray and Gupta, 2013). Generally, in CAM plants, osmolite accumulation is one of the main approaches to deal with water deficit stress (Rontein et al., 2002; Chen and Murata, 2002; Delatorre-herrera et al., 2010).

5. Conclusion

In agricultural systems, final yield highly depends on environmental conditions so that abiotic stresses are the main limiting factors. Although water deficit stress could affect *A. vera* yield, proper management can diminish adverse effect of water deficit stress. For instance, zeolite application. Based on the findings of this experiment, it could be concluded that zeolite application significantly decreases water deficit stress effects and improved plant growth and yield. On the other hand, crop yield is correlated with osmotic adjustment mechanisms. In addition, WUE increased due to zeolite application so that the positive effect of zeolite increased over the time.

Acknowledgments

The authors would like to acknowledge the financial support received from the project via the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

References

Al-Busaidi, A., Yamamoto, T., Tanigawa, T., Rahman, H.A., 2011. Use of zeolite to alleviate water stress on subsurface drip irrigated barley under hot environments. Irrig. Drain. 60, 473–480.

Balibrea, M.E., Parra, M., Bolarin, M.C., Perez-Alfocea, F., 1999. PEG-osmotic treatment in tomato seedlings induces salt-adaptation in adult plants. Aust. J. Plant Physiol. 26, 781–786.

Bates, L.S.. Waldren, R.P., Tear, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 207, 205–207.

Beppu, H., Kawai, K., Shimpo, K., Chihara, T., Tamai, I., Ida, C., Ueda, M., Kuzuya, H., 2004. Studies on the components of Aloe arborescens from Japan - Monthly variation and differences due to part and position of the leaf. Biochem. Syst. Ecol. 32, 783–795.

Chen, T.H.H., Murata, N., 2002. Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr. Opin. Plant Biol. 5, 250–257.

Christopher, J.T., Holtum, J. A.M., 1996. Patterns of carbon partitioning in leaves of crassulacean acid metabolism species during deacidification. Plant Physiol. 112, 393–399.

Cousins, S.R., Witkowski, E.T.F., 2012. African aloe ecology: A review. J. Arid Environ. 85, 1–17.

Delatorre-herrera, J., Delfino, I., Salinas, C., Silva, H., Cardemil, L., 2010. Irrigation restriction effects on water use efficiency and osmotic adjustment in Aloe Vera plants (*Aloe barbadensis* Miller). Agric. Water Manag. 97, 1564–1570.

Geerts, S., Raes, D., 2009. Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. Agric. Water Manag. 96, 1275–1284.

Gholamhoseini, M., AghaAlikhani, M., Dolatabadian, A., Khodaei-Joghan, A., Zakikhani, H., 2012. Decreasing nitrogen leaching and increasing canola forage yield in a sandy soil by application of natural zeolite. Agron. J. 104, 1467–1475.

Gholamhoseini, M., Ghalavand, A., Dolatabadian, A., Jamshidi, E., Khodaei-Joghan, A., 2013. Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. Agric. Water Manag. 117, 106–114.

Gholizadeh, A., Amin, M.S.M., Anuar, A. R., Saberioon, M.M., 2010. Water stress and natural zeolite impacts on phisiomorphological characteristics of moldavian balm (*Dracocephalum moldavica* L.). Aust. J. Basic Appl. Sci. 4, 5184–5190.

Hamman, J.H., 2008. Composition and applications of *Aloe vera* leaf gel. Molecules. 13, 1599–1616.

Hazrati-Yadekori, S., Tahmasebi-Sarvestani, Z., 2012. Effects of different nitrogen fertilizer levels and hormone benzyl adenine (BA) on growth and ramet production of *Aloe vera* L. Iran. J. Med. Aromat. Plants 28, 210–223.

Herrera, A., 2009. Crassulacean acid metabolism and fitness under water deficit stress: If not for carbon gain, what is facultative CAM good for? Ann. Bot. 103, 645–653.

Joyce, P., Aspinall, D., Paley, L., 1992. Photosynthesis and the accumulation of proline in response to water deficit. Aust. J. Plant Physiol. 19, 249–261.

Lucini, L., Pellizzoni, M., Molinari, G. Pietro, 2013. Anthraquinones and β-polysaccharides content and distribution in Aloe plants grown under different light intensities. Biochem. Syst. Ecol. 51, 264–268.

Lüttge, U., 2004. Ecophysiology of Crassulacean Acid Metabolism (CAM). Ann. Bot. 93, 629–652.

Moreira, L.R.S., Filho, E.X.F., 2008. An overview of mannan structure and mannan-degrading enzyme systems. Appl. Microbiol. Biotechnol. 79, 165–178.

Murillo-Amador, B., Córdoba-Matson, M.V., Villegas-Espinoza, J.A., Hernández-Montiel, L.G., Troyo-Diéguez, E., García-Hernández, J.L., 2014. Mineral content and biochemical variables of *Aloe vera* L. under salt stress. PLoS One 9, 1–9.

Najafinezhad, H., Tahmasebi -Sarvestani, Z., Modarres Sanavy, S. A. M., & Naghavi, H.2014. Evaluation of yield and some physiological changes in corn and sorghum under irrigation regimes and application of barley residue, zeolite and superabsorbent polymer. Arch. Agron. Soil Sci. 61, 891–906.

Nobel, P.S., Zutta, B.R., 2007. Carbon dioxide uptake, water relations and drought survival for *Dudleya saxosa*, the "rock live-forever", growing in small soil volumes. Funct. Ecol. 21, 698–704.

Paez, A., Michael Gebre, G., Gonzalez, M.E., Tschaplinski, T.J., 2000. Growth, soluble carbohydrates, and aloin concentration of *Aloe vera* plants exposed to three irradiance levels. Environ. Exp. Bot. 44, 133–139.

Polat, E., Karaca, M., Demir, H., Onus, A. N., 2004. Use of natural zeolite (clinoptilolite) in agriculture. J. Fruit Ornam. Plant Reserarch 12, 183–189.

Radha, M.H., Laxmipriya, N.P., 2015. Evaluation of biological properties and clinical effectiveness of *Aloe vera*: A systematic review. J. Tradit. Complement. Med. 5, 21–26.

Rahi, T.S., Singh, K., Singh, B., 2013. Screening of sodicity tolerance in *Aloe vera* : An industrial crop for utilization of sodic lands. Ind. Crop. Prod. 44, 528–533.

Rahimi-Dehgolan, R., Tahmasebi Sarvestani, Z., Rezazadeh, S.A., Dolatabadian, A., 2012. Morphological and physiological characters of *Aloe vera* subjected to saline water irrigation. J. Herbs. Spices Med. Plants. 18, 222–230.

Ray, A., Dutta Gupta, S., Ghosh, S., 2013a. Isolation and characterization of potent bioactive fraction with antioxidant and UV absorbing activity from *Aloe barbadensis* Miller gel. J. Plant Biochem. Biotechnol. 22, 483–487.

Ray, A., Gupta, S.D., 2013. A panoptic study of antioxidant potential of foliar gel at different harvesting regimens of *Aloe vera* L . Ind. Crop. Prod. 51, 130–137.

Ray, A., Gupta, S.D., Ghosh, S., Aswatha, S.M., Kabi, B., 2013b. Chemometric studies on mineral distribution and microstructure analysis of freeze-dried *Aloe vera* L . gel at different harvesting regimens. Ind. Crop. Prod. 51, 194–201.

Rodríguez-García, R., Rodríguez, D.J. De, Gil-Marín, J. A., Angulo-Sánchez, J.L., Lira-Saldivar, R.H., 2007. Growth, stomatal resistance, and transpiration of *Aloe vera* under different soil water potentials. Ind. Crops Prod. 25, 123–128.

Rodríguez-González, V.M., Femenia, A., Minjares-Fuentes, R., González-Laredo, R.F., 2012. Functional properties of pasteurized samples of *Aloe barbadensis* Miller: Optimization using response surface methodology. LWT - Food Sci. Technol. 47, 225–232.

Rontein, D., Basset, G., Hanson, A.D., 2002. Metabolic engineering of osmoprotectant accumulation in plants. Metab. Eng. 4, 49–56.

Sahu, P.K., Giri, D.D., Singh, R., Pandey, P., Gupta, S., 2013. Therapeutic and Medicinal Uses of *Aloe vera* : A Review. Pharmacol. Pharm. 2013, 599–610.

Sankar, B., Jaleel, C. A., Manivannan, P., Kishorekumar, A., Somasundaram, R., Panneerselvam, R., 2007. Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench . Acta Bot.Croat. 66, 43–56.

SAS Institute Inc., 2002. The SAS System for Windows. In: Release 9.0. Statistical Analysis Systems Institute., Cary, NC, USA.

Sepaskhah, A.R., Barzegar, M., 2010. Yield , water and nitrogen-use response of rice to zeolite and nitrogen fertilization in a semi-arid environment. Agric. Water Manag. 98, 38–44.

Silva, H., Sagardia, S., Ortiz, M., Franck, N., Opazo, M., Quiroz, M., Baginsky, C., Tapia, C., 2014. Relationships between leaf anatomy, morphology, and water use efficiency in *Aloe vera* (L) Burm f. as a function of water availability. Rev. Chil. Hist. Nat. 87(13), 1-10.

Silva, H., Sagardia, S., Seguel, O., Torres, C., Tapia, C., Franck, N., Cardemil, L., 2010. Effect of water availability on growth and water use efficiency for biomass and gel production in Aloe Vera (*Aloe barbadensis* M.). Ind. Crops Prod. 31, 20–27.

Sturm, K., Koron, D., Stampar, F., 2003. The composition of fruit of different strawberry varieties depending on maturity stage. Food Chem. 83, 417–422.

Waller, T. a, Pelley, R.P., Strickland, F.M., 2004. Industrial processing and quality control of Aloe barbadensis (Aloe vera) gel. genus Aloe Ed. by Tom Reynolds CRC Press.

Winter, K., Aranda, J., Holtum, J. a. M., 2005. Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. Funct. Plant Biol. 32, 381-388.

Zapata, P.J., Navarro, D., Guillén, F., Castillo, S., Martínez-romero, D., Valero, D., Serrano, M., 2013. Characterisation of gels from different *Aloe* spp . as antifungal treatment: Potential crops for industrial applications. Ind. Crop. Prod. 42, 223–230.

Table1

Soil Physicochemical properties used in the experiments.

Texture	Filed capacity	Wilting	pH	EC	O.C[1]	T.N[2]	P	M. N[3]	K	Sulfur
	% by volume			(dS m $^{-1}$)			(g kg^{-1})			
Sandy loam	20.87	7.61	7.6	1.76	10. 3	1	0.020	0.020	0.42	0.49

1, 2 and 3 denotes the organic matter, total nitrogen and mineral nitrogen, respectively.

Table 2

Physicochemical properties of zeolite used at the experiments.

CaO	MgO	Na_2O	K_2O	AL_2O_3	SiO_2	P_2O_5	SO_3	Cl	Fe_2O_3	MnO	TiO_2
						g kg^{-1}					
23	1	10.8	30	120. 2	650	0. 1	-	-	15	0. 4	0. 3

CEC = 200 meq $(100g)^{-1}$

Table 3

The influence of irrigation regime and application of zeolite on some growth and yield characteristics of *A. vera* (n=4).

		Days after treatment (date)											
		90 (22 September) or summer				180 (21 December) or autumn				270 (21 March) or winter			
Irrigation regime (after depleting %FC)	Zeolite (g kg^{-1} soil)	Number of leaves/plant	Number of pup/ plant	fresh weight of Leaf (g)	fresh weight of gel (g)	Number of leave/ plant	Number of pup/ plant	fresh weight of Leaf (g)	fresh weight of Gel (g)	Number of leave/ plant	Number of pup/plant	fresh weight of leaf (g)	fresh weight of Gel (g)
20	0	13b^{c}	4.00^{b}	195.20^{cdef}	117.63^{bcd}	17.75ab	2.50^{b}	317.80^{cd}	180.00^{cd}	22^{bc}	3.25^{a}	399.41^{def}	232.99^{cd}
	4	13.75^{ab}	4.00^{b}	222.60^{bcd}	132.70^{bcd}	18.75a	3.83^{a}	303.17^{cd}	180.28^{cd}	22.3^{bc}	3.25^{a}	436.50^{cde}	256.53^{cde}
	8	14.25^{a}	4.25^{b}	296.46^{a}	185.17^{a}	18ab	3.25^{a}	405.80^{a}	241.30^{ab}	24.25^{a}	3.50^{a}	566.12^{ab}	353.88^{ab}
40	0	13^{bc}	3.75^{bc}	240.15^{bc}	144.08^{abc}	18.25ab	3.00^{a}	399.25^{a}	245.15^{a}	21.5^{cde}	3.25^{a}	490.87^{bc}	294.45^{bc}
	4	14^{a}	4.25^{b}	266.57^{ab}	159.60^{ab}	17.75ab	3.25^{a}	340.05^{bc}	208.85^{abc}	21.75^{bcd}	3.25^{a}	456.15^{cd}	271.58^{cd}
	8	14^{a}	6.75^{a}	248.15^{b}	134.58^{ab}	17.5bc	4.00^{a}	381.62^{ab}	219.23^{ab}	22.5^{b}	3.83^{a}	624.13^{a}	397.17^{a}
60	0	10.5^{f}	3.00^{cd}	178.62^{defg}	112.40^{bcd}	15.75de	2.50^{b}	271.52^{de}	161.00^{de}	20.5^{f}	3.25^{a}	348.57^{efg}	203.20^{def}
	4	12.25^{cd}	4.67^{b}	179.57^{defg}	103.25^{cd}	16.5cd	3.50^{a}	305.82^{cd}	178.10^{cd}	21^{def}	3.50^{a}	424.27^{cde}	307.30^{bc}
	8	12.5^{c}	4.25^{b}	200.05^{cde}	123.78^{bcd}	15.75de	3.50^{a}	333.32^{bc}	206.18^{bc}	21.5^{cde}	3.50^{a}	480.87^{bcd}	299.80^{bc}
80	0	10.25^{f}	1.75^{e}	150.42^{fg}	90.85^{d}	14.25f	2.00^{b}	221.50^{e}	139.00^{e}	20.75^{ef}	1.75^{b}	282.05^{g}	154.55^{f}
	4	11^{ef}	2.75^{d}	145.57^{g}	90.68^{d}	15.5de	2.00^{b}	234.72^{e}	144.20^{de}	20.75^{ef}	2.00^{b}	350.65^{efg}	203.53^{def}
	8	11.5^{de}	2.75^{d}	166.77^{efg}	104.98^{cd}	15.25ef	2.25^{b}	270.97^{de}	165.38^{de}	20.75^{ef}	2.25^{b}	332.05^{fg}	191.25^{ef}
Mean		12.50^{C}	3.84^{A}	207.51^{C}	124.97^{C}	16.75^{B}	2.96^{B}	315.46^{B}	189.054^{B}	21.64^{A}	3.048^{B}	432.64^{A}	263.85^{C}

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). Different capital letters amongst seasons show significant differences.

Table 4
The influence of applied water stress and zeolite on soluble sugars and proline of *A. vera* (n=3).

		Days after treatment (date)								
		90 (22 September) or summer			180 (21 December) or autumn			270 (21 March) or winter		
Irrigation regime (after depleting %FC)	Zeolite (g kg^{-1} soil)	Fructose	Glucose	Proline (mg [g $FW]^{-1}$)	Fructose	Glucose	Proline (mg [g $FW]^{-1}$)	Fructose	Glucose	Proline (mg [g $FW]^{-1}$)
		(mg [g $DW]^{-1}$)			(mg [g $DW]^{-1}$)			(mg [g $DW]^{-1}$)		
20	0	71.83^{e}	30.92^{fg}	0.75^{de}	46.60^{f}	29.70^{cd}	0.77^{bcd}	78.13^{efg}	35.53^{f}	0.74^{c}
	4	63.19^{fg}	29.34^{g}	0.71^{de}	43.47^{f}	25.33^{ef}	0.54^{e}	45.40^{hl}	37.30^{ef}	0.79^{c}
	8	40.12^{h}	23.05^{h}	0.62^{ef}	40.93^{f}	19.60^{g}	0.60^{de}	44.27^{l}	28.40^{g}	0.54^{d}
40	0	77.88^{d}	31.34^{fg}	0.75^{de}	69.57^{e}	27.40^{de}	0.85^{bc}	82.60^{ef}	37.73^{ef}	0.80^{c}
	4	56.14^{g}	29.46^{g}	0.71^{de}	70.07^{e}	22.07^{fg}	0.61cde	62.13^{ghl}	35.27^{f}	0.78c
	8	69.26^{ef}	29.05^{g}	1.21^{b}	65.83^{e}	23.63^{efg}	0.86^{bc}	65.97f^{g}	34.20^{f}	1.03^{b}
60	0	101.68^{c}	38.99^{d}	1.45^{a}	94.17^{c}	33.90^{c}	1.35^{a}	108.77^{d}	46.73^{d}	1.24^{a}
	4	99.08^{c}	34.52^{ef}	1.18^{b}	91.90^{cd}	30.30^{cd}	1.25^{a}	106.10^{d}	40.33^{e}	1.04^{b}
	8	83.86^{d}	37.00^{de}	0.62^{ef}	65.07^{e}	25.07^{ef}	0.31^{f}	87.20^{e}	40.87^{e}	0.40^{d}
80	0	234.71^{a}	79.83^{a}	1.45^{a}	178.33^{b}	70.17^{a}	0.98^{b}	249.87^{a}	87.30^{a}	1.04^{b}
	4	230.67^{a}	75.00^{b}	1.18^{b}	210.27^{a}	67.97^{a}	0.92^{b}	230.93^{b}	80.23^{b}	0.97^{b}
	8	177.92^{b}	68.28^{c}	1.21^{b}	78.47^{de}	61.73^{b}	0.87^{b}	187.73^{c}	73.53^{c}	0.99^{b}
	Mean	108.86^{A}	42.23^{B}	0.97^{A}	87.89^{C}	36.40^{C}	0.83^{B}	112.42^{A}	48.11^{A}	0.86^{B}

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). Different capital letters amongst seasons show significant differences.

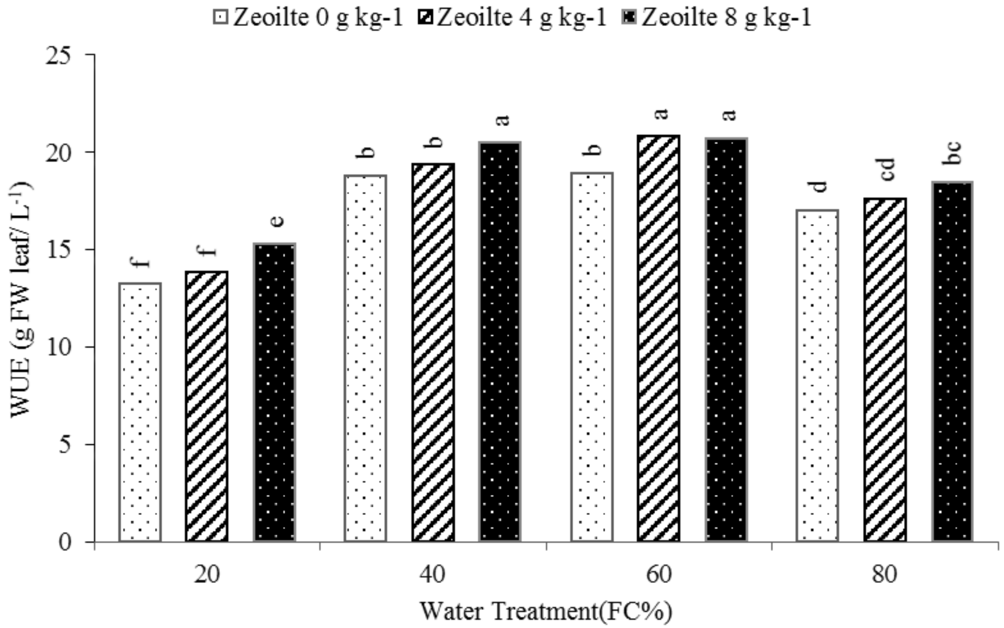

Fig. 1. Interaction of irrigation regimes (FC %) × zeolite (Z) treatments on water use efficiency (WUE) leaf fresh weight at different times. FC (%): Irrigation after depleting 20, 40, 60 and 80% of the filed capacity. Means within a column followed by the same letter are not significantly different at the level of 5%.

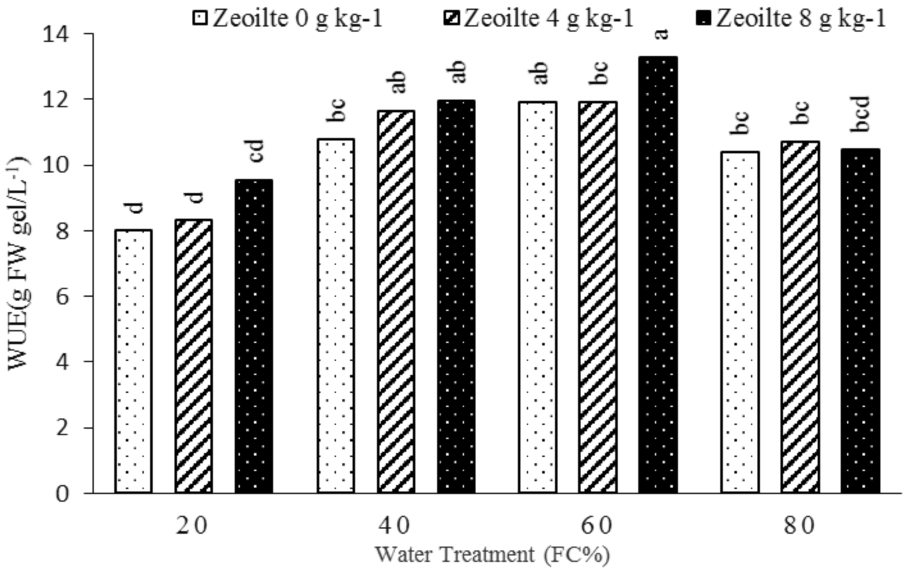

Fig. 2. Interaction of irrigation regimes (FC %) × zeolite (Z) treatments on WUE gel fresh weight at different times.
FC (%): Irrigation after depleting 20, 40, 60 and 80% of the filed capacity. Means within a column followed by the same letter are not significantly different at the level of 5%.

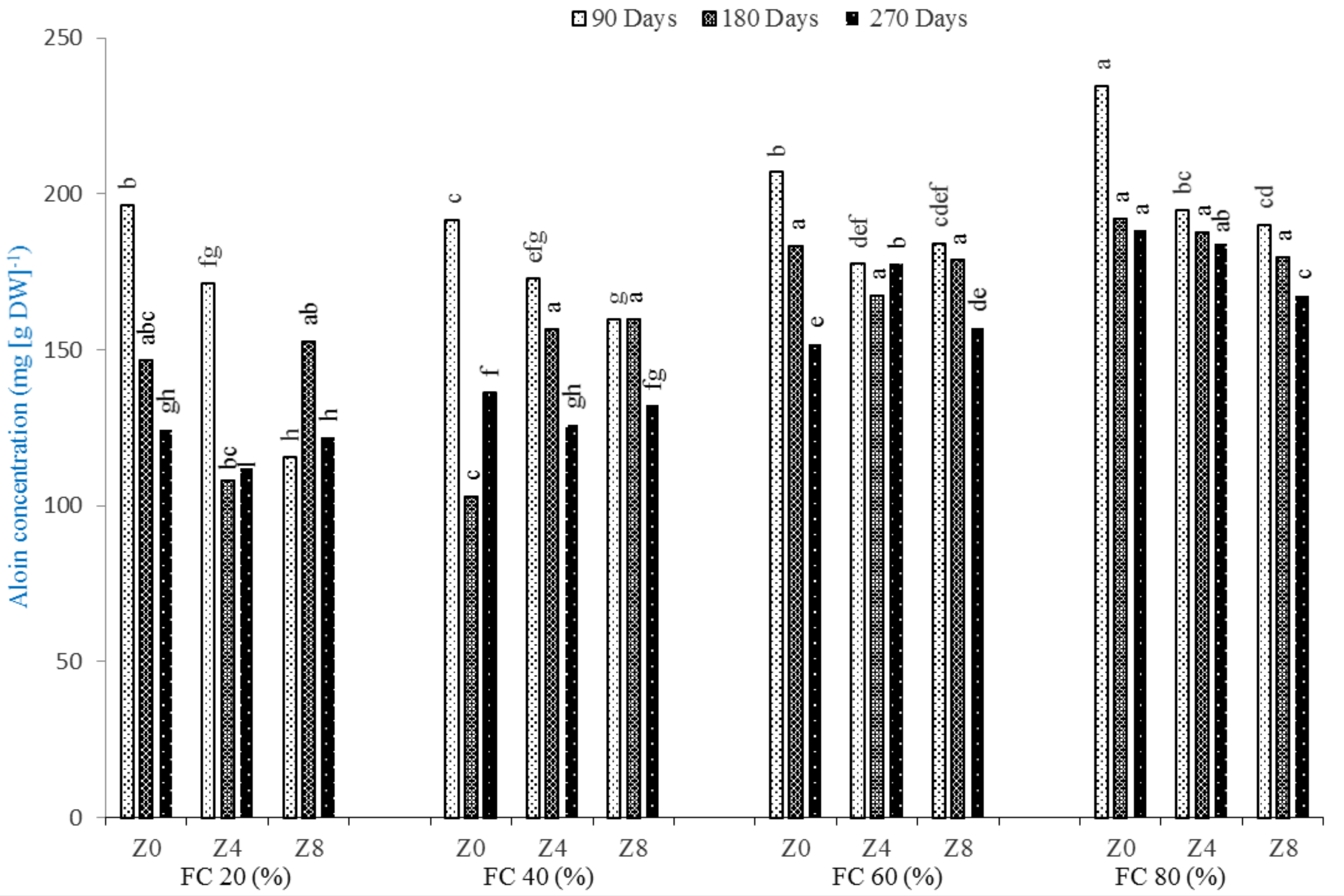

Fig. 3. Interaction of irrigation regimes (FC %) × zeolite (Z) treatments on aloin concentration at different times.
FC (%): Irrigation after depleting 20, 40, 60 and 80% of the filed capacity, Z: Zeolite, 0, 4, and 8 g kg^{-1} soil. Means within a column followed by the same letter are not significantly different at the level of 5%.