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Surfactant and Limited Irrigation Effects on Forage and Seed Production and Water Use Efficiency in Alfalfa (*Medicago sativa* L.)

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Abstract

Alfalfa is one the most important forage resources in arid and semiarid regions of the world. To evaluate the response of alfalfa to limited irrigation and surfactant application, an experiment was conducted at Research Farm of College of Agriculture, University of Tehran, Iran, during 2013 and 2014 growing seasons. The experimental treatments were arranged as split plots based on a complete randomized block design with three replications. The limited irrigation treatments comprised of replenishment of 100%, 75% and 50% of weekly evaporation and plant water requirements assigned to the main plots. Water treatments of control (water alone) and water + surfactant, assigned to the subplots. The quantitative and physiological characteristics of alfalfa forage were recorded at 10% flowering stage. The seed yield of alfalfa was measured after the plants reached full physiological maturity stage. The result of the experiment showed that as the severity of limited irrigation increased, plant height, tiller number per plant, RWC, total forge yield and seed yield followed a decreasing trend. Across all the limited irrigation systems, surfactant application increased plant height, RWC, seed yield and total forge yield. As the severity of limited irrigation increased, water use efficiency (WUE) in forage yield followed a significant increasing trend. The highest forage (7500 kg/ha) and seed yield (820 kg/ha) under limited irrigation treatments were achieved at 75% weekly evaporation and plant water requirements + surfactant, while the highest irrigation water use efficiency for forage (1.5 kg/m^3) and seed (0.16 kg/m^3) production was observed in limited irrigation treatment of 50% weekly evaporation and plant water requirements + surfactant.

Keywords: alfalfa, limited irrigation, surfactant, vegetative growth traits, forage yield, seed yield, water use efficiency

1. Introduction

Drought is one of the most common and critical issues in arid and semi-arid regions of the world. Water shortage for human and agricultural consumption is a vital matter in some parts of the world. According to some predictions, global warming and precipitation decrement will be more violent in the near future (Farre & Faci, 2006). Water is known as the most important factor in crop yields and freshwater sources conservation plays an important role in sustainable agriculture. By the year 2020 the number of countries that will be faced to water scarcity, reaches 35 (Morid et al., 2004). So, more attention is needed to improve the management of water consumption in countries located in arid and semi-arid regions of the world. Based on the research literature, limited irrigation treatments increases the pure income of farm (Chaichi et al., 2015). The goal of limited irrigation is to increase irrigation water use efficiency (IWUE) by reducing the amount of water in irrigation or by reducing the number of irrigation events (Kirda, 2002). By inducing limited irrigation methods not only water consumption will be reduced but also the area under cultivation will be increased (Safai et al., 2011).

Surfactant is the abbreviation form of "Surface Active Agent". It composes of two polar molecules which include a hydrophilic head (hydrophilic) and a hydrophobic tail (hydrophobic) (Turcios, 2007). The major effect of surfactant is on the surface tension of the air-water interface. Because of these characteristics by application of surfactant to water, the speed of water penetration into the soil will be increased. Additionally, with the specific volume of water, broader profile of soil will be wetted (Leinauer, 2002). Surfactants also help uniformity of soil moisture distribution and root zone moisture holding capacity and as a consequence will improve crop yield (Wolkowski et al., 1985). Surfactant provides the plants with the better establishment by enhancing excess water absorption in the soil and makes it available for plants in the appropriate situation. Consequently production of plants will be increased (Ahmed & Verplancke, 1994). Economical evaluation have shown that using surfactant increased yield production cost, however, the yield increment could compensate surfactant price and consequently higher profit could be achieved. By surfactant application in limited irrigation systems, higher yields could be produced (Chaichi et al., 2015).

The demand for the food will reach two times more than the current level till the year of 2030 (Eshghizadeh et al., 2007). Development of animal husbandry industry to meet the needs of population growth for protein products requires a serious approach for supplying forage and livestock feed (Dastjerdi, 2012). Alfalfa (*Medicago sativa* L.) is known as the best forage among different forages for livestock. This plant has a great nutritional value compared to other forage sources (Khodabandeh, 2009). The superiority of this plant among other forage crops is due to its very high yield, broad compatibility with weather conditions, nitrogen-fixing ability, resistance to the grazing and good re-growth ability during the growing season (Changizi & Moaveni, 2007). All these characteristics along with alfalfa potential drought tolerance make it the best forage crop to be produced under limited water conditions in arid and semi-arid regions of the world. No experiment has studied the effect of surfactant application and limited irrigation on alfalfa. There is a need to evaluate the application of new technologies on alfalfa response to drought stress to find new solutions for higher forage quality along with more efficient water consumption in the arid and semi-arid regions of the world.

This experiment was conducted to evaluate the response of alfalfa to limited irrigation and surfactant application.

2. Materials and Methods

2.1 Site Description

A 2-yr (2013 and 2014) experiment was conducted at the Research Farm of the College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (N35°56′N, E50°58′E). The climate type of this site is considered as arid to semi-arid with long-term (50-yr) air temperature of 13.5 °C, soil temperature of 14.5 °C, and 262 mm of annual rainfall. The weather condition at the experimental site during the two growing seasons is shown in Table 1 and Figure 1.

Table 1. Monthly relative humidity, evaporation, precipitation and mean air temperature during 2013 and 2014 growing seasons

Month	Relative Humidity (%)		Evaporation (mm)		Precipitation (mm)		Mean Air Temperature (ºC)	
	2013	2014	2013	2014	2013	2014	2013	2014
May	45.3	37.9	7.8	10	21.2	18.4	18.7	22.1
June	38.2	30.7	13.2	13	1.5	11.6	24.1	26.2
July	39.5	30.9	14.0	14	0.0	8	27.1	28.7
August	42.2	27.0	9.9	12	3.9	0.0	25.3	28.5
September	32.7	33.7	9.9	11	0.0	0.0	25.3	24.0

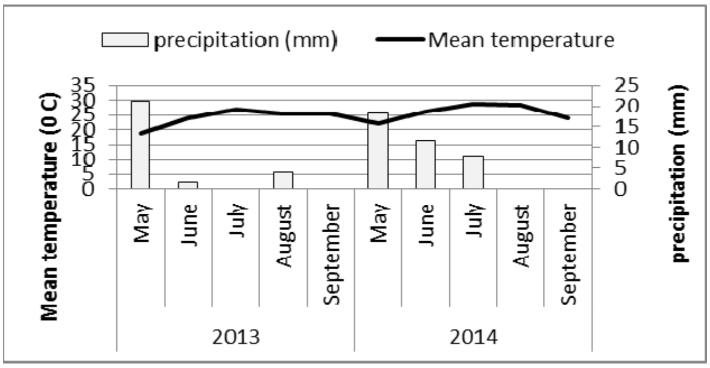

Figure 1. Ambriothermic curve of experimental site base on mean temperature and precipitation of weather station of Karaj (2013-2014)

According to the USDA classification (Soil Survey Staff, 1999), the soil at the site is classified as a typic haplocambid (Mirkhani et al., 2010). Prior to planting, soil samples were taken from 0 to 30 cm soil depth and analyzed for selective physical and chemical properties which included soil textures, pH , EC, total nitrogen (N), available phosphorus (P), and available potassium (K). All soil characteristics of the experimental site are presented in Table 2.

Table 2. General properties of the soil of the experimental site (depth of 0–30 cm)

Year	Soil texture	pH	EC (ds m^{-1})	Total N (%)	Available P	Available K
					-------------mg kg^{-1}------------	
2013	Clay loam	8	1.86	0.09	8.87	225
2014	Clay loam	7.9	1.96	0.07	9.0	202

2.2 Experimental Design and Factors

The statistical design of the experiment was split plot based on a randomized complete block design (RCBD) with three replications. The experimental treatments comprised of three levels of irrigation systems and two types of water treatments. Main plots were allocated to irrigation regimes comprised of normal irrigation (replenishment of 100% of weekly evaporation and plant water requirements) (I_{100}) and limited irrigations including I_{75} (replenishment of 75% of weekly evaporation and plant water requirements) and I_{50} (replenishment of 50% of weekly evaporation and plant water requirements). Sub plots were assigned to water treatments of control (water alone irrigation) and water + surfactant (1 ppm) irrigation. The study was carried out in plot sizes of 4 × 2 = 8 m^2, which consisted of 4 rows of cropping, 50 cm apart. The Alfalfa (domestic Hamedani cultivar) was cultivated at the rate of 25 kg of seed per hectare.

To prepare a suitable seedbed, the land was cultivated by a deep plough in autumn and a light one in the spring of each year. The final preparation was achieved after 2 vertical and horizontal disks were applied. Seedbed preparation was accomplished on 1st May, 2013 and 3rd May, 2014 for the first and second experimental periods, respectively.

The vermi-compost (2 tons ha^{-1}) fertilizer was incorporated to the soil before land cultivation. Before sowing, the alfalfa seed was inoculated by biological fertilizer comprised of a mixture of different probiotic bacteria (20cc bacterial solution per 1kg seed) according to Somasegaran and Hoben (1994). The blend bio-fertilizer comprised of different probiotics of Azotobacter + Azosperilium + Mycorrhiza + Bacilus and Rhizobium bacterial was provided by the Soil Microbiology Lab. of Department of Soil Science, College of Agriculture, University of Tehran.

2.3 Irrigation

In both years, all experimental plots were irrigated normally until plants reached full establishment (4-6 leaf stage). Times of irrigation in the normal irrigation regime were scheduled based on the common practice in the area, which consisted of irrigating at 7 day intervals. At the trigger of the second step of irrigation (limited irrigation), all experimental plots were protected by pile of soil to preserve the measured irrigated water during the seasons. Likewise, the timing of the irrigation treatments ($IR_{100,}$ IR_{75} and IR_{50}) were scheduled once a week and started on 22nd May and 25th April in 2013 and 2014 (when plants reached 4 to 6-leaf growth stage), respectively.

Actual crop water requirements for alfalfa were determined according to the crop evapotranspiration (ETc), estimated from the potential evapotranspiration (ETo), and using the crop coefficients (Kc) by the following equation:

$$ETc = ETo \times Kc \qquad (1)$$

ETo was calculated by the Penman–Monteith method (Allen et al., 1998) using daily data of synoptic weather station at Research Farm of College of Agriculture located in Karaj, Iran. The Kc is defined as the ratio of the crop evapotranspiration rate to the reference evapotranspiration rate. The localized step-wise Kc for alfalfa was 0.9 in Karaj according to FAO, 2012 report. The water requirement for individual plots was measured in gallon per week then it was converted to liter per week. The amount of water applied based for each treatment was calculated by:

$$I_n = \frac{0.623 \times A \times K_c \times ETo}{IE} \qquad (2)$$

Where I_n is the volume of irrigation water (gallons), 0.623 the constant of equation, A the canopy surface area (sq. ft.) in each plot, Kc the crop coefficient, ETo the accumulative weekly potential evapotranspiration (in.) and IE the irrigation efficiency. The surface area of each plot was 8 m^2 and the irrigation use efficiency was assumed 80% in both years (Howell, 2003). A counter meter was used for accurate water measurement and control. The total amount of irrigation water used during the plant life cycle were as follows: IR_{100} = 5150 m^3 ha^{-1}, IR_{75} = 3910 m^3 ha^{-1} and IR_{50} 2575 m^3 ha^{-1} during the first year, and IR_{100} = 9000 m^3 ha^{-1}, IR_{75} = 6750 m^3 ha^{-1} and IR_{50} = 4500 m^3 ha^{-1} during the second year for normal, moderate and severe limited irrigation regimes, respectively. To reach the physiological maturity the different irrigation regimes continued until 25th Aug. and 8th Sep. in 2013 and 2014, respectively. In this study non-ionic surfactant (10% alkyl polyglycoside, 7% EO/PO block copolymer and 83% water) was applied at the rate of 1 ppm which was added to the irrigation water treatments during irrigation (Karcher & Landreth, 2003; Mitra et al., 2006).

2.4 Measurements

The quantitative forage characteristics of Alfalfa were recorded at 10% flowering stage. The seed yield of Alfalfa was measured after the plants reached full physiological maturity stage.

Water use efficiency of irrigation for forage and seed yield (kg seed/m^{-3}) was calculated by dividing the yield (kg ha^{-1}) by the volume of irrigation water used (m^3 ha^{-1}).

To calculate the RWC, ten random leaf samples were taken from the lower, middle and top layers of the canopy of the alfalfa in each plot at early flowering stage (before the pollination). The sampling was repeated two times during the early flowering season. The leaf samples were taken in early morning and were carried out in sealed containers to be weighted in the laboratory. In the lab. the samples were put in the water for 24 hours and weighted again for saturated weight. To measure the dry weight, leaves were transferred to the oven, 70 Centigrade temperature, for 72 hours. RWC was computed by the following equation:

$$RWC = F_w - D_w / S_w - D_w \times 100 \tag{3}$$

Where, the F_w is fresh weight, D_w is the dry weight and the S_w is saturated weight (Ritchi et al., 1990).

2.5 Statistical Analysis

Data were analyzed with SAS software (V9.2). Mean comparison implemented using Duncan's multiple range test at the 95% level of probability. All differences reported are significant at $P \leq 0.05$ unless otherwise stated. Graphs were designed by using Microsoft Office Excel.

3. Results and Discussions

3.1 Plant Height

The height of the plant decreased by increasing the severity of limited irrigation, however, it significantly increased across all irrigation treatments with surfactant application (Table 3).

The interaction of irrigation systems and water treatments showed that the surfactant application at each irrigation treatment, significantly increased the plant height compared to control (no surfactant application) (Figure 2). These results support the previous researches which showed that the corn height was enhanced with the surfactant application under limited irrigation conditions (Mehrvarz, 2013).

Table 3. Plant height, leaf/stem ratio, number of tillers per plant and RWC of alfalfa as affected by limited irrigation systems and water treatments

Treatments	**Plant height (cm)**	**Leaf/stem ratio**	**Tiller number per plant**	**RWC (%)**	**Total forage yield (kg ha^{-1})**	**Seed yield (kg ha^{-1})**
Irrigation system						
100%	48.50a	2.40b	5.10a	56.88a	8354.8a	919.93a
75%	43.20b	3.12a	4.82a	51.38b	6970.3b	719.02b
50%	41.02c	2.50b	4.41a	50.29c	5921.7c	552.97c
Water treatment						
Water	42.12b	2.70a	4.67a	50.77b	6543.40b	660b
Water+ surfactant	46.28a	2.69a	4.88a	54.90a	7421.25a	801.1a

Note. Mean comparison was implemented using Duncan test at the 95% level of probability. All differences reported are significant at $P \leq 0.05$ unless otherwise stated.

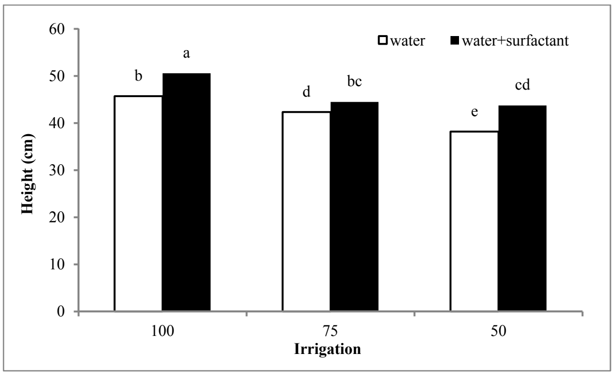

Figure 2. The interaction of irrigation systemes and water treatments (with and without surfatant) on the height of alfalfa (*Medicago sativa* L.) (mean 2013 and 2014)

3.2 RWC (Relative Water Content)

Across water treatments (surfactant application), the RWC followed a decreasing trend as the severity of limited irrigation increased However, water treatments (surfactant application) resulted in a great effect on RWC at 75% and 50% irrigation treatment which increased by 8% and 12% respectively (Figure 3). Very interestingly, by saving 25% water in I_{50} we would have equal RWC to I_{75}. According to these results, it seems that surfactant can help the plants to conserve more water in severe drought conditions which well demonstrates the advantage of wetting agent application in harsh situations. Since RWC has a great correlation with soil moisture, by surfactant application more water will reach the plant roots in deeper soil layers and help to save plants in drought stress conditions. Due to the effect of surfactant on the surface tension of the air-water interface, by application of surfactant to water, the speed of water penetration into the soil will be increased. Though, less water will be evaporated due to the environmental factors like wind and sun irradiations. In this way, with the specific volume of water, broader soil profile will be wetted (Leinauer, 2002). Surfactants also help uniformity of soil moisture distribution and root zone moisture holding capacity and as a consequence will improve crop yield and water use efficiency (Wolkowski et al., 1985). Consequently, in all treatments with surfactant application the RWC rate, which is a characteristic for assessing the crops tolerance to drought stress, was more than the control (no surfactant application).

RWC of *Psyllium* sp. was decreased by increasing drought stress (Afsharmanesh, 2008). Treatments of limited irrigation in diverse genotypes of red bean reduced the RWC, dry yield, harvest index and plant height (Sadeghipour, 2009).

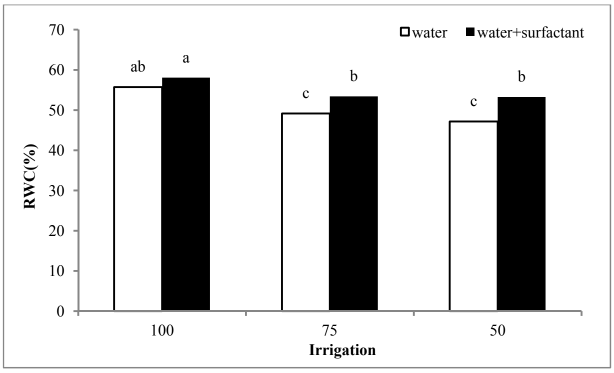

Figure 3. The interaction of irrigation systemes and water treatments (with and without surfatant) on the RWC of alfalfa (*Medicago sativa* L.) (mean 2013 and 2014)

3.3 Total Forage Yield

As the severity of the limited irrigation increased, total forage yield followed a decreasing trend. Moderate limited irrigation (I_{75}) and also the Sever limited irrigation (I_{50}) increased the total yield by 11% and 13% when received surfactant. However, 25% of irrigation water was saved by I_{75} + surfactant treatment while alfalfa yield did not suffer compared to control (I_{100}) (Figure 4).

It is important to note that in both water treatments (with and without surfactant application) the total forage yield did not experience significant changes when severity of limited irrigation increased from I_{75} to I_{50}. This could be explained by the alfalfa well root development especially in the second year of the experiment. The good performance of water treatment (surfactant application) at I_{75} irrigation treatment indicates that in a dry region like Karaj, by saving 25% of irrigation water in I_{75}+ surfactant treatment, we are still able to gain almost the same forage yield as control with no water stress (I_{100} + no surfactant application) (Figure 4). In I_{50} + surfactant the same yield as I_{75} + no surfactant application, was achieved. The positive effect of surfactant is explained by its role in increasing water infiltration into the soil which can reduce the run off rate as well as better water distribution in the soil profile (Mitra et al., 2006). Kostka (2000) stated that surfactant as a moisture absorbent can be used as a management method to increase the efficiency of water use. Increasing water stress from optimum irrigation (I_{100}) to moderate (I_{75}) and severe limited irrigation (I_{50}), resulted in 20% and 34% reduction in forage dry matter yield. Jahanzad et al. (2013) showed a stepwise reduction in total dry matter (TDM) and grain yield (GY) of sorghum in both treatments of with and without surfactant as the limited water severity increased. However, surfactant application at any irrigation level resulted in higher grain yield (GY) and total dry matter (TDM) compared to no-surfactant treatments (Chaichi et al., 2015). Application of nonionic surfactant, (0.36 liter ha^{-1}) on soybean, resulted in 37% increment in forage production (Mccauley, 1993).

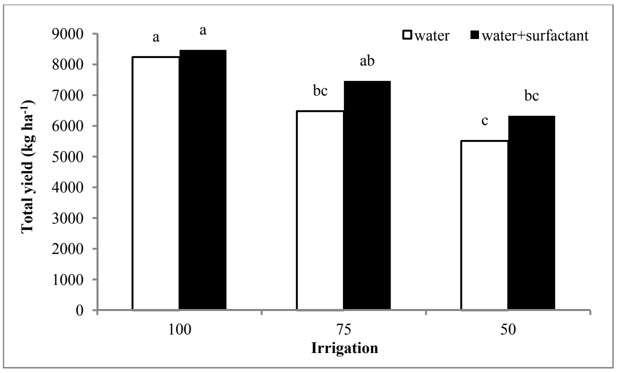

Figure 4. The interaction of irrigation systemes and water treatments (with and without surfatant) on the total yield of alfalfa (*Medicago sativa* L.) (mean 2013 and 2014)

3.4 Seed Yield

As the severity of the limited irrigation increased, seed yield followed a decreasing trend (Table 3). In all treatments with surfactant application, seed yield was more than the control (no surfactant utilization). The highest seed yield was gained at I_{100} + surfactant. Considering the results obtained in I_{75} + surfactant treatment, it could be concluded that by conserving 25% of irrigation water, the same seed yield of control (I_{100}) could be achieved. Moreover, at 50% irrigation water with surfactant treatments the same seed yield as in I_{75} + no surfactant application was obtained. In this case 25% of irrigation water will be saved with no reduction in seed yield compare to I_{75} + no surfactant treatment (Figure 5). Our results supports the findings of the other researchers like Naemi et al. (2009) who reported that the seed yield of canola was decreased under limited irrigation by about 30% which occurred due to the shattering of siliques under stress condition. By increasing the severity of limited irrigation for soybean, the seed number per plant, 1000 seed yield, seed yield, biological yield and harvest index were reduced (Rustaei et al., 2012).

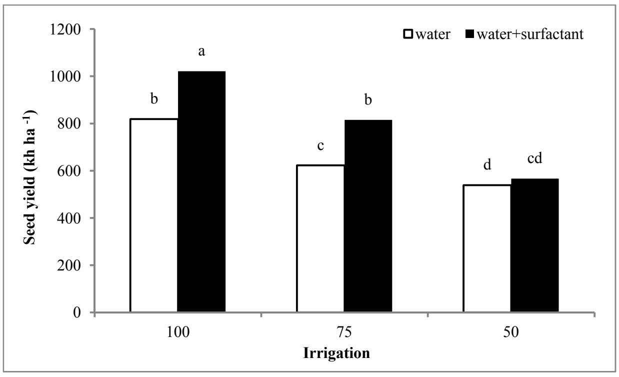

Figure 5. The interaction of irrigation systems and water treatments (with and without surfatant) on the seed yield of alfalfa (*Medicago sativa* L.) (mean 2013 and 2014)

3.5 Effect of Irrigation Regime and Surfactant Application on Irrigation Water Use Efficiency (IWUE) in Forage Production

The WUE is expressed as total above ground biomass to total evapotranspiration. IWUE remarkably increased under both limited irrigation systems (I_{75} and I_{50}) and surfactant application (Table 4).The effect of surfactant was quietly significant in I_{50} + surfactant treatment which increased the IWUE by 12% (Figure 6). These results corresponds with findings of Kiani and Reisi (2013) on soybean. IWUE is influenced by crop yield potential, method of irrigation and climatic characteristics of a region. It has been demonstrated that WUE in different crops have increased in water stress condition (Zegada-Lizarazu & Ijima, 2005; Nagaz et al., 2009).

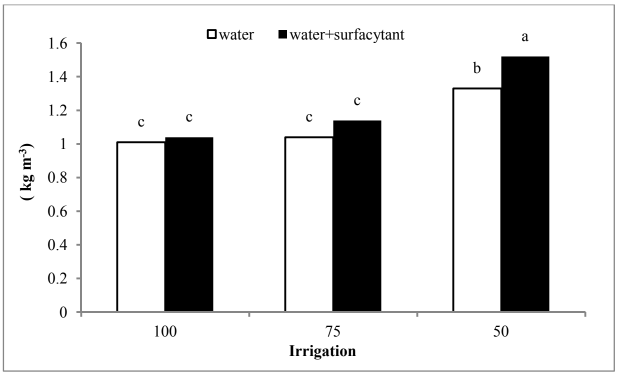

Figure 6. The interaction of irrigation systems and water treatments (with and without surfatant) on the IWUE in alfalfa (*Medicago sativa* L.) forage production (mean 2013 and 2014)

3.6 Water Use Efficiency (WUE) for Seed Yield

The highest IWUE (2 kg m^{-3}) was gained in I_{50} + surfactant treatment. The surfactant treatment showed a significant influence on IWUE in all treatments (Figure 7). These results are supported by the previous reports by Chaichi et al. (2015) who explained that by application of surfactant in irrigation water, both IWUE and GY (grain yield) of corn increased.

Water use efficiency (WUE) in both limited irrigation treatments of I_{75} + surfactant application and I_{50} + surfactant application increased by 12% and 5%, respectively, compared to control (no surfactant application) which clearly displays the positive role of surfactant application in deficit water conditions. Thus, the outcome of this research indicated that by increasing the severity of limited irrigation, the amount of irrigation water that could be available for plants in limited irrigation will be increased by surfactant utilization and subsequently, IWUE for forage yield would be raised (Chaichi et al., 2015). The highest IWUE for grain yield have been reported in 50% ETc treatment (3.25 kg m^{-3}) for corn by Di Paolo and Rinaldi (2008) which supports our findings.

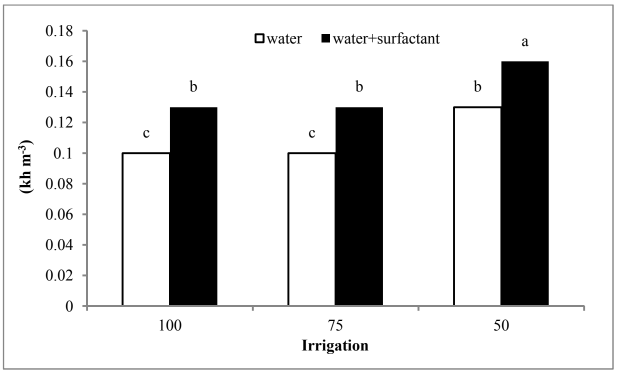

Figure 7. The interaction of irrigation systems and water treatments (with and without surfatant) on the IWUE in alfalfa (*Medicago sativa* L.) seed production (mean 2013 and 2014)

Table 4. IWUE for total forage and seed yield of alfalfa as influenced by irrigation systems and water treatments

Treatments	IWUE for total forage (kg m^{-3})	IWUE for seed yield (kg m^{-3})
Irrigation system		
100%	1.02b	0.11b
75%	1.09b	0.12b
50%	1.40a	0.14a
Water treatments		
Water	1.13b	0.11b
Water+ surfactant	1.23a	0.13a

Note. Mean comparison was implemented using Duncan test at the 95% level of probability. All differences reported are significant at $P \leq 0.05$ unless otherwise stated.

4. Conclusion

With increasing the severity of limited irrigation, plant growth characteristics, forage and seed yield followed a decreasing trend. Across all the irrigation systems, surfactant application modified the adverse effects of limited irrigation and significantly increased water use efficiency in forage and seed production in alfalfa. According to the results of this experiment, surfactant application at moderate limited irrigation treatment (75% weekly evaporation and plant water requirement + surfactant) in dry regions could be agronomic and economically point of view justified by 25% water conservation along with securing forage and seed yield equal to 100% irrigation treatment without surfactant application.

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